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Recent advances in the pharmacology, neurotoxicology and epidemiology of the adenosine A_{2A} receptor have provided evidence that A_{2A} receptor antagonists (including caffeine) may offer therapeutic benefits in Parkinson's disease (PD) at multiple levels. Not only does A_{2A} receptor blockade reduce the symptomatic psychomotor slowing characteristic of PD, but based on recent preclinical data on rodents and non-human primates A_{2A} receptor blockade potentially can attenuate neurotoxin-induced dopaminergic neuron loss and the development of maladaptive (dyskinetic) responses to chronic dopaminergic therapy. The conference and post-conference publication have been organized to systematically explore the role of the A_{2A} receptor in PD through sequential themes leading from $A_{2A}R$ neurobiology to the development of clinical trials for A_{2A} antagonists in PD. ***The purpose of our post-conference publication -- a special supplement issue of the journal Neurology -- is to broadly disseminate the information generated by the conference to a wide audience of basic and clinical neuroscientists in academics, government and industry.*** Given this journal's high profile and direct distribution of 20,000 as well as PubMed indexing, the publication will markedly enhance the dissemination of information coming out of the conference.

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TRANSLATING ADENOSINE A_{2A} RECEPTOR BIOLOGY INTO NOVEL THERAPIES FOR PARKINSON'S DISEASE

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Guest Editors

LIPPINCOTT WILLIAMS & WILKINS

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Dedication

Introduction

Keynote Address

Articles

I. THE BIOLOGY OF A_{2A} RECEPTORS IN THE BASAL GANGLIA

II. A_{2A} RECEPTOR MODULATION OF MOTOR SYSTEMS FOR SYMPTOMATIC THERAPY IN PD

III. A_{2A} RECEPTORS IN NEUROPROTECTION OF DOPAMINERGIC NEURONS

This supplement to *Neurology* was produced by Lippincott Williams & Wilkins, Baltimore, Maryland. The **United States Department of Army — Neurotoxin Exposure Treatment Research Program** sponsored its publication.

This supplement to based upon an international research conference entitled, *Translating Adenosine A_{2A} Receptor Biology into Novel Therapies for Parkinson's Disease*, held in Boston, USA, September 25–27, 2002 and co-chaired by Drs. Michael Schwarzschild and Tom Chase. The conference was sponsored by the **National Institute of Neurological Disorders and Stroke**, with additional support from the National Institute on Aging, the National Institute of Environmental Health Sciences, the Department of the Army (NETRP), Kyowa Hakko Kogyo, Aventis, Vernalis, Schering-Plough, Biogen, Merck, Roche, Pfizer.

J. Stephen Fink, MD, PhD (1950–2002)

Our friend, our colleague, and a pioneer of research on the adenosine A_{2A} receptor in the service of improved neurotherapeutics, Dr. J. Stephen Fink, died December 30, 2002 (figure). Steve originally accepted an invitation to chair a session at this symposium but later reluctantly informed us he would be unable to participate because of the recently diagnosed glioblastoma that would claim his life only 3 months later. His death deeply saddens us and is a painful loss to the adenosine receptor and Parkinson's disease (PD) research communities. Steve was a gifted scientist who seamlessly combined his basic and clinical research skills to advance our knowledge of the A_{2A} receptor and PD.

Steve trained as a movement disorder specialist at Massachusetts General Hospital and became an international leader in clinical research of PD. Despite his growing success and responsibilities, he maintained his clinical practice and was beloved by his patients for his gracious caring manner. He was also an outstanding neuroscientist with considerable expertise in molecular analysis of neuropsychiatric disorders, particularly disorders of the basal ganglia. His contributions to adenosine A_{2A} receptor research began when he cloned (at approximately the same time as did Dr. Parmentier's group in Belgium) the rat A_{2A} receptor. His work, along with others', next showed that A_{2A} receptors are colocalized with dopamine D_2 receptors in striatopallidal neurons (at approximately the same time as Dr. Schiffmann in Belgium). This body of work established a strong anatomic basis for adenosine-dopamine interaction, an important step leading to the development of the concept of A_{2A} antagonists as a novel treatment strategy for PD.

After the cloning and CNS localization of A_{2A} receptors, Steve quickly embarked on a series of pharmacologic studies to explore the potential of A_{2A} antagonists as a new antiparkinsonian treatment strategy. Together with several other laboratories, his laboratory's work demonstrated that A_{2A} antagonists could synergize with dopamine D_2 agonists to enhance motor function and c-*fos* expression in animal models of PD. His work also helped demonstrate cross-talk between the A_{2A} receptor and the dopamine D_1 receptor.

He also contributed to the concept of A_{2A} antagonists for PD therapy through his important role in initiating a research project in late 1994 to generate A_{2A} receptor knockout mice in his laboratory. Later,

J. Stephen Fink, MD, PhD (1950–2002)

while pursuing an opportunity in industry to advance a neurotransplantation treatment for PD, he continued to contribute his expertise to the behavioral and neurochemical characterization of the A_{2A} receptor knockout mice, which highlighted the potential of A_{2A} receptor antagonists to serve as neuroprotectants in ischemia, Huntington's disease, and PD.

At the beginning of 2000, Steve accepted the position of chair of the Department of Neurology at Boston University School of Medicine. He was genuinely excited about returning to basic science and clinical research on the A_{2A} receptor in the development of improved treatments for neurologic disorders. One of his immediate goals was to establish an active research center at the department with a strong emphasis on A_{2A} receptor neurobiology. Even during his illness,

Steve continued to direct an A_{2A} receptor project. Although we knew his time remaining would be far too short, his sudden passing left us stunned once again.

Steve's strong support and encouragement were instrumental in launching our adenosine research. That his death occurred at a time when the translation of A_{2A} receptor biology into neurotherapeutic application is accelerating only serves to amplify the loss to our research community. For those of us who were privileged to work with and know Steve, this was particularly painful. His demeanor was defined by exceptional poise, warmth, and enthusiasm that live on in the memories of his students, colleagues, and patients. Steve is deeply missed as a superb scientist and a kind man.

Jiang-Fan Chen, MD, PhD
Thomas Chase, MD
Michael Schwarzschild, MD, PhD

A_{2A} antagonists for PD

A prime example of translational neuroscience

Michael A. Schwarzschild, MD, PhD; Jiang-Fan Chen, MD, PhD; and Thomas N. Chase, MD

Last autumn, academic and industry investigators from around the world, US government research program officers, and patient advocates convened in Boston to consider the broadening potential of adenosine A_{2A} receptor antagonists as therapeutic agents for Parkinson's disease (PD). Based on three decades of steadily accumulating preclinical data on the behavioral pharmacology, neuroanatomy, and molecular biology of the A_{2A} receptor, it has emerged as an attractive target of efforts to ameliorate the pathophysiology of PD. The Boston conference and its proceedings (which are reviewed in the articles of this supplement) reflect a prime example of translational research. A_{2A} receptor antagonists are moving from informative biologic reagents to leading candidates in the search for nondopaminergic influences on the symptoms and possibly the progression of PD.

The **neurobiological background** for targeting the A_{2A} receptor is laid out in the first section. Bertil Fredholm, who gave the keynote address of the conference, together with Per Svenningsson trace the evolution of the concept that motor function of the basal ganglia reflects a balance between the neuromodulatory influences of adenosine and dopamine. Rosin et al. then review the anatomic specificity of CNS A_{2A} receptors for and within the basal ganglia. This unique pattern of restricted expression suggests a low potential for CNS side effects of A_{2A} antagonists, underscoring a key conceptual advantage of A_{2A} antagonists over existing nondopaminergic antiparkinsonian drugs. (See figure on page S56.)

Fuxe et al. describe recent findings of functional heterodimeric interactions between the A_{2A} receptors, dopamine D_2 receptors, and metabotropic glutamate mGluR5 receptors. These fundamental insights raise the possibility of powerful synergistic pharmacologic approaches to leveraging the potential therapeutic benefits of A_{2A} antagonists for PD. Schiffmann et al. review the cellular physiology of how the A_{2A} receptor modulates striatal gene expression, long-term potentiation, and synaptic plasticity, and thus they highlight its potential involvement in long-term responses to repeated dopaminergic stimulation.

The second section conveys the preclinical basis for the **symptomatic motor stimulant actions** of A_{2A} antagonists in PD. Peter Jenner systematically reviews the substantial evidence that A_{2A} antagonists can reverse motor deficits in rodent and primate models of PD, without inducing dyskinesias in the latter. Morelli et al. present a hemiparkinsonian rat model of PD that strengthens the argument that A_{2A} receptor blockade may offer adjunctive antiparkinsonian effects without producing maladaptive motor activity. Akihisa Mori and Tomomi Shindou summarize multiple lines of data that convincingly implicate the modulation of striatopallidal GABAergic transmission as the mechanism by which A_{2A} antagonists reverse hypodopaminergic motor dysfunction.

A remarkable convergence of epidemiologic and laboratory data has raised the possibility that **neuroprotective effects** of A_{2A} receptor blockade may compliment its symptomatic benefits for PD; this is covered in the next section of the supplement. Ascherio et al. review recent prospective epidemiologic studies confirming previous suggestions that the common consumption of caffeine, a nonspecific adenosine antagonist, is associated with a decreased chance of later developing PD. Schwarzschild et al. summarize the complementary animal model data demonstrating neuroprotection by caffeine and more specific A_{2A} antagonists against toxin-induced dopaminergic neuron death, and they speculate on possible mechanisms. Castagnoli, Jr. et al. then relate a serendipitous finding that a subset of xanthine-based A_{2A} antagonists unexpectedly also possesses potent monoamine oxidase (MAO) B inhibitory activity, and they discuss the potential therapeutic application of such dual mechanism compounds as novel antiparkinsonian agents. Popoli et al. close this section by providing evidence that reduced release of glutamate and thus attenuated excitotoxicity may explain the neuroprotective effects of A_{2A} antago-

From the Molecular Neurobiology Laboratory (Dr. Schwarzschild), Massachusetts General Hospital, Charlestown, MA; Neurology and Molecular Neuropharmacology Laboratory (Dr. Chen), Boston University School of Medicine, Boston, MA; and National Institute of Neurological Disorders and Stroke (Dr. Chase), Bethesda, MD.

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nists across a range of neurotoxicity models of neurodegenerative disease.

The supplement places the aforementioned antiparkinsonian effects of A_{2A} antagonists into the context of their expected **effects on other important aspects of CNS physiology and PD pathophysiology**. Chen et al. elaborate on the critical role played by the A_{2A} receptor in the potentially maladaptive behavioral and neurochemical responses to repeated dopaminergic stimulation. Conversely, A_{2A} antagonists may be able to prevent or reverse the maladaptive basal ganglia plasticity underlying the occasionally disabling dyskinetic complications of long-term levodopa therapy in patients with PD. Vaugeois et al. make the case for antidepressant effects of specific A_{2A} antagonists (in contrast to caffeine). Despite the caveats inherent in modeling depression in mice, their findings have major implications for the management of depression in the general population and for those with PD (which is associated with a high incidence of depression). Weiss et al. take on the important question of A_{2A} antagonist effects in models of psychosis. Although the established opposing effects of A_{2A} and D_2 receptors have supported the idea that A_{2A} *agonists* may possess antipsychotic potential, A_{2A} antagonists do not necessarily induce psychotomimetic side effects. Urade et al. highlight the role of the A_{2A} receptor in sleep and, conversely, the possibility that A_{2A} antagonists may display arousal activity.

Finally, the supplement presents perspectives on the **translational steps from development of lead compounds for human use to early clinical trials**. Hiroshi Kase recounts one company's major contributions to the conceptual and pharmaceutical development of currently the most advanced A_{2A} antagonist candidate for PD, which has now successfully moved to phase II clinical trials. Dourish et al. present another company's discovery and progress in preclinical development of unique nonxanthine A_{2A} antagonists targeted to PD. Chase et al. close the supplement with an overview of how an A_{2A} antagonist has advanced steadily from rodent to monkey to human studies, with the latter clinical trials validating the preclinical evidence for antiparkinsonian benefits. These perspectives reflect the overarching translational theme of the conference and this supplement, which have adopted the fitting symbol of Boston's nearly completed Lenny Zakim Bunker Hill Bridge. The striking images (figure and throughout) by the photographer Don Eyles capture the construction of Boston's new landmark gateway. They also provide a metaphor for the building of a bridge that spans our knowledge of A_{2A} receptor neurobiology and the promise of improved therapeutics for patients with PD.

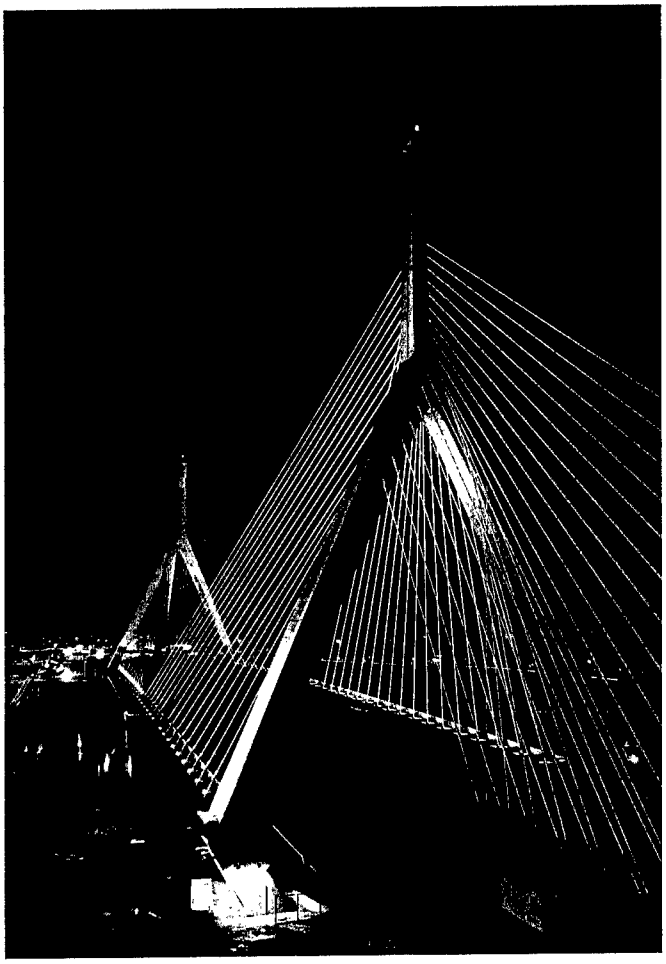

Figure. Boston's Leonard P. Zakim Bunker Hill Bridge under construction in November 2001, photographed by Don Eyles.

Keynote Address

Adenosine–dopamine interactions

Development of a concept and some comments on therapeutic possibilities

Bertil B. Fredholm, PhD; and Per Svenningsson, MD, PhD

Abstract—This brief review presents a personal perspective on the historical development of the current knowledge about the biologically important concept of functional antagonism between adenosine A_{2A} and dopamine D_2 receptors in caudate-putamen, accumbens, and tuberculum olfactorium. In the 1970s, studies of dopamine actions suggested an unexpected role of adenosine. Developments during the next decade substantiated this finding and demonstrated that a subform of adenosine A_2 receptors was enriched in the basal ganglia. Cloning of adenosine receptors provided better tools for cellular localization and showed that A_{2A} receptors are closely associated with D_2 receptors. Distinct functional interactions at several levels were discovered, and there is now strong evidence that A_{2A} receptors are tonically active and modified by dopamine acting at D_2 receptors. Development of selective antagonists and knockout mice have highlighted the potential usefulness of A_{2A} antagonists in decreasing symptoms and progression of Parkinson's disease—something that has also been vindicated by careful epidemiologic studies. There are issues of efficacy and potential side effects that need to be resolved, but the future looks bright.

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After the demonstration by Arvid Carlsson that dopamine is an important transmitter in the basal ganglia, with a particular role in Parkinson's disease (PD), the finding from Greengard's group[1] that dopamine can stimulate adenylate cyclase attracted much attention. This attention led to the discovery that adenosine might be intimately involved.

In 1974, Kjell Fuxe and Urban Ungerstedt showed, using animals with unilateral 6-OH-dopamine lesions, that theophylline could itself induce the same type of rotation behavior that was induced by drugs that directly or indirectly stimulated dopamine receptors and that it could markedly enhance dopamine-mediated effects.[2] In that study, the effect was interpreted as secondary to blockade of phosphodiesterase (PDE) and was therefore taken as evidence for an important role of cyclic adenosine monophosphate (cAMP) as a mediator of dopamine actions. However, in a follow-up study in which I (B.F.) was involved, several different PDE inhibitors were examined, and it was found that the potency of the drugs to induce rotation fitted much better with their potency as adenosine antagonists (or enhancers) than with their potency as PDE inhibitors.[3] Together these studies showed that methylxanthines, probably by blocking adenosine receptors, could potentially be used as treatment for patients with PD.

Studies in two laboratories of dopamine-stimulated adenylyl cyclase in the brain also showed that methylxanthines could decrease "basal" enzyme activity and that adenosine could stimulate it.[4,5] This was observed in dopamine-rich areas of the brain, including caudate-putamen and tuberculum olfactorium, but not in other brain areas. This finding suggested that these parts of the brain might have a different set of adenosine receptors than other brain areas (figure, A).

This contention received support during the following decade as methods to study receptors using binding techniques were developed. The first studies used relatively nonselective radioligands but pharmacologic means to discriminate between multiple binding sites.[6-9] Later studies used a rather selective ligand for A_{2A} receptors, including CGS 21680.[10-14] Altogether these studies vindicated the belief that a special form of adenosine receptors, the A_{2A} receptor, is enriched in dopamine-rich areas of the brain and that this offers a rationale for examining the role of adenosine in mediating or modulating behaviors and traits traditionally associated with dopamine.

The availability of more selective adenosine receptor agonists and antagonists also reinforced the idea that behavioral consequences of A_{2A} receptor- and dopamine receptor-mediated effects tended to be opposite.[15-19]

The interactions between adenosine and dopamine

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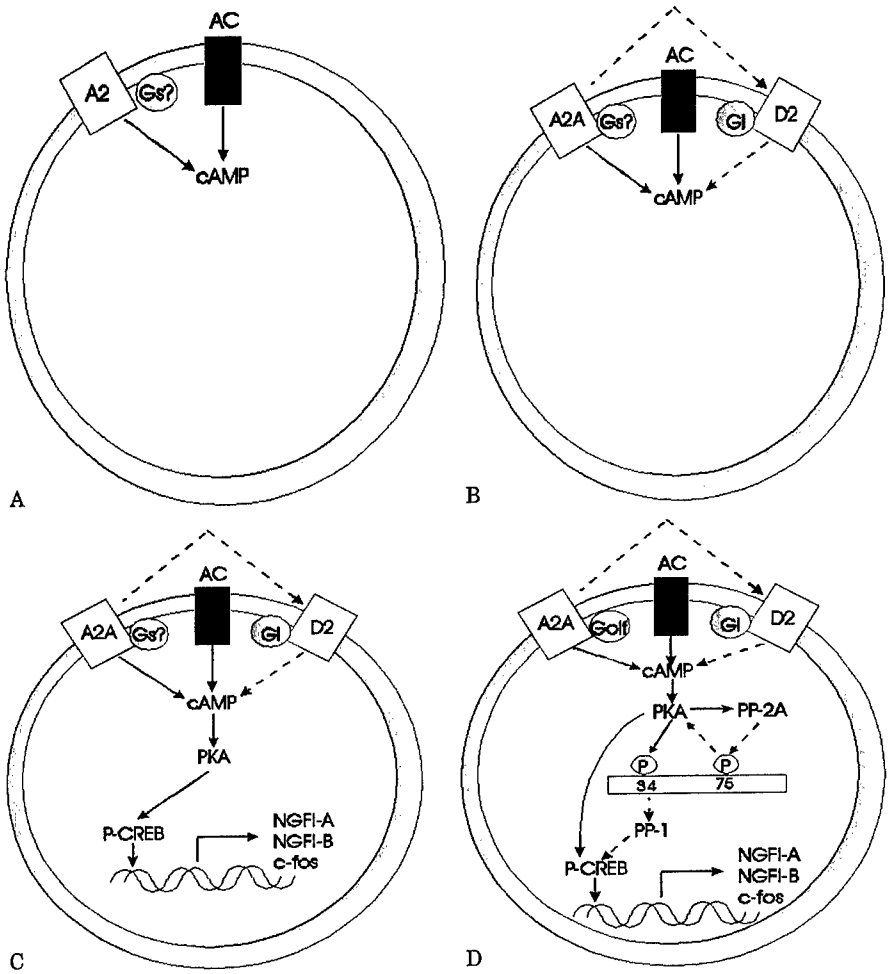

Figure. Graphic description of the development of our knowledge of A_{2A}-D_2 interactions. Illustration (A) shows the situation in the 1970s; (B) depicts the knowledge around 1991; (C) shows the same in the late 1990s; and (D) demonstrates our current understanding. For further details, see text.

receptors in the striatum continued to be studied at the biochemical level. It was demonstrated that there were interactions between adenosine A_{2A} receptors at several levels. The A_{2A} receptor, being coupled to a member of the G_s family of G proteins, and the D_2 receptor, coupled to a G_i protein, would interact negatively at the level of second messengers and beyond. Binding studies revealed that high-affinity binding of D_2 agonists could be reduced by stimulation of adenosine A_{2A} receptors.[20] This finding suggested that there were interactions directly between the receptors (figure, B), an issue that has been forcefully pursued by Fuxe et al. (see Fuxe et al., page S19).

The next major conceptual advance was the cloning of several adenosine receptors. Four novel members of the G protein-coupled receptor family were cloned from a canine thyroid library.[21] Of these, one was the adenosine A_{2A} receptor,[22] and another was the canine A_1 receptor.[23] Once these first structures were obtained, the same receptors were soon cloned from other mammals, including humans. Furthermore, the adenosine A_{2B} receptor was cloned.[24] More surprisingly, a fourth adenosine receptor, denoted A_3, was cloned, first as an orphan[25] and later as a bona fide methylxanthine-insensitive adenosine receptor.[26]

These findings not only conclusively proved that there are two distinct adenosine A_2 receptors but also provided a set of novel tools that proved useful. In situ hybridization was used to pinpoint the cells that express A_{2A} receptors in the brain. Using increasingly sophisticated methods, it was proven that the bulk of A_{2A} expression is confined to one set of neurons in the striatum, namely those GABAergic output neurons that constitute the so-called indirect pathway.[27-33] These cells also express the bulk of the dopamine D_2 receptors. Hence, the link between A_{2A} and dopamine D_2 receptors was further strengthened.

Techniques with a cellular resolution were also used to try to determine the roles of adenosine A_{2A} receptors in the intact striatum. This was based on early findings showing that expression of immediate early genes (IEGs) could be used to pinpoint changes in neuronal activity or signal transduction.[34] We observed that stimulatory doses of caffeine and selective A_{2A} receptor antagonists caused a decrease in the expression of IEGs, known to be regulated by the cAMP/CREB cascade, in striatopallidal neurons (figure, C).[35,36] These and subsequent studies[37-40] provide strong evidence that adenosine, via A_{2A} receptors, exerts a robust tonic activation on the cAMP/CREB/IEG cascade in striatopallidal neurons. Moreover, this result also provided evidence that multiple D_2 receptor-mediated effects of dopamine can be attrib-

uted to the antagonism of this adenosine-mediated activation of striatopallidal neurons.

To increase our understanding of the interactions of adenosine and dopamine at the signal transduction level, we proposed a collaborative project with Paul Greengard to study the effects of adenosine A_{2A} selective compounds and caffeine on the phosphorylation of dopamine and cAMP phosphoprotein of 32 kDa (DARPP-32). DARPP-32 is highly enriched in all striatal GABAergic medium-sized projection neurons and is an important mediator of dopaminergic signaling.[41] Its function is determined by its relative phosphorylation state at several different threonine/serine residues, of which the most studied is a protein kinase A (PKA) site at Thr34. When this residue is phosphorylated, it converts DARPP-32 into an inhibitor of protein phosphatase-1, which in turn regulates the activity of multiple transcription factors, including CREB, ion channels, and ionotropic receptors (figure, D). In initial studies conducted in brain slices prepared from striatum, it was found that CGS 21680 potently increases phosphorylation at Thr34.[42] This effect was additive to that of SKF81297, a selective D_1 agonist, and could be counteracted by quinpirole, a selective D_2 agonist.[43] This result identified adenosine, via A_{2A} receptors, as a key regulator of the phosphorylation state of DARPP-32 in striatopallidal neurons. Subsequently, we developed a method to reliably detect DARPP-32 phosphorylation in vivo and could demonstrate that the A_{2A} antagonist used, SCH 58261, significantly counteracted the increase in DARPP-32 phosphorylation that was observed after treatment with selective D_2 receptor antagonists.[44] Likewise, the ability of D_2 antagonists to increase DARPP-32 phosphorylation was dramatically reduced in A_{2A} receptor knockout mice. Therefore, these data provided further support for the notion that adenosine acting on A_{2A} receptors is an important mediator for establishing a basal cAMP level, which is necessary for many effects of dopamine's action via D_2 receptors. To address the involvement of DARPP-32 in the behavioral actions of caffeine and selective adenosine A_{2A} receptor compounds, we administered such compounds to DARPP-32 knockout mice and studied effects on locomotor behavior. As expected from the biochemical data, we found that the ability of CGS 21680 to induce hypolocomotion was attenuated in DARPP-32 knockout mice.[45] Similarly, the ability of caffeine and SCH 58261 to induce hyperlocomotion was attenuated in DARPP-32 knockout mice. In this article, an additional effect of A_{2A} receptors on DARPP-32 phosphorylation was shown, namely that A_{2A} agonism via cAMP-dependent mechanisms increases the phosphorylation of Thr34–DARPP-32 but decreases the phosphorylation at Thr75–DARPP-32. Conversely, caffeine and SCH 58261 increase phosphorylation at Thr75–DARPP-32. This site has recently been shown to be phosphorylated by Cdk5, and when this happens, DARPP-32 is converted into an inhibitor of PKA.[46] Therefore, by increasing the phosphorylation of Thr75–DARRP-32, caffeine and selective A_{2A} receptor antagonists will further increase the inhibition of PKA. This feed-forward mechanism, which is also used by D_2 receptor agonists, will potentiate the inhibitory influence of adenosine on the cAMP/PKA/CREB/IEG signaling pathway in striatopallidal neurons (see figure, D).

In parallel with the development of an increasingly clear understanding of the biochemical and molecular underpinning of the adenosine–dopamine interactions, there has been extensive work on the effectiveness of adenosine A_{2A} antagonists in various experimental models of PD. Extensive review of these results is beyond the scope of this commentary (see Chase et al., page S107; Kase et al., page S97; Jenner et al., page S32; and Weiss et al., page S101). However, a recent study showed that A_{2A} receptor antagonism could reduce not only symptoms of PD but also the loss of dopamine neurons induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP).[47] Furthermore, it was shown that persistent L-dopa effects require A_{2A} receptors.[48]

Therefore, the results of many studies over the years have strongly developed the concept that A_{2A} and D_2 receptors interact in such a way that A_{2A} antagonists could prove useful in treating patients with PD. However, there are concerns. One potential concern is related to tolerance. It is well known that some actions of caffeine develop rapid tolerance.[49,50] However, caffeine effects in PD models do not,[51] and there is also no tolerance to selective A_{2A} antagonists in models that show tolerance to caffeine.[52]

Another, and perhaps more serious, concern is related to the fact that A_{2A} receptors regulate other things in addition to activity in striatopallidal neurons. It has long been known that adenosine regulates platelet activation,[53,54] and now we know that A_{2A} receptors are responsible for this.[55,56] Similarly, A_{2A} receptors are critically important in regulating neutrophil leukocyte activity[57] and activity of macrophages.[58] Even more importantly, A_{2A} receptors regulate inflammatory reactions in general.[59,60] Therefore, long-term blockade of adenosine A_{2A} receptors may cause undesirable peripheral morbidity.

A potential way to attack this problem was afforded when it was discovered that A_{2A} receptors in striatum are coupled to G_{olf} proteins,[61] whereas on platelets, neutrophils, and lymphocytes, G_s mediates the A_{2A} effects. If it proves possible to find agents that selectively affect A_{2A}-G_{olf}, more selective drugs may be found.

Already in the early 1970s Fuxe and Ungerstedt suggested that methylxanthines might be used for management of PD. Since then, substantial progress has been made in understanding why, and now the prospect looks good that A_{2A} antagonists may prove of value as part of the therapeutic armamentarium.[62] The potential may be particularly strong in compounds that have more than one potentially beneficial action.[63]

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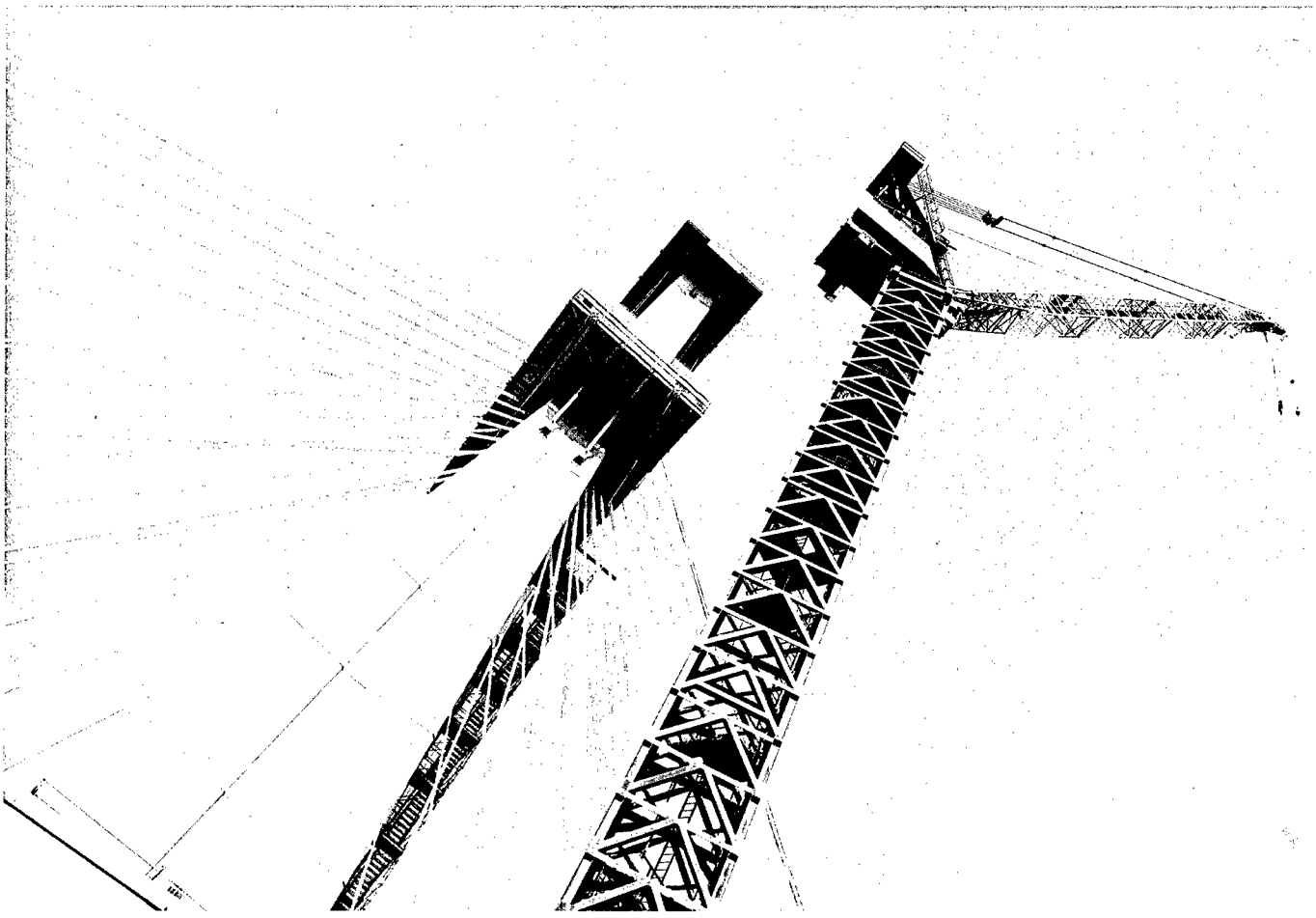

Don Eyles **April 2000**

I. The biology of A_{2A} receptors in the basal ganglia

Exciting news about A_{2A} receptors

Per Svenningsson, MD, PhD; and Bertil B. Fredholm, PhD

Our understanding of the fundamental actions of adenosine A_{2A} receptors has increased significantly during the past years, and this was well illustrated by the lectures in the session "The Biology of A_{2A} Receptors in the Basal Ganglia." The existence of a strong interaction between adenosine A_{2A} and dopamine D_2 receptors is now firmly established at the anatomic, biochemical, and functional levels. In each of the lectures, novel evidence for important interactions between adenosine A_{2A} receptors and excitatory glutamatergic neurotransmission was presented.

Using A_{2A} receptor-selective antibodies and immunohistochemistry at the light and electron microscopic levels, Rosin et al. have performed detailed anatomic work that unambiguously shows that A_{2A} receptors are highly enriched in medium-sized spiny GABAergic striatal neurons.[1,2] Within these neurons, A_{2A} receptors are found in most cellular compartments, i.e., dendrites, terminals of axon collaterals, and in soma. However, from a quantitative standpoint, a pronounced subcellular enrichment of A_{2A} receptors is found in dendrites and dendritic spines, which form asymmetric synapses. These synapses receive input from glutamatergic terminals and are of excitatory nature. This postsynaptic localization of A_{2A} receptors implies that A_{2A} receptors may play an important role in the regulation of synaptic plasticity. The excitatory glutamatergic inputs to striatum are derived predominantly from the cerebral cortex and thalamus. In an ongoing effort, Rosin et al. are trying to define the anatomic origin of the excitatory glutamatergic inputs that innervate the A_{2A} receptor-containing dendritic spines. For this purpose, colabeling studies with A_{2A} receptor antibodies and VGLUT1, which is located on cortical terminals, or VGLUT2, which is located on thalamic terminals, are performed (see Rosin et al., page S12). It is anticipated that detailed information of the synaptology of the A_{2A} receptor-containing neurons will be available in the near future.

Fuxe and Schiffmann presented evidence for the functional importance of A_{2A} receptors in modulating excitatory glutamatergic neurotransmission. By combining biochemical and anatomic techniques, Fuxe et al.[3] have demonstrated that A_{2A} receptors form heterodimers with metabotropic glutamate 5 receptors (mGluR5). Interestingly, mGluR5 receptors have been shown to be highly concentrated postsynaptically at excitatory synapses and thus exhibit a subcellular distribution similar to A_{2A} receptors. The heterodimerization between A_{2A} receptors and mGluR5 receptors is specific and cannot be found between A_{2A} receptors and mGluR1 receptors. Studies in cell lines transiently transfected with A_{2A} receptors and mGluR5 receptors have shown that costimulation of these receptors has a synergistic action on mitogen-activated protein kinase (MAPK) activation and c-fos gene transcription. The precise mechanisms underlying this synergy remain to be clarified, but it appears not to involve cyclic adenosine monophosphate (cAMP) or Ca^{2+} accumulation (see Fuxe et al., page S19).

Schiffmann et al. have extensively used adenosine A_{2A} receptor knockout mice to study the influence of A_{2A} receptors on electrophysiologic properties of striatal neurons and on gene regulation. Using patch-clamp methodology and brain slices, Schiffmann et al. have now demonstrated that long-term potentiation in the ventral part of the striatum, nucleus accumbens, is significantly attenuated in A_{2A} receptor knockout mice.[4] Similar results were obtained when striatal slices from wild-type mice were treated with the selective A_{2A} receptor antagonist ZM241385 (see Schiffman et al., page S24). This finding adds further evidence that A_{2A} receptors are tonically activated by endogenous adenosine. Moreover, this effect on synaptic plasticity can be viewed as a functional correlate to the anatomic finding of Rosin et al., who found that A_{2A} receptors are enriched in dendritic spines that receive excitatory inputs.

As expected from previous work using caffeine and A_{2A} receptor antagonists, a reduction of immediate early gene and neuropeptide expression has been found throughout the striatum in A_{2A} receptor knockout mice.[5] This is probably not the result of a developmental deficit because A_{2A} receptors are first expressed late in ontogeny. Importantly, these changes are not confined to striatopallidal neurons

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but also involve striatonigral neurons and nonstriatal regions. This finding clearly demonstrates that A_{2A} receptors, despite being restrictively expressed on striatopallidal neurons, strongly influence the physiology of neurons that do not express A_{2A} receptors. The extremely tight coupling between neuronal populations in the striatum has been described and provides a good explanation for these findings. It is of course also known that activity in striatal outputs affects many other brain regions. Therefore, there is no discrepancy between the fact that A_{2A} receptors have a restricted distribution and the fact that affecting them will influence many parts of the brain.

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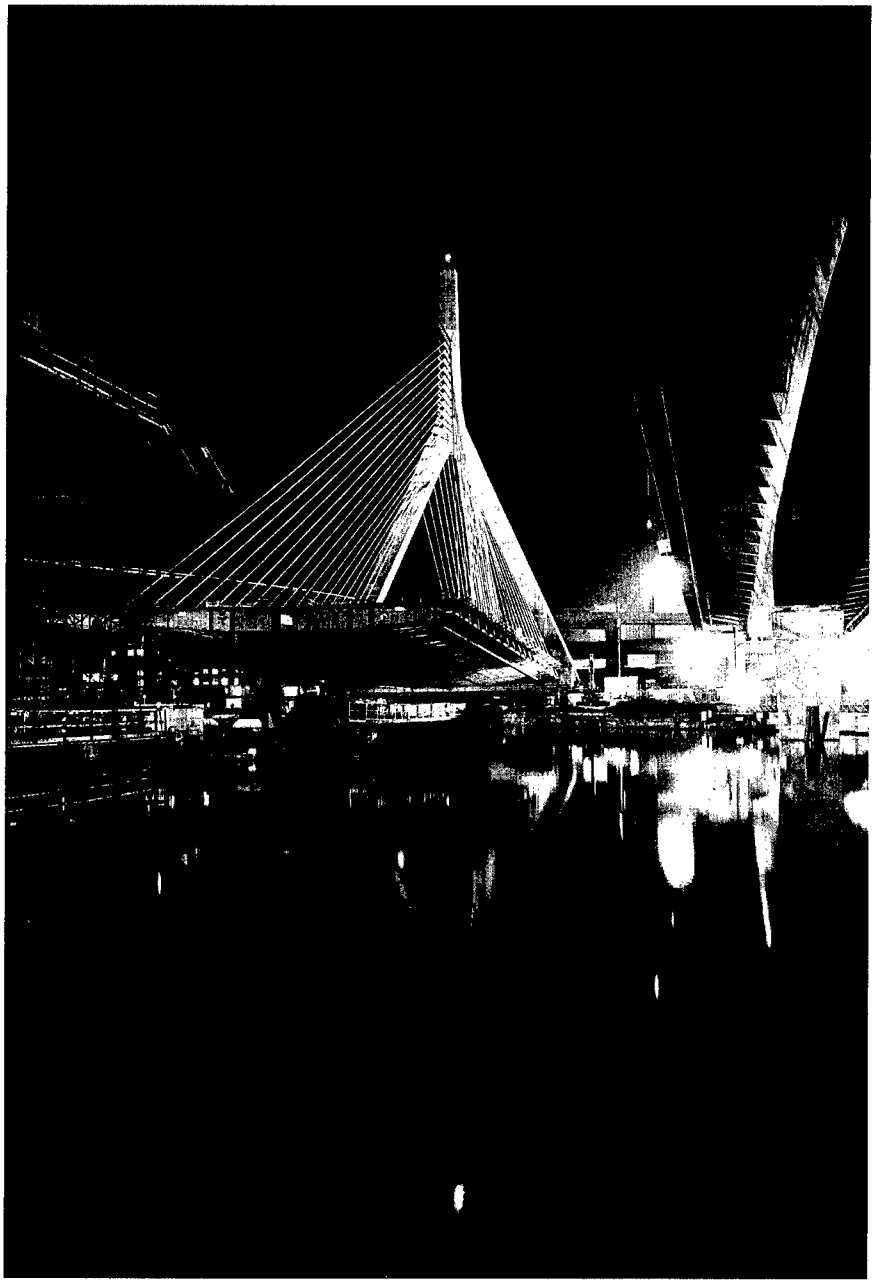

Don Eyles **November 2000**

Anatomy of adenosine A_{2A} receptors in brain

Morphological substrates for integration of striatal function

Diane L. Rosin, PhD; Barbara D. Hettinger, PhD; Amy Lee, PhD; and Joel Linden, PhD

Abstract—A_{2A} adenosine receptors (A_{2A}Rs) are expressed with the greatest abundance in the striatum and other nuclei of the basal ganglia. The segregated expression of A_{2A}Rs on the GABAergic striatopallidal medium spiny neurons, where A_{2A}R and D_2 dopamine receptor mRNAs are colocalized, and the opposing functional interaction between adenosine and dopamine suggest that A_{2A}Rs may be an important therapeutic target. To further explore the role of A_{2A}Rs in the synaptic organization of the basal ganglia, the authors developed an antibody directed against the purified A_{2A}R. Immunohistochemical studies in rat brain showed dense labeling of the neuropil in the striatum, nucleus accumbens, and olfactory tubercles with lighter labeling of terminals in the globus pallidus (GP), where A_{2A}R transcript is not detected. Stimulation of A_{2A}Rs on GP terminals may facilitate GABAergic signaling and contribute to the overactivation observed in Parkinson's disease (PD). Analysis at the ultrastructural level allowed a more detailed characterization of the mechanism(s) of A_{2A}-mediated control of striatal output. In the striatum, terminals expressing A_{2A}Rs accounted for 25% of the labeled elements. These presynaptic receptors may facilitate excitatory glutamatergic, inhibitory GABAergic, and possibly cholinergic striatal transmission. However, the majority of striatal A_{2A}R immunoreactivity was found on postsynaptic elements, including dendrites of striatopallidal neurons, in which A_{2A}R and GABA immunoreactivity is colocalized. Activation of these receptors may promote GABAergic signaling in striatopallidal output neurons and their local axon collaterals in the striatum. Many of the A_{2A}-labeled dendrites were contacted by terminals forming asymmetric (excitatory) possibly glutamatergic synapses. Using the vesicular glutamate transporters (VGLUTs) as markers of glutamatergic terminals, the authors have found that VGLUT1-immunoreactive (ir) terminals make asymmetric contacts on A_{2A}-ir spines and spine heads in the striatum, suggesting that regulation of striatal output by A_{2A}R stimulation may involve facilitation of the cortical glutamatergic excitatory input to striatopallidal neurons. These ultrastructural findings suggest several pathways through which A_{2A} receptor blockade may act to dampen the elevated striatopallidal GABAergic signaling that occurs in PD.

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Adenosine A_{2A} receptors (A_{2A}Rs), which are highly expressed in the basal ganglia, have become a target of therapeutic interest for a number of diseases, including Parkinson's disease (PD),[1,2] mostly because of their discrete anatomic localization and biochemical interaction with dopamine D_2 receptors.[3] The progressive degeneration of nigrostriatal neurons that occurs in PD results in a loss of dopaminergic input to striatal output neurons and enhanced striatopallidal GABAergic signaling, resulting in the motor disturbances that are characteristic of the disease. With declining levels of dopamine, which normally regulates the direct (striatonigral) and indirect (striatopallidal) output pathways of the basal ganglia, an imbalance occurs in the outflow of inhibitory GABAergic projection neurons. Replacement of dopaminergic input by administration of exogenous L-dopa has been the primary therapeutic strategy for the management of PD for decades. The finding that the mRNAs for A_{2A} and D_2 receptors are colocalized in striatopallidal neurons,[4,5] where they are physically and biochemically poised to mediate adenosine–dopamine antagonistic interactions,[3,6] has focused attention on blocking A_{2A}Rs as another potential means for resetting the motor imbalance in PD. We have taken the approach of studying the subcellular localization of A_{2A}Rs to enhance understanding of adenosine's role as a neuromodulator in the basal ganglia. This article will briefly review the anatomy of A_{2A}Rs in striatum and will integrate our recent findings in the context of striatal circuitry with a wealth of published data on A_{2A}Rs to suggest a speculative model for multiple mechanisms by which these receptors may modulate striatal function.

Distribution of A_{2A} receptors in CNS. Beginning with some of the earliest studies of the distribution of A_{2A}Rs in the brain, studies of receptor

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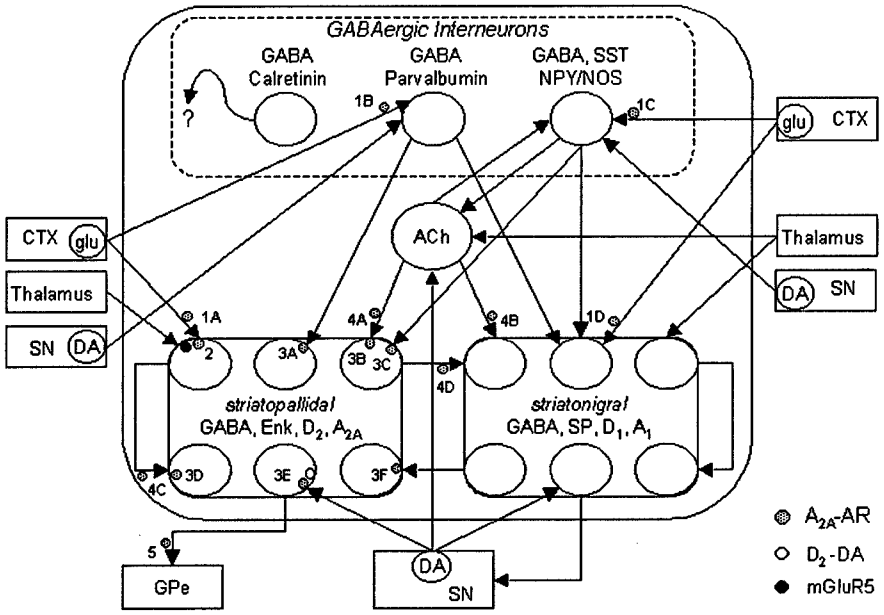

Figure 1. Working model for the morphologic basis of A_{2A} action in basal ganglia: potential sites for modulation of striatal function. A simplified drawing of the striatal output neurons (GABAergic projection neurons of the indirect pathway that express enkephalin [enk], dopamine D_2 receptors [D_2], and A_{2A} adenosine receptors [A_{2A}Rs] and of the direct pathway that express substance P [SP], dopamine D_1 receptors [D_1], and adenosine A_1 receptors [A_1]) and the four interneuronal cell types (GABAergic interneurons, classified based on their histochemical profiles as labeled, and cholinergic interneurons that express acetylcholine [ACh]) illustrates the known extrinsic inputs and established connections in the striatum. Putative and postulated sites of subcellular localization of A_{2A}Rs are given based on our ultrastructural findings and biochemical, physiologic, and anatomic results in the literature. The diagram is not meant to show all the neurochemical messengers and their receptors in the striatum but emphasizes the role of the A_{2A}Rs in modulating the output pathways of the striatum. CTX = cortex; DA = dopamine; glu = glutamate; GPe = globus pallidus, external segment; mGluR5 = metabotropic glutamate receptor; NOS = neuronal nitric oxide synthetase; NPY = neuropeptide Y; SN = substantia nigra; SST = somatostatin.

binding have shown that A_{2A}Rs are abundantly expressed in striatum. Membrane binding and ligand binding autoradiographic studies in brain slices using agonist and antagonist radioligands have demonstrated high levels of A_{2A}Rs in the striatum, nucleus accumbens, olfactory tubercles, and globus pallidus (GP) of rat and human brain.[7-13] Lower levels of extrastriatal binding sites have been revealed in some studies,[14,15] but these will not be discussed here. We have corroborated these findings immunohistochemically using an antibody directed against purified recombinant human A_{2A}R.[16] The antibody epitope has been mapped to a region of the receptor in the third intracellular loop that is conserved in multiple species, including humans, rats, and mice. No specific immunoreactivity is found in brains derived from mice in which the A_{2A}R gene has been deleted. In wild-type mice and rats, dense A_{2A}R-like immunoreactivity was detected in the neuropil of the striatum, nucleus accumbens, olfactory tubercles, and portions of the extended amygdala. Lighter labeling was found in the nucleus of the solitary tract and in the GP, presumably in terminals of vagal afferents and striatal projection neurons, respectively.

In situ hybridization studies describing the distribution of A_{2A}R mRNA in brain[4,5,17-21] have demonstrated that A_{2A}R mRNA in striatum is found almost exclusively in medium spiny neurons that also express preproenkephalin[17] and dopamine D_2 receptor mRNA[4,18,20,22] and is not colocalized (or only to a limited extent) in striatal neurons that express substance P and dopamine D_1 receptor mRNA. A_{2A}R mRNA has not been detected in GP or on striatal GABAergic interneurons, but the expression in cholinergic interneurons, and similarly the ability of A_{2A}Rs to regulate acetylcholine (ACh) release, has been controversial.[2,15,23,24] The segregated expression of A_{2A}Rs in the striatopallidal pathway and the colocalization with dopamine D_2 receptors have formed the basis for a large body of work on adenosine–dopamine interactions in striatum and potential applications to neurologic and neuropsychiatric disorders.[3,25-28]

A_{2A}Rs and striatal circuitry. The localization of A_{2A}Rs in the brain within the context of striatal circuitry is illustrated in figure 1. This working hypothetical model proposes potential sites of regulation of striatal function by A_{2A}Rs and is based on our findings and published results from other groups. A complete survey of all the relevant data on A_{2A}Rs that support this diagram is beyond the scope of this article, although reviews can be found elsewhere.[2,15] Figure 1 also draws on a wealth of studies that have defined the anatomy of the striatum[29-35] and is meant to focus on the primary types of striatal neurons, the sources of extrinsic and intrinsic input, and the targets of the projection neurons, but it does not illustrate the extended feedback circuitry of the basal ganglia. The majority of cells in the striatum (95%) are the medium-sized GABAergic spiny neurons, which can be divided into two subpopulations—the striatopallidal and striatonigral neurons—based on their projection patterns and neurochemical phenotype, as shown in figure 1. Cholinergic and GABAergic interneurons constitute the remaining striatal neurons, and the GABAergic interneurons can be di-

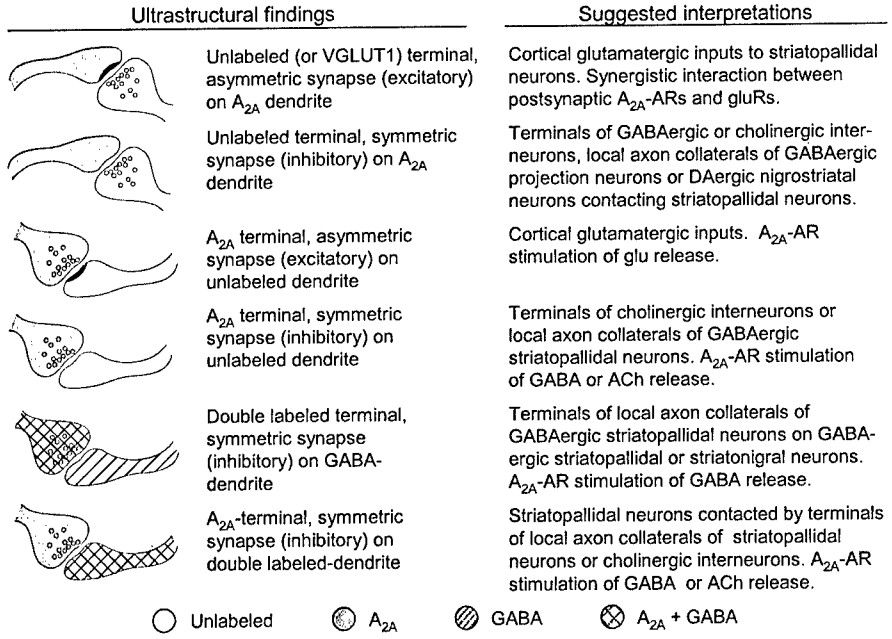

Figure 2. Summary of ultrastructural studies of A_{2A} adenosine receptors ($A_{2A}R$) and GABA localization in striatum.

vided into three subtypes based on the coexpression of other neurotransmitters and neuromodulators. A_{2A}Rs are highly expressed in the striatopallidal neurons within the striatum. A somewhat lower level of expression in the GP can likely be attributed to receptors on terminals of the striatopallidal neurons. Localization of A_{2A}Rs on cholinergic interneurons and terminals of corticostriatal neurons in the striatum, although controversial, would be consistent with a role for adenosine in regulation of ACh and glutamate release in the striatum. Each of these sites will be discussed in more detail in reference to our ultrastructural findings.

Ultrastructural localization: A_{2A}Rs and GABA. To further elucidate mechanisms by which stimulation of A_{2A}Rs can modulate the activity of striatal neurons, we examined the ultrastructural localization of A_{2A}Rs in the striatum.[36] Our initial experiments revealed that A_{2A}Rs are found predominantly at postsynaptic sites in the striatum (70% of labeled profiles were dendrites and dendritic spines, and 3% were soma) but that presynaptic (23%) and glial (3%) receptors are also present. The somatodendritic localization of receptors most likely represents GABAergic striatopallidal neurons. To determine anatomic substrates for A_{2A}R–GABA interactions, we then examined the colocalization of A_{2A}R and GABA. The majority of double-labeled profiles were dendrites (77%) with double-labeled soma (11%) and axons (11%) accounting for the remainder.

Examination of synaptic relationships in the single- and double-labeling experiments revealed that the majority of contacts involving A_{2A}R-labeled profiles contained asymmetric synapses, which are indicative of synapses formed with excitatory presynaptic elements. These contacts included unlabeled terminals synapsing on A_{2A}R dendrites, spines, and soma and A_{2A}R terminals contacting unlabeled somatodendritic sites. These results suggest that A_{2A}Rs are crucially poised at the cellular level to modulate the excitatory glutamatergic cortical input to the striatum. Symmetric or inhibitory contacts, although fewer in number, were also observed and could originate from extrinsic D_2 dopamine inputs or local axon terminals of cholinergic or GABAergic striatal interneurons or GABAergic projection neurons. These two main categories of symmetric and asymmetric synapses can be further divided according to the identity of the labeled elements as shown in figure 2. In figure 2, we summarize our findings and assign possible interpretations for each observed synaptic relationship. Some of the key points of figure 2 will be discussed below. The increase in neuronal activity in the striatopallidal pathway and the decrease in the output from the GP produced by A_{2A}R activation in the basal ganglia are likely the sum total of the effects at presynaptic and postsynaptic sites (i.e., on soma, dendrites, or nerve terminals). Whether directly at presynaptic terminals or indirectly by increased impulse flow, we speculate that facilitation of neurotransmitter release by A_{2A}R activation, secondary to stimulating cyclic adenosine monophosphate (cAMP) accumulation and activating protein kinase A, plays prominently in the integration of A_{2A}R effects.

Several studies using different techniques have shown variously that A_{2A}R stimulation either increases or decreases GABA release in the GP or striatum.[37-44] The reason for this discrepancy remains unresolved. Although it seems unlikely that activation of one receptor subtype could have inhibitory and stimulatory effects on neurotransmitter re-

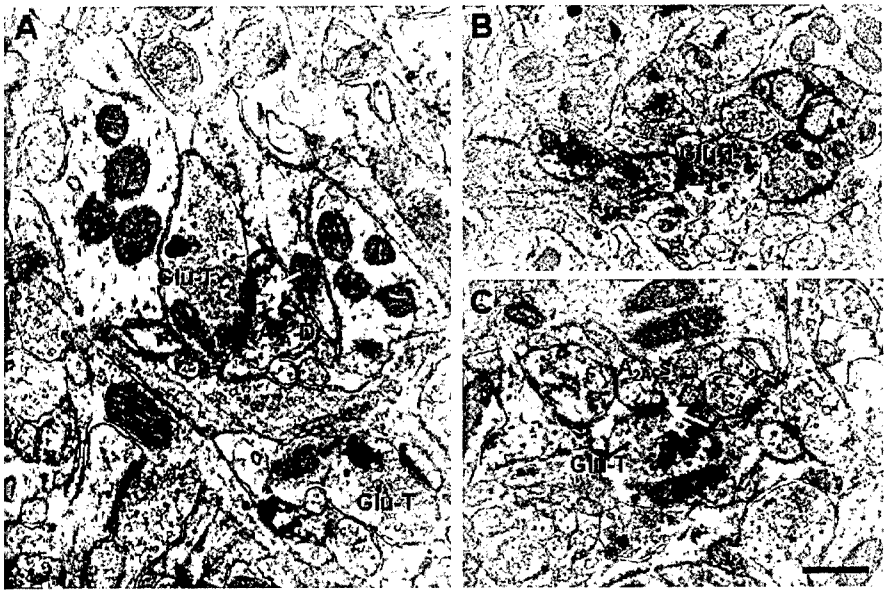

Figure 3. Electron micrographs showing glutamatergic (vesicular glutamate transporter [VGLUT] 1-containing) terminals (Glu-T) labeled with silver-intensified immunogold making asymmetric synapses (arrows) on A_{2A}-immunoreactive dendrites (A; A_{2A}-D) and dendritic spines (B, C; A_{2A}-s) containing immunoperoxidase reaction product. Scale = 0.3 μm.

lease, the integration of the physiologic effects of activation of receptors at multiple sites may account for inconsistent observations. We found A_{2A}R-labeled terminals in GP (Rosin et al., unpublished observations) that are likely terminals of striatopallidal neurons where A_{2A}R activation facilitates GABAergic transmission.[37,39,43,44] By contrast we found A_{2A}Rs (or the colocalization of A_{2A}Rs and GABA) in striatum at somatodendritic sites and in terminals forming symmetric synapses on GABAergic dendrites or soma indicative of prejunctional and postjunctional sites of A_{2A}R-mediated regulation of GABA release in striatum. One may predict that activation of presynaptic A_{2A}Rs should facilitate GABA release in the striatum as it does in the GP. At postsynaptic sites stimulation of striatal somatodendritic A_{2A}Rs could directly stimulate the activity of GABAergic neurons, which could lead to enhanced GABA release from terminals in the GP and from terminals of local axon collaterals in the striatum. Inhibitory effects of locally released GABA on other medium spiny neurons could subsequently lead to decreased GABA release from local axon collaterals of the affected neurons. These conflicting effects may account for inconsistent experimental observations. Therefore, the output of the medium spiny neuron after A_{2A}R activation would likely involve an integration of signals, including direct effects on striatopallidal neurons and inhibitory input from recurrent collaterals. Ultrastructural evidence for synaptic connections between striatal projection neurons[45-47] is now supported by physiologic evidence for a functional role of lateral inhibition in striatal output.[48]

Ultrastructural localization: A_{2A}Rs and glutamate. Our ultrastructural findings that many A_{2A}-immunoreactive (ir) dendritic profiles in striatum receive asymmetric synaptic contacts suggest this is a site for A_{2A}R modulation of excitatory input (likely the corticostriatal glutamatergic neurons) to striatal GABAergic neurons. Recent studies demonstrated a functional synergistic interaction between A_{2A}Rs and both NMDA receptors[49-51] and metabotropic glutamate receptors (mGluR5),[52] including evidence for A_{2A}R/mGluR5 heteromerization.[52,53] These studies support the hypothesis that excitatory neurons contact striatopallidal neurons at subcellular domains containing A_{2A}Rs that act to facilitate glutamatergic transmission. The observation of A_{2A}R stimulation of glutamate release[54] supports the presence of presynaptic A_{2A}Rs on glutamatergic terminals. Therefore, we were prompted to directly examine an adenosine–glutamate interaction in striatum by dually labeling for A_{2A}Rs and glutamate terminals.

The histologic identification of glutamate cell bodies and terminals using specific reagents has been vastly simplified by the discovery of three unique proteins identified as vesicular glutamate transporters (VGLUT).[55-61] The majority of known glutamatergic neurons in brain express either VGLUT1 or VGLUT2,[58,61,62] whereas VGLUT3 was found in small populations of cells in the cortex, hippocampus, striatum, and raphé.[60,63,64] It is not clear yet if each of these different transporters serves a unique function in glutamate neurons or if they identify different subtypes of glutamate neurons (aside from their anatomic localization).

Using antibodies directed against these transporters as markers of glutamatergic terminals, we have begun to examine at the ultrastructural level the notion that regulation of striatal output by A_{2A} receptor stimulation may involve enhancement of excitatory input from the cortex to striatal projection neurons. Our preliminary studies show that VGLUT1-labeled terminals make asymmetric synapses on A_{2A}-labeled dendrites in the striatum (figure 3A). This is the first direct anatomic evidence for A_{2A}R modulation of excitatory glutamate input to striatopallidal neurons. Particularly interesting is the observation that some of these VGLUT1 terminals synapse on the head of A_{2A}-ir spines (figure 3, B

and C), in keeping with the idea that this excitatory input originates from the cortex. This interpretation is consistent with the highly specific topography of synaptic inputs onto medium spiny neurons.[29] Specifically, cortical afferents form asymmetric synapses predominantly on the head of striatal spines at the distal portion of the dendritic tree. Excitatory signal can be transmitted directly to the dendritic shaft unopposed, or it may be modulated by an inhibitory signal in cases where dopaminergic afferents synapse on the neck of the same spine. The juxtaposition of A_{2A}Rs with cortical and nigral afferents in the latter case would situate A_{2A}Rs ideally for integrating glutamatergic and dopaminergic input to GABAergic striatal neurons.

Although it is known that the striatum receives a topographic distribution of inputs from specific areas of the cortex, there is no evidence yet for whether these excitatory neurons show any phenotypic differences (e.g., if there is a segregation of neurons based on expression of vesicular glutamate transporter). And although terminals from all three types of VGLUT-expressing neurons have been found in striatum,[58,60] it is not known which types contact striatopallidal neurons, much less A_{2A}R-containing somatodendritic domains of striatopallidal neurons. Given the differential distribution of each of the VGLUTs, it will ultimately be important to determine if striatal output neurons receive input preferentially from neurons expressing one of these transporters or if terminals from all three VGLUT-expressing neuronal phenotypes make synaptic contact on A_{2A}R-containing cellular domains.

Multiple mechanisms for integration of striatal function. The ability of adenosine to alter basal ganglia function likely depends on the localization of A_{2A}Rs at multiple sites that modulate the inputs to and output activity of GABAergic striatopallidal projection neurons. Returning to our working model, we summarize our ultrastructural findings and examine multiple mechanisms by which A_{2A}Rs could modulate striatal function (see figure 1). We use this model to discuss five key sites of localization (as identified numerically in figure 1; numbers in parentheses below refer to these numbered sites) and to suggest interpretations and physiologic relevance of these findings, as follows.

1. Presynaptic A_{2A}Rs are occasionally found on excitatory terminals forming asymmetric synapses on unlabeled dendrites (12% of all A_{2A}R-containing synaptic contacts). These could be glutamatergic corticostriatal terminals contacting GABAergic projection neurons (1A, D) or GABAergic interneurons (1B, C) where A_{2A}Rs could stimulate glutamate release.
2. More prominent is the observation that somatodendritic A_{2A}Rs are frequently found postsynaptically to unlabeled terminals forming asymmetric synapses (83% of all A_{2A}R-containing synaptic contacts), suggesting a site for modulation of excitatory input (possibly glutamatergic corticostriatal neurons) to GABAergic striatopallidal neurons. Results showing VGLUT1-labeled terminals contacting A_{2A}R dendrites, and specifically A_{2A}R spines, although still preliminary, provide additional evidence for glutamatergic input from cortex to A_{2A}R-expressing striatal neurons and provide the morphologic basis for a synergistic interaction of A_{2A}Rs with mGluR5 receptors postsynaptic to glutamate terminals.
3. The prevalence of somatodendritic A_{2A}Rs in ultrastructural studies (~70% of all A_{2A}R-labeled profiles)—either in single-labeled profiles or colocalized with GABA—is consistent with the localization of A_{2A}Rs on GABAergic striatopallidal neurons. Receptors have been found postsynaptically to inhibitory (symmetric) input from unlabeled or GABA-labeled terminals that could originate from GABAergic interneurons (3A, C), cholinergic interneurons (3B), local axon collaterals from striatopallidal (3D) or striatonigral (3F) neurons, or dopaminergic inputs from substantia nigra (3E). The latter case would, of course, form the basis for sites of antagonistic interactions between A_{2A}Rs and dopamine D_2 receptors.
4. Presynaptic A_{2A}Rs are occasionally found on terminals forming symmetric synapses (~3% of all A_{2A}R-containing synaptic contacts) with GABA-labeled or unlabeled soma or dendritic profiles. Single-labeled A_{2A}R-containing terminals could originate from cholinergic interneurons (although this has been a controversial topic) where A_{2A}Rs could modulate the release of ACh onto GABAergic projection neurons (4A, B). More likely is the possibility that A_{2A}Rs are found presynaptically at terminals of GABAergic striatopallidal local axon collaterals where they could enhance the release of GABA onto either type of GABAergic projection neuron, resulting in functional inhibition (4C, D).
5. A_{2A}R-expressing GABAergic neurons in the striatum that project to the GP express A_{2A}Rs at their terminals in the GP where they could enhance the release of GABA.

Although some of these points remain to be proven experimentally, it is hoped that this model can provide a framework for development of hypotheses and strategies for future studies on the role of adenosine in modulating striatal function.

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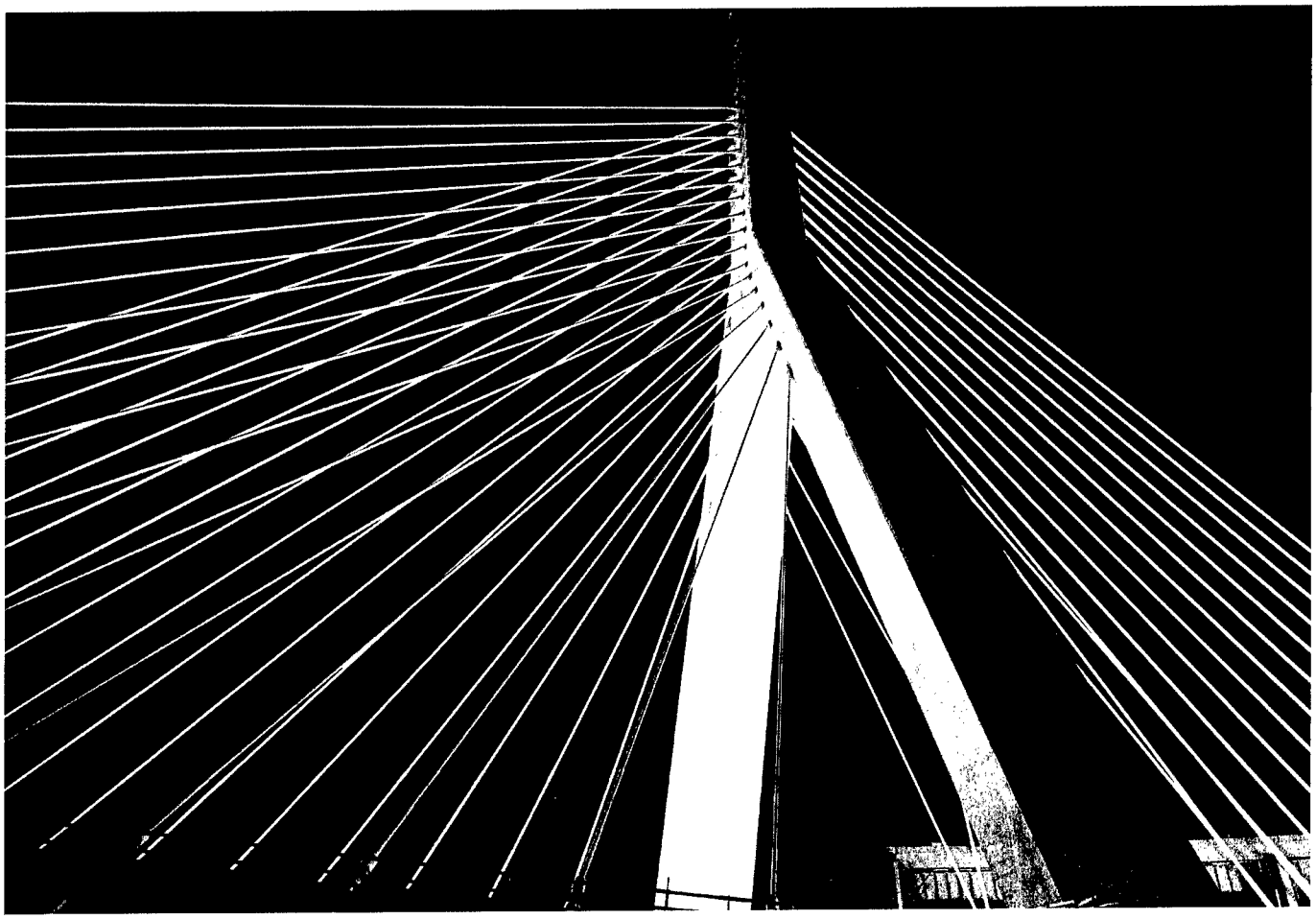

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Receptor heteromerization in adenosine A_{2A} receptor signaling

Relevance for striatal function and Parkinson's disease

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Abstract—Recently evidence has been presented that adenosine A_{2A} and dopamine D_2 receptors form functional heteromeric receptor complexes as demonstrated in human neuroblastoma cells and mouse fibroblast Ltk$^-$ cells. These A_{2A}/D_2 heteromeric receptor complexes undergo coaggregation, cointernalization, and codesensitization on D_2 or A_{2A} receptor agonist treatments and especially after combined agonist treatment. It is hypothesized that the A_{2A}/D_2 receptor heteromer represents the molecular basis for the antagonistic A_{2A}/D_2 receptor interactions demonstrated at the biochemical and behavioral levels. Functional heteromeric complexes between A_{2A} and metabotropic glutamate 5 receptors (mGluR5) have also recently been demonstrated in HEK-293 cells and rat striatal membrane preparations. The A_{2A}/mGluR5 receptor heteromer may account for the synergism found after combined agonist treatments demonstrated in different in vitro and in vivo models. D_2, A_{2A}, and mGluR5 receptors are found together in the dendritic spines of the striatopallidal GABA neurons. Therefore, possible D_2/A_{2A}/mGluR5 multimeric receptor complexes and the receptor interactions within them may have a major role in controlling the dorsal and ventral striatopallidal GABA neurons involved in Parkinson's disease and in schizophrenia and drug addiction, respectively.

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The A_{2A}/D_2 heteromeric receptor complex. Evidence has accumulated that intramembrane antagonistic receptor–receptor interactions between adenosine A_{2A} and dopamine D_2 receptors exist in dorsal and especially in the ventral striatum as studied in biochemical and receptor autoradiographic experiments.[1-4] Both receptors are located on the dendritic spines of the striatopallidal GABA neurons.[5,6] In 2002, it became possible to demonstrate the existence of A_{2A}/D_2 heteromeric receptor complexes in membrane preparations from human D_2 receptor (long-form) stably transfected SH-SY5Y neuroblastoma cells and from mouse fibroblast Ltk$^-$ cells stably transfected with human D_2 (long-form) receptors and transiently cotransfected with the A_{2A} receptor double tagged with hemagglutinin.[7] It was observed that the A_{2A}/D_2 heteromeric receptor complexes existed in the absence of exogenous A_{2A} and D_2 receptor agonists and therefore represented constitutive heteromers. Experiments using bioluminescence resonance energy transfer (BRET) and fluorescence resonance energy transfer (FRET) techniques indicate that the A_{2A}/D_2 heteromeric receptor complex represents a heterodimer (Canals et al., unpublished observations).

Based on these observations, it is likely that the A_{2A} receptor agonist-induced reduction of D_2 receptor affinity, mainly involving the high-affinity state, is caused by an activation of the A_{2A} receptor in the heteromeric receptor complex causing a conformational change in the binding pocket of the D_2 receptor.[8] A_{2A} receptor activation also leads to a reduction of the G-protein coupling of the D_2 receptor as seen from an antagonism of the GTP-induced cross-regulation of the D_2 receptor with a disappearance of the high-affinity state.[9] These events result in a reduction of D_2 receptor signaling as inferred from the ability of A_{2A} receptor agonists to counteract the reduction of adenylyl cyclase activity[7] and the changes in intracellular Ca^{2+} levels[8,10] induced by D_2 receptors. Conversely, D_2 receptor activation antagonizes A_{2A} receptor signaling by a G_i-mediated inhibition of the A_{2A}/G_{olf}-activated adenylyl cyclase, which seems to be particularly pronounced in the dorsal striatum.[11] The existence of the A_{2A}/D_2 heteromeric receptor complex is probably also the molecular

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mechanism underlying the demonstrated coaggregation, cointernalization, and codesensitization of A_{2A} and D_2 receptors on A_{2A} or D_2 receptor agonist treatments.[7] It is of substantial interest that combined D_2 and A_{2A} receptor agonist treatment markedly enhances the cointernalization and codesensitization of the A_{2A} and D_2 receptors. Therefore, the A_{2A}/D_2 heteromeric receptor complex may make A_{2A}/D_2 receptor cotrafficking possible.

Relevance of the A_{2A}/D_2 heteromeric receptor complex for PD and its management. Based on the above, it seems likely that the recently demonstrated antiparkinsonian actions of A_{2A} receptor antagonists in humans (see Chase, page S107) are to a substantial degree caused by blocking the action of endogenous adenosine on A_{2A} receptors of the A_{2A}/D_2 receptor heteromer, leading to enhancement of D_2 receptor signaling.[1,3] This may permit the reduction of the L-dopa dose and thus reduces the development of the L-dopa–induced dyskinesias related to a change in the phenotypic character of striatal GABAergic neurons with overexpression of prodynorphin and glutamic acid decarboxylase (GAD) mRNA levels[12] (see Chen et al., page S74).

Carta et al.[13] (see Carta et al., page S39) have shown that combined treatment with an A_{2A} receptor antagonist and a low dose of L-dopa did not produce the possibly deleterious long-term increases in GAD, dynorphin, and enkephalin mRNA levels. By contrast, repeated treatment with a higher dose of L-dopa alone (which produced the same acute motor stimulant effect as did the combination of L-dopa plus A_{2A} antagonist) led to a significant increase in striatal GAD, dynorphin, and enkephalin expression. This absence of striatal gene inductions with repeated L-dopa plus A_{2A} antagonist was correlated with a stable turning response, in contrast to the sensitized turning response that developed after repeated treatment with L-dopa alone in this hemiparkinsonian model in rats.[13] It is of note that A_{2A} receptor antagonists alone produce antiparkinsonian effects without dyskinesias in parkinsonian monkeys.[14-16] It has also been indicated that long-term L-dopa therapy requires A_{2A} receptors for persistent behavioral sensitization as studied in A_{2A} receptor knockout mice.[17]

The loss of inhibition of A_{2A} receptor signaling by the reduced D_2 receptor signaling in patients with Parkinson's disease (PD) adds to the parkinsonian symptoms, and thus antiparkinsonian actions of A_{2A} receptor antagonists can be related not only to an enhancement of D_2 receptor signaling but also to the blockade of increased A_{2A} receptor signaling of the hypodopaminergic state.[11,18] A_{2A} receptor antagonists can counteract motor inhibitory and cataleptic effects after genetic and pharmacologic disruptions of D_2 receptor-mediated transmission.[19,20]

Data on A_{2A}/D_2 receptor cotrafficking[7] suggest that increased A_{2A}/D_2 receptor cointernalization in response to long-term L-dopa therapy, in combination with increased striatal adenosine tone,[7] may contribute to the deterioration of the therapeutic action of L-dopa. Simply stated, the desensitization may result from a decreased membrane presence of the D_2 receptor.

There is also the possibility that A_{2A} receptor antagonists can show neuroprotective activity because a higher coffee and caffeine intake was associated with reduced risk for PD.[21,22] A_{2A} receptor antagonists may reduce excitotoxicity[23] because, for example, the stimulation of striatal glutamate release by metabotropic glutamate 5 receptor (mGluR5) agonists involves A_{2A} receptors.[24]

In our opinion, an important aspect to be further investigated to reach a better understanding of the sensitization/desensitization process of the D_2 receptors may reside in the stoichiometry of the A_{2A}/D_2 receptor heterodimers vs the A_{2A} and D_2 receptor monomers and homodimers present at the plasma membrane level. In particular, long-term L-dopa therapy may induce internalization of A_{2A} and D_2 receptors when associated as heterodimers, whereas it may not affect A_{2A} receptors existing as monomers or homodimers.[7] Therefore, the relative amount of D_2 receptors in the two forms may be of importance in controlling the manifold aspects (efficacy and potency of D_2 signaling trafficking, sensitization, and desensitization) of the neuronal response to long-term L-dopa therapy.

The A_{2A}/mGluR5 heteromeric receptor complex. Our interest in A_{2A}/mGluR5 receptor interactions started with the demonstration that A_{2A} and group I mGluR receptor agonists could synergistically reduce affinity of D_2 receptor in striatal membranes.[25] Recently it has been possible to show the existence of heteromeric receptor complexes between A_{2A} receptors and the group I mGluR receptor subtype mGluR5 in coimmunoprecipitation experiments in HEK-293 cells cotransfected with Flag-A_{2A} and hemagglutinin mGluR5 receptors and in rat striatal membrane preparations involving native A_{2A} and mGluR5 receptors.[26] In contrast, there was a lack of coimmunoprecipitation between mGluR5 and mGluR1β (an isoform of another group I mGluR receptor) receptors. In agreement, it was found that the A_{2A} and mGluR5 receptors were colocalized on the membrane surface of cotransfected HEK-293 cells as shown with confocal laser microscopy after transient cotransfections. Furthermore, in preliminary fluorescence microscopy experiments with optical sectioning techniques (using the exhaustive photon reassignment process) we have found evidence for a strong A_{2A}/mGluR5 receptor colocalization in rat striatal primary cultures (figure). The detailed quantitative analysis of these results is in preparation (only the images corresponding to the pictures in the figure have been analyzed). It is presently unknown if adapter proteins, such as Homer proteins or the Shank family of scaffold proteins, link mGluR5 and A_{2A} receptors together in the het-

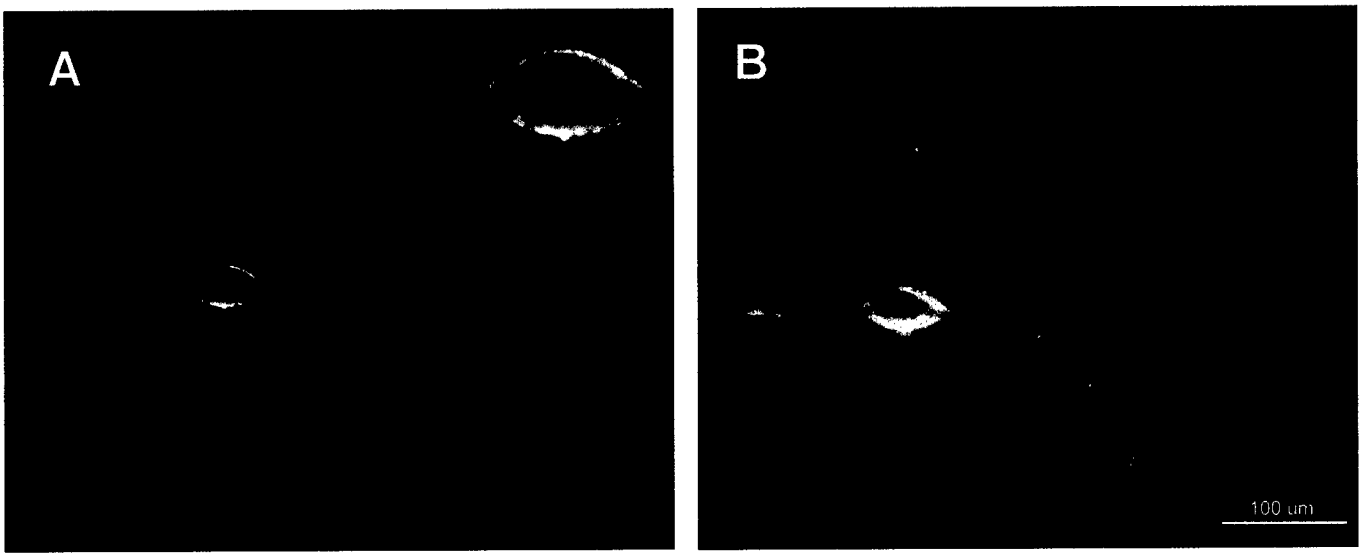

Figure. Colocalization of A_{2A} and metabotropic glutamate 5 (mGluR5) receptor immunoreactivities in the soma (A) and soma and dendrites (B) of striatal neurons in primary cultures (day 15 in vitro). Cryopreserved primary striatal neurons were obtained from QBM Cell Science (R-Cp-502; Ottawa, Canada). Cells were stored in liquid nitrogen until use. Cells were immunostained with rabbit anti-mGluR5 receptor (1:100; Upstate Biotechnology, Lake Placid, NY) and mouse anti-A_{2A} receptor (1:1000) antibodies.[40] Goat antirabbit Alexa-Fluor 488 (1:400; Molecular Probes, Eugene, OR) and goat anti-mouse CY3 (1:400; Sigma Chemical Co., St. Louis, MO) conjugated antibodies were used as secondary antibodies. The immunostaining procedure is the same as described in detail elsewhere for permeabilized cells[41] (the A_{2A} receptor antibody is directed against an intracellular epitope[40]) with some modifications. Cells were analyzed by double immunofluorescence with confocal-like microscopy. Superimposition of the red (CY3) and green (Alexa-Fluor 488) images reveals the A_{2A}/mGluR5 receptor colocalization in yellow (magnification bar, 100 μm). The "Boolean colocalization" (overlap of the field areas between A_{2A} and mGluR5 receptors) at soma level was equal to approximately 33% of the entire soma field area, whereas the "yellow colocalization" (overlap of the field areas between A_{2A} and mGluR5 receptors where red and green emission showed similar intensity of emission) at soma level was equal to approximately 4.5% of the Boolean colocalization. This yellow colocalization most probably represents the area with the highest ratio of A_{2A}/mGluR5 receptor heteromeric complexes vs A_{2A} and mGluR5 homomeric (or monomeric) receptor complexes (for detailed description of the method, see elsewhere[42]).

eromeric receptor complex. The Homer proteins can bind to the C-terminal part of mGluR5 receptor and produce their clustering, and the Shank proteins link together the mGluR5 with the NMDA receptors. The A_{2A}/mGluR5 receptor heteromer was also present in the absence of exogenous agonists and appeared to be preformed, like the A_{2A}/D_2 receptor heteromer.

In the HEK-293 cells, it was possible to give a functional correlate to the A_{2A}/mGluR5 heteromeric receptor complex.[26] Therefore, A_{2A}/mGluR5 receptor coactivation produced a synergistic interaction at the level of extracellular signal-regulated kinase 1/2 (ERK) phosphorylation and c-fos expression. These and other results suggested that the A_{2A}/mGluR5 heteromeric receptor complex is involved in striatal neuron plasticity, including long-term potentiation and depression.[26] This synergism may be brought about by independent signals interacting at the level of the mitogen-activated protein kinase (MAPK) cascade.[27] There is also the possibility that on coactivation of the heteromeric receptor complex, a multireceptor complex may be assembled with receptor tyrosine kinases or nonreceptor tyrosine kinase Src, leading to ERK activation.[28-32] Another possible mechanism involved in the synergism between A_{2A} and mGluR5 receptors could be the modulation of mGluR5 receptor desensitization, which has been demonstrated for NMDA/mGluR5 and group II mGluR/mGluR5 receptor interactions.[33,34] In these cases, the modulation seems to depend on the activation of phosphatase 2B, which reverses the agonist-induced protein kinase C-mediated desensitization of mGluR5 receptors.[33,34] However, this mechanism would imply synergistic interactions at the second-messenger level (Ca^{2+} mobilization),[33,34] which could not be demonstrated in A_{2A}/mGluR5 receptor-cotransfected HEK-293 cells.[26]

A similar A_{2A}/mGluR5 receptor synergism could be demonstrated in the rat striatum for c-fos expression correlated with a synergistic A_{2A}/mGluR5-mediated counteraction of phencyclidine-induced motor activity.[26] It is well known that this motor activity depends on D_2 receptor activity and has so far been blocked only by a high degree of D_2 receptor blockade. Therefore, the results suggest that A_{2A}/mGluR5 receptor costimulation can override a strong D_2 receptor-mediated transmission at the behavioral level. Long-term, but not short-term, treatment with an mGluR5 receptor antagonist can reverse the akinetic deficit in a model of PD.[35] It seems possible that this can in part be caused by an internalization and downregulation of the A_{2A}/mGluR5 heteromeric re-

ceptor complex removing the D_2 receptor from inhibition of its signaling. In addition, there may also exist an acute mGluR5 receptor antagonist-induced counteraction of D_2 receptor blockade with haloperidol as seen from reduced rigidity and catalepsy[36] that may in part be exerted at the network level (e.g., at the level of the subthalamic nigral glutamate system).

The possible existence of A_{2A}/D_2/mGluR5 multimeric receptor complexes. The A_{2A}/mGluR5 heteromeric receptor complexes appear to be preferentially located at dendritic spines of striatopallidal GABAergic neurons[20,37] like the A_{2A}/D_2 heteromeric receptor complexes. Based on the aforementioned observations, it seems a reasonable hypothesis that there exist A_{2A}/D_2/mGluR5 multimeric receptor complexes in the dendritic spines of the striatopallidal GABA neurons.

Observations supporting this hypothesis:

1. A_{2A} and group I mGluR synergistically reduced the affinity of the high-affinity state of the striatal D_2 receptors in membrane preparations.[25]
2. Group I mGluR activation synergistically potentiated the ability of the A_{2A} receptor agonist CGS21680 to counteract D_2 receptor agonist (quinpirole)-induced contralateral rotational behavior.[25]
3. The mGluR5 receptor agonist CHPG reduced the affinity of the high-affinity state of the D_2 receptor, an action potentiated by CGS21680.[38]
4. CHPG inhibited contralateral rotational behaviors induced by quinpirole, an effect potentiated by CGS21680.[38]
5. The mGluR5 receptor agonist CHPG in the nucleus accumbens increased GABA release in the ventral pallidum, an action strongly potentiated by coperfusion with CGS21680.[39]
6. Coperfusion with quinpirole counteracted the increases in ventral pallidal GABA levels by CGS21680 and CHPG.[39]

These results are compatible with the existence of A_{2A}/D_2/mGluR5 multimeric complexes that are important in regulation of the dorsal and ventral striatopallidal GABA neurons and are of high relevance for management of PD (dorsal system) and schizophrenia and drug dependence (ventral system).

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A_{2A} receptor and striatal cellular functions

Regulation of gene expression, currents, and synaptic transmission

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Abstract—A_{2A} receptor is highly coexpressed with enkephalin and D_2 receptor in striatopallidal neurons. A_{2A} antagonists acutely enhance motor behavior in animal models of Parkinson's disease (PD) and are therefore considered potential PD therapeutic agents. Analysis of gene expression regulation using pharmacologic tools or A_{2A} receptor-deficient mice ($A_{2A}^{-/-}$) shows that the A_{2A} receptor positively and tonically controls the expression of enkephalin and immediate early genes in striatopallidal neurons. Because this regulation strictly mirrors the effect of D_2 receptor, these data strongly support the hypothesis that A_{2A} antagonists reduce the activity of striatopallidal neurons in models of PD. However, analysis of $A_{2A}^{-/-}$ mice suggests additional effects of A_{2A} receptor in the control of striatal physiology. Unexpectedly, these animals exhibited a reduction in exploratory activity and a 50% reduction in substance P expression. This was associated with a 45% decrease in the striatal extracellular dopamine concentration, suggesting that chronic absence of A_{2A} receptor results in a functional hypodopaminergic state in the striatum. The A_{2A} receptor controls inhibitory synaptic transmission negatively in the striatum and positively in the globus pallidus; this further supports the efficacy of A_{2A} antagonists in reducing the activity of striatopallidal neurons in PD. The A_{2A} receptor does not modulate basal α-amino-3-hydroxy-5-methyl-4-isoxazole proprionic acid (AMPA)-mediated excitatory corticoaccumbal synaptic transmission during normal physiologic conditions. However, genetic inactivation or pharmacologic blockade of the A_{2A} receptor significantly reduced long-term potentiation (LTP) at this synapse. Therefore, this receptor is implicated in the induction of corticoaccumbal LTP, an effect that could be related to its involvement in long-term behavioral sensitization to repeated dopaminergic treatment.

NEUROLOGY 2003;61(Suppl 6):S24–S29

Medium-sized spiny neurons of the striatum give rise to the output pathways of the basal ganglia system. Two distinct populations of these efferent GABAergic neurons have been recognized. Neurons projecting to the globus pallidus express enkephalin and form the striatopallidal pathway, whereas neurons projecting to the substantia nigra pars reticulata and entopeduncular nucleus express substance P and dynorphin and form the striatonigral pathway.[1] Although still controversial, dopamine receptors appear to be largely segregated in these two subpopulations with D_1 receptor expressed by striatonigral and D_2 receptor mostly expressed by striatopallidal neurons.[1-3] Striatonigral and striatopallidal pathways have been recognized as excitatory and inhibitory, respectively, to thalamocortical activity,[4] although this scheme has been refined more recently.[5] Adenosine A_{2A} receptors are particularly abundant in the striatum in most mammalian species studied so far,[2,3,6-10] although they are also expressed at a much lower level in other brain areas.[11,12] In the striatum, A_{2A} receptor expression is restricted to enkephalin- and D_2 receptor-containing striatopallidal neurons,[2,3,6] and this coexpression supports most, if not all, functional interactions between these receptors.

Regulation of striatal gene expression. Neuronal immediate early gene (IEG) expression is rapidly and strongly induced in response to a variety of stimuli. Quantitative assessment of IEG expression is frequently used as a neuroanatomic tool to examine neuronal activity because this expression is considered a robust indicator of changes in neuronal activity in most systems.[13] The products of IEGs mostly function as transcription factors to activate or repress other genes and are critical in mediating stimulus-induced neuronal plasticity.

In the striatum, neuronal activity and ultimately neuropeptide and IEG expression are controlled through complex interactions between many neurotransmitters and their receptors. Among these, the

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dopaminergic nigrostriatal pathway differentially influences gene expression in both striatal subpopulations. Reduction in dopamine input into the striatum decreases substance P expression in striatonigral neurons, whereas it increases enkephalin expression in striatopallidal neurons; these alterations can be reversed by activation of D_1 and D_2 receptors, respectively.[1] Conversely, expression of IEG (i.e., c-fos and zif-268 or NGFI-A) is increased or induced in striatonigral neurons through activation of D_1 receptors and in striatopallidal neurons after blockade of D_2 receptors or dopamine depletion.[14,15] D_1 antagonists and D_2 agonists, respectively, counteract these inductions in IEG expression and may even reduce their constitutive expression.[16]

A_{2A} receptor and neuropeptide gene expression. The reversal of the dopamine depletion-induced increase in enkephalin gene expression by D_2 agonists,[1] the A_{2A}/D_2 coexpression in striatopallidal neurons,[2,3,6] and the opposite actions of these receptors on signaling cascades have strongly suggested a putative role for A_{2A} receptors in the control of enkephalin gene expression. More precisely, it was expected that blockade of A_{2A} receptors on striatopallidal neurons might counteract the increase in enkephalin expression induced by dopamine depletion. This hypothesis was first strongly supported by the observation that a 15-day treatment with caffeine, a nonspecific adenosine receptor antagonist, reduced the level of enkephalin expression in the ipsilateral striatum of unilaterally 6-hydroxydopamine (6-OHDA)–lesioned rats (figure 1).[3] The specificity of this effect on striatopallidal neurons was suggested by the absence of any change in substance P expression, which remained strongly decreased (see figure 1).[3] The involvement of the A_{2A} receptor in the control of enkephalin expression was supported further using more selective A_{2A} antagonists. Molecules such as CGS15943A or KF17837 were able to counteract the dopamine depletion- or D_2 receptor blockade-induced increase in enkephalin expression.[17-19] Although strongly suggestive, the pharmacologic analysis of A_{2A} receptor function is hindered by the partial specificity and efficacy, as well as poor solubility and brain penetration, of available adenosinergic ligands. Therefore, these questions were further addressed by disrupting the A_{2A} receptor gene in mice.[20,21] Supporting a role of A_{2A} receptor activation in the control of gene expression in striatopallidal neurons, enkephalin expression is slightly but significantly reduced in mice lacking this receptor ($A_{2A}^{-/-}$) (figure 2).[20,22-24] This decreased expression mainly occurs in the lateral striatum.[22,23] Interestingly, specific A_{2A} antagonist[25] or genetic inactivation of A_{2A} receptor[24] counteracted the increase in enkephalin expression detected in the striatum of D_2 receptor-deficient mice ($D_2^{-/-}$) and reversed the parkinsonian-like motor behavior observed in these mice.[25] In the same way, deficiency in A_{2A} receptor strongly but partially reduced the ability of haloperidol to induce enkephalin

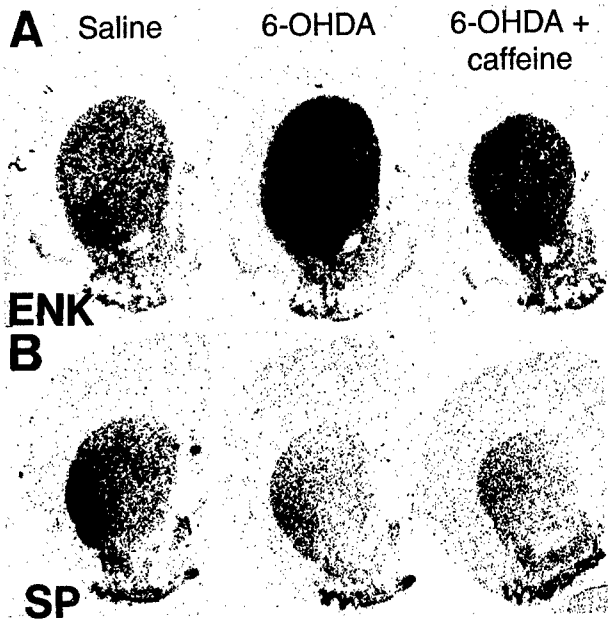

Figure 1. Enkephalin (A) and substance P (B) expression detected by in situ hybridization in the striatum of saline-treated rats (left) and of 6-hydroxydopamine (6-OHDA)–lesioned animals subsequently treated with saline (middle) or with caffeine (right). The 6-OHDA–lesion-induced increase in enkephalin mRNA was partially reversed by caffeine (A), which did not affect the decrease in substance P mRNA (B).

expression and catalepsy.[24] Altogether, these data demonstrate that postsynaptic A_{2A} receptors on striatopallidal neurons positively regulate the expression of enkephalin, in balance with the negative action of D_2 receptor. They also strongly suggest that this control is at least partly independent of the dopamine D_2 receptor.

Available data on control of substance P expression are rather puzzling. A_{2A} antagonists did not modify substance P expression in rat[19] or wild-type mice,[25] whereas they increased substance P expres-

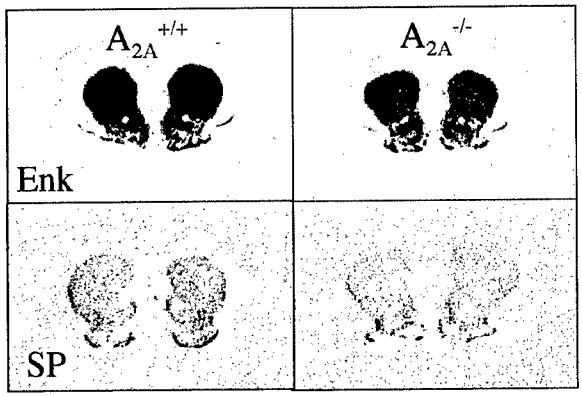

Figure 2. Expression of enkephalin mRNA and substance P mRNA was significantly reduced in the striatum of $A_{2A}^{-/-}$ mice (right) compared with the wild-type mice (left). Reprinted from Neuroscience, vol 107, D'Alcantara et al., pp 455–464, Copyright 2001, with permission from Elsevier Science.

sion in $D_2^{-/-}$ mice.[25] Moreover, substance P expression was strongly decreased in $A_{2A}^{-/-}$ mice (see figure 2).[20,22,23] In agreement with this latter observation, caffeine used at a dose acting principally on A_{2A} receptor (see below) also decreased substance P expression.[22] Further studies are required to explain these discrepancies. Undoubtedly, a complex interplay exists between neurotransmitters such as dopamine, acetylcholine, and glutamate, whose release is regulated by A_1 and A_{2A} presynaptic receptors.

A_{2A} receptor and immediate early gene expression. First clues for a role of adenosine receptors in the regulation of IEG expression came from data showing that a high dose of caffeine dramatically induced their expression in striatum.[26] Because some IEG, such as zif-268, are constitutively expressed at a significant level in the striatum, it was further demonstrated that caffeine exerts a biphasic effect. Similar to its biphasic action on locomotor behavior (stimulatory at low doses and inhibitory at high doses), caffeine decreased striatal IEG expression at low doses and increased it at high doses.[10,27] Interestingly, blockade of A_{2A} receptors by specific molecules (SCH58261) led to locomotor stimulation and inhibition of IEG expression in striatopallidal neurons.[10,28] This strongly suggests that the blockade of A_{2A} receptors underlies the behavioral activation by low dose of caffeine through a decreased activity of these striatopallidal neurons. Several pharmacologic studies extended this concept by showing that A_{2A} agonists (CGS21680) stimulated c-fos expression in striatal neurons of 6-OHDA–lesioned rats,[29] that the reserpine-induced increase in c-fos expression was reduced by coapplication of D_2 agonist and A_{2A} antagonists,[30] and that the haloperidol-induced or 6-OHDA–induced increase in c-fos mRNA expression in striatopallidal neurons was reduced by SCH58261.[31,32] This role of A_{2A} receptor was definitively demonstrated in $A_{2A}^{-/-}$ mice, in which the low-dose effects of caffeine (decreased IEG expression and stimulated locomotor behavior) were abolished, whereas the high-dose effects persisted.[20,22,33] Altogether, these data demonstrate that the A_{2A} receptor exerts positive tonic regulation of IEGs in striatopallidal neurons, whereas the D_2 receptor exerts negative tonic regulation.

Conversely, the increase in IEG expression and the depressant effect of high-dose caffeine were mimicked by the A_1 antagonist DPCPX[28,33,34] and have therefore been attributed to blockade of A_1 receptor. This increase in IEG expression occurred in both neuronal subpopulations through different mechanisms involving an increase in release of neurotransmitters such as dopamine and acetylcholine.[34]

Finally, it is noteworthy that the basal level of IEG expression in $A_{2A}^{-/-}$ mice was strongly decreased not only in striatopallidal neurons but also in all striatal neurons and in the cerebral cortex and hippocampus.[22,23] This decrease is related to a reduction in dopamine release[23,35] and leads to the hypothesis of a functional hypodopaminergic activity in these mice.[23] This is also supported by a paradoxical reduction in their locomotor behavior[20,24,33,36] and by the attenuated effect of psychostimulant agents.[36]

Regulation of currents and synaptic transmission. An intricate balance between excitatory and inhibitory inputs controls the activity of striatal neurons. These inputs can be modulated during the short term or long term by many neurotransmitters. Although the regulation of synaptic transmission and neuronal ion currents by A_1 receptor has been thoroughly described in most brain areas, including the striatum,[37,38] fewer data are available regarding the action of A_{2A} receptors. Three types of actions have been considered so far: 1) the regulation of inhibitory synaptic transmission in the striatum and the globus pallidus; 2) the regulation of the NMDA current; and 3) the modulation of excitatory synaptic transmission and long-term plasticity at the corticostriatal synapse.

A_{2A} receptor and inhibitory synaptic transmission. Using patch-clamp whole-cell recording in brain slices, it has been shown that an A_{2A} agonist can reduce $GABA_A$-mediated inhibitory postsynaptic currents (IPSCs) evoked in medium-sized spiny neurons by an intrastriatal stimulation.[39] This effect was counteracted by A_{2A} antagonists and mimicked by cyclic adenosine monophosphate (cAMP) analogs. Moreover, because CGS21680 decreased the frequency of miniature IPSCs without modification of their amplitude, the effect was interpreted to be mediated at a presynaptic site[39] by either recurrent terminals of these projection neurons (feedback circuit) or terminals of GABAergic interneurons (feedforward circuit). However, these data are in opposition to the previous demonstration that stimulation of the A_{2A} receptor stimulates GABA release in the striatum.[40] Conversely, the same patch-clamp approach revealed a stimulating effect of A_{2A} agonists on $GABA_A$-mediated IPSCs evoked in principal neurons of the globus pallidus.[41,42] This effect also involved the cAMP pathway and occurred presynaptically on terminals of striatopallidal neurons. This latter effect confirms the positive regulation of GABA release in the globus pallidus by the A_{2A} receptor in vitro and in vivo.[40,43-45] Because it is difficult to assume that two terminals of the same neuron are regulated in opposite directions by an agonist acting at the same receptor, the inhibitory effect observed in the striatum probably occurs at terminals of GABAergic interneurons rather than on the recurrent collaterals of spiny neurons. Nevertheless, this latter hypothesis could not be definitively ruled out if we assume that a disynaptic pathway including recurrent collaterals and interneurons could also be involved. Although further work is needed to reconcile all data, they converge to the concept that activation of A_{2A} receptor in the striatum or the globus pallidus increases the inhibitory action of striatopallidal neurons on their targets.

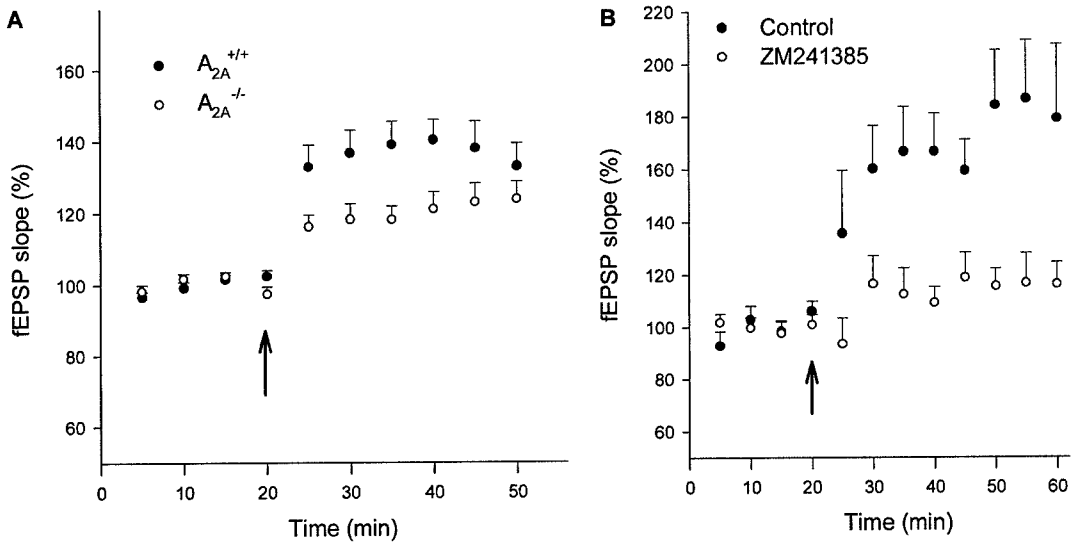

Figure 3. Long-term plasticity evoked by high-frequency stimulation of the corticoaccumbal synaptic pathway is regulated by A_{2A} receptor. The level of long-term potentiation of the field excitatory postsynaptic potential (EPSP) slope was decreased in slices from $A_{2A}^{-/-}$ mice compared with wild-type mice (left) and in ZM241385-treated wild-type slices compared with wild-type slices recorded in control conditions (right). Reprinted from Neuroscience, vol 107, D'Alcantara et al., pp 455–464, Copyright 2001, with permission from Elsevier Science.

A_{2A} receptor and NMDA-AMPA glutamate currents. A_{2A} receptor activation reduced the amplitude of NMDA-mediated inward currents in a subpopulation of spiny neurons.[46] This postsynaptic effect is independent of the cAMP–protein kinase A pathway but appears to involve a phospholipase C–induced stimulation of the Ca^{2+}/calmodulin kinase II pathway.[47] To our knowledge, stimulation of this cascade by A_{2A} receptors has not been reported elsewhere;[48] instead, NMDA currents are increased on activation of the cAMP–protein kinase A pathway.[49] In the same paradigm, an A_{2A} agonist did not modify α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA)-induced inward currents.[47,50]

A_{2A} receptor and excitatory synaptic transmission. A_1 receptor activation strongly depresses corticostriatal or corticoaccumbal transmission through a presynaptic mechanism.[37,38] In contrast, activation of A_{2A} receptors does not modify the excitatory postsynaptic currents (EPSCs) or excitatory postsynaptic potentials (EPSPs) recorded during basal conditions by patch-clamp or extracellular field recording.[37,38,50,51] Note that in these conditions (including Mg^{2+} in the bath), the EPSP fully depends on AMPA receptor activation. The lack of A_{2A} effect was demonstrated not only by using pharmacologic tools on slices from wild-type animals[37,38,50,51] but also by the observation that there was no difference between wild-type and $A_{2A}^{-/-}$ mice in this condition.[51] Because long-term interactions between A_1 and A_{2A} receptors in controlling synaptic transmission have been described in other systems, different protocols were considered in which the drugs were applied at different times. However, they all failed to show any effect of A_{2A} receptors on the basal synaptic transmission.[51] Recently, it was shown that A_{2A} receptors are able to modulate this basal excitatory synaptic transmission only on slices recorded in the presence of 4-aminopyridine (see Popoli et al., page S69). This observation is consistent with in vivo microdialysis studies suggesting that adenosine acting through the A_{2A} receptor stimulates glutamate release in the striatum.[52,53]

Because a specific transmitter pathway may regulate synaptic plasticity even if it does not appear to control basal synaptic transmission, the role of A_{2A} receptors in long-term potentiation (LTP) of the AMPA receptor-mediated EPSP was analyzed in the accumbens nucleus.[51] LTP could be elicited in wild-type and $A_{2A}^{-/-}$ mice. However, LTP appeared to be quantitatively modulated by the A_{2A} receptor pathway because the level of potentiation was reduced in $A_{2A}^{-/-}$ mice and in slices of wild-type mice in which the A_{2A} receptor pathway was blocked (figure 3).[51] The involvement of protein kinase A was supported by a reduced level of potentiation in slices of wild-type mice treated with an inhibitor of this enzyme. Although this latter experiment is suggestive of a postsynaptic site of action, the involvement of presynaptic regulation of dopamine or glutamate release is not ruled out. Therefore, adenosine acting at the A_{2A} receptor is implicated in events directly or indirectly related to LTP induction in the accumbens, whereas it is barely involved in the regulation of basal AMPA receptor-mediated excitatory synaptic transmission. Because long-term synaptic plasticity and particularly the equilibrium between long-term depression (LTD) and LTP in the nucleus accumbens are supposed to play important roles in motor learning and reward processes, the regulation of LTP induction by adenosine A_{2A} receptor should

have important and specific behavioral consequences. Several observations in the literature support this hypothesis. First, adenosine acting at A_{2A} receptors inhibits central reward processes, particularly during the adaptation associated with chronic cocaine-induced neuronal activation.[54] In addition, A_{2A} receptors are required for the persistence of behavioral sensitization to repeated L-dopa therapy.[55]

Conclusions. Roles and mechanisms of action of A_{2A} receptor in basal ganglia have been extensively studied in the past decade through the analysis of A_{2A} receptor-mediated regulation of striatal gene expression, ion currents and synaptic transmission, and plasticity using pharmacologic tools and knockout mice. These studies provide major clues for targeting this receptor in PD therapy. The main locus of the A_{2A} receptor-mediated action is the striatopallidal neuron. The ability of the A_{2A} receptor to regulate enkephalin and IEG expression in this neuron and to modulate its inhibitory input and output constitutes the basis for the efficacy of A_{2A} antagonists in models of PD.

However, inactivation of the A_{2A} receptor gene in mice leads to additional alterations in striatal and extrastriatal gene expression and in locomotor activity that were unexpected based on the A_{2A}/D_2 coexpression in striatopallidal neurons. These alterations may be related to a functional hypodopaminergic state; therefore, further work is needed to address this question with regard to long-term treatment with A_{2A} antagonists.

A_{2A} receptors are also involved in the induction of long-term synaptic plasticity in the accumbens. Therefore, specific behavioral consequences of A_{2A} receptor blockade are expected because synaptic plasticity in the nucleus accumbens is involved in motor learning and reward processes. Although these consequences remain to be identified, relationships with the mechanisms of persistence of behavioral sensitization to repeated L-dopa therapy or with long-term side effects of L-dopa therapy, such as dyskinesia, could be suspected.

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II: A_{2A} receptor modulation for symptomatic therapy in Parkinson's disease

Adenosine A_{2A} receptor modulation of motor systems in PD

David G. Standaert, MD, PhD

The modern era of treatment for Parkinson's disease (PD) began with the work of Cotzias et al.,[1] who demonstrated that sustained improvement in the symptoms of the disease could be obtained by long-term therapy with L-dopa. Although L-dopa remains the most effective agent available for the management of PD, it was not long before the shortcomings of this approach became apparent. The most common problems are related to the development of "motor complications:" wearing off and dyskinesias. These are observed in at least 40% of patients with PD after 4 to 6 years of treatment with L-dopa and in up to 70% of patients after 10 years of treatment.[2] With time, the magnitude of beneficial effect that can be achieved with L-dopa therapy decreases, and motor complications become a major source of disability.[3]

The pathogenesis of motor complications is not well understood,[4] but the existing evidence suggests a complex interplay between the pharmacologic agents administered and adaptive responses of neural circuits. Degeneration of dopaminergic neurons seems to be important; persons who do not have PD but receive L-dopa for other indications do not develop motor complications. The intermittent stimulation of dopaminergic receptors produced by oral administration of medications is thought to be a key factor.[5] Experiments in patients using controlled administration of dopaminergic drugs support the view that such intermittent stimulation results in persistent changes in brain function.[6-8] The downstream mechanisms for these adaptive changes likely include modification of striatal NMDA glutamate receptors[9,10] and changes in dopaminergic signaling, opioid neuropeptides, and other mechanisms.[11]

Clinicians have adopted a variety of strategies in attempt to prevent or reduce the occurrence of motor complications of L-dopa. Two recent large clinical trials have suggested that initial therapy with the dopamine agonist drugs pramipexole or ropinirole, which have longer plasma half-lives than L-dopa, may be associated with reduced development of wearing off and dyskinesias.[12,13] However, the beneficial effect of these drugs may be less than that of L-dopa, and some have questioned whether the methodologies of these studies are adequate to warrant a change in clinical practice.[14] Other approaches to the management of motor complications have been the use of inhibitors of catechol-O-methyl-transferase, an important pathway for the catabolism of L-dopa,[15] and the use of amantadine, a drug with actions at NMDA glutamate receptors and other properties.[16] Intractable motor complications are one of the main indications for surgical intervention, using pallidotomy[17] or implanted deep brain stimulators.[18] On the whole, however, none of the currently available approaches are satisfactory, and motor complications remain a major problem for patients with PD.

It is hoped that drugs acting on the A_{2A} receptor ($A_{2A}R$) may fill this unmet need by either permitting management of PD without inducing motor complications or attenuating motor complications once they develop. In the following articles, Dr. Jenner discusses the role of $A_{2A}Rs$ in the circuitry of the basal ganglia and the efficacy of an $A_{2A}R$ antagonist as symptomatic therapy in rodent and primate models of parkinsonism. Morelli et al. describe the ability of an A_{2A} antagonist to augment the effectiveness of dopaminergic treatment in a rodent model without induction of sensitization or the associated downstream alterations in neuropeptide expression. Mori and Shindou provide evidence for a novel mechanism of $A_{2A}R$ action in the basal ganglia through presynaptic inhibition of GABAergic transmission. All of these are important steps toward a better understanding of the role of $A_{2A}Rs$ in the function of the basal ganglia and the eventual clinical use of drugs acting on $A_{2A}Rs$ in patients with PD.

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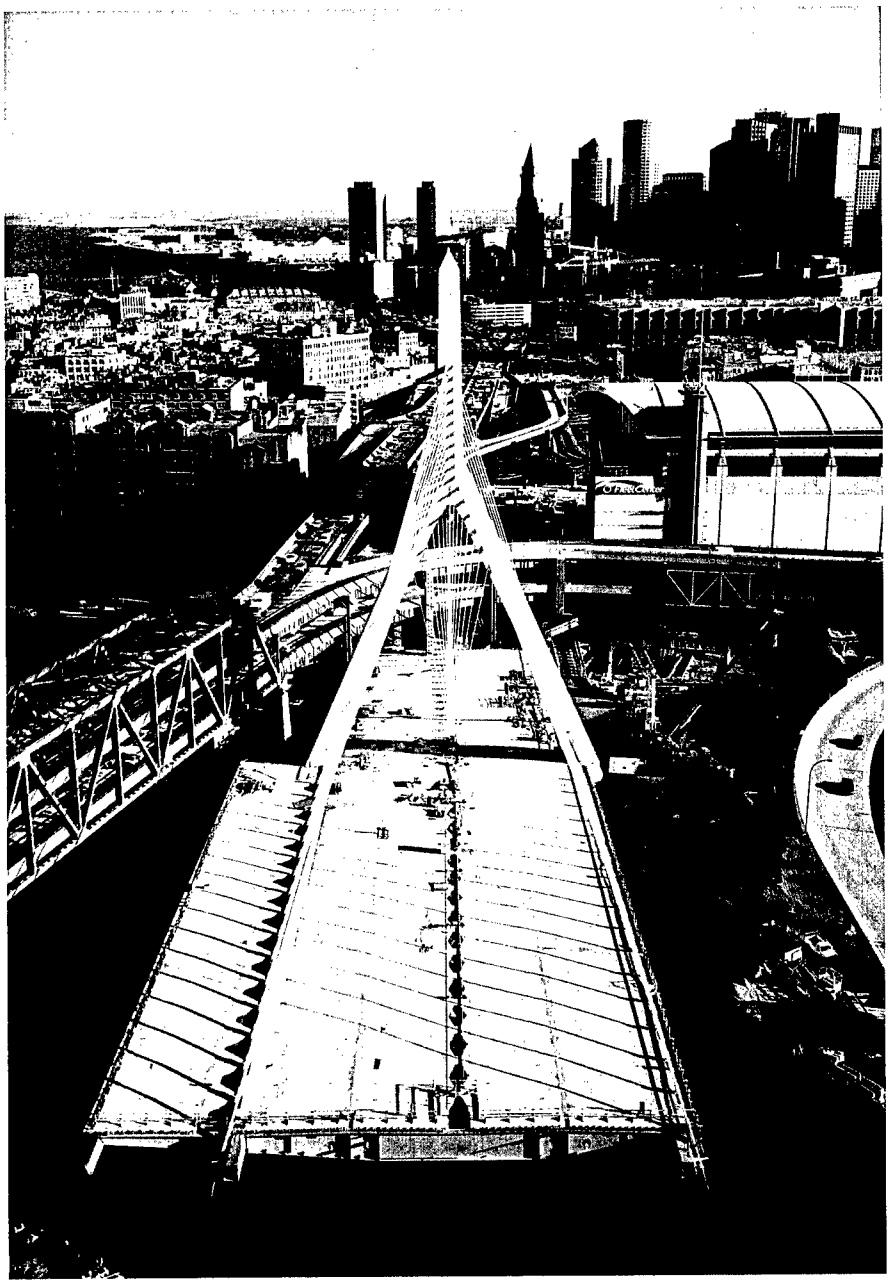

Don Eyles **February 2001**

A_{2A} antagonists as novel non-dopaminergic therapy for motor dysfunction in PD

Peter Jenner, DSc

Abstract—The future management of Parkinson's disease (PD) requires pharmacologic agents that do not lose efficacy with disease progression or induce dyskinesia and that are free of other dopaminergic side effects. A_{2A} receptor antagonists may provide an opportunity to introduce nondopaminergic management of PD. A_{2A} receptors are selectively localized in basal ganglia and to the indirect output pathway where they control GABA and acetylcholine release. The A_{2A} antagonists are effective in rodent models of PD, reversing motor deficits in haloperidol-treated, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated, or reserpinized mice, and potentiating L-dopa–induced rotation in 6-hydroxydopamine–lesioned rats without inducing dyskinesia. Importantly, the selective A_{2A} antagonist KW6002 reverses motor disability and increases locomotor activity in MPTP-treated primates without provoking dyskinesia established by previous exposure to L-dopa. In addition, KW6002 shows additive antiparkinsonian activity with L-dopa and the D_2 agonist quinpirole in MPTP-treated primates without enhancing the intensity of dyskinesia. The data available suggest that A_{2A} antagonists, such as KW6002, may be effective as monotherapy for the management of PD and that they will also produce additional benefit when administered in combination with L-dopa or dopamine agonist therapy.

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The current management of Parkinson's disease (PD) is based on dopaminergic therapy aimed at reversing the effects of striatal dopamine depletion induced by the destruction of the nigrostriatal pathway.[1-3] Symptomatic management of PD with L-dopa and dopamine agonist drugs dominates therapy and is highly effective in managing the early stages of the disorder. However, the introduction of dopaminergic drugs is associated with acute side effects, such as nausea, vomiting, and hypotension, and with a series of long-term, treatment-related complications that increase in severity with disease progression. These include a loss of drug efficacy ("wearing-off" and "on-off" phenomena), the onset of dyskinesias, and the occurrence of psychosis. There are also aspects of PD, such as postural instability, that do not respond to dopamine replacement therapy. In addition, all current therapies are symptomatic and do not significantly modify disease progression. As a consequence, there is a therapeutic need for novel pharmacologic approaches to the management of PD. New approaches should produce agents that are antiparkinsonian throughout the course of the disease without a loss of drug efficacy and that do not prime the basal ganglia for the appearance of dyskinesia. They should not provoke the expression of involuntary movements in those patients already exposed to L-dopa or induce psychosis. There should be ameliorative effects on nonmotor symptoms that do not respond to current therapy without the occurrence of acute peripheral side effects. An additional advantage would be an effect in slowing or stopping disease progression by producing a neuroprotective or neurorestorative action.

A major asset in the search for novel therapeutic strategies for the management of PD is the availability of the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated primate model of the disorder. MPTP-treated primates show a selective degeneration of nigral dopaminergic cells leading to caudate-putamen dopamine deficiency and the onset of most of the cardinal symptoms of PD.[4,5] Such animals exhibit akinesia or bradykinesia, rigidity, and postural abnormalities, although they seldom show the rest tremor characteristic of PD and instead exhibit postural tremor. The MPTP-treated primate is only a partial model of PD because there is no loss of serotonin (5HT)-containing neurons in the raphe nuclei or noradrenaline-containing neurons in the locus ceruleus. Lewy body pathology is not found in the substantia nigra, and the loss of dopaminergic neurons is not progressive. However, the model has the advantage of being responsive to all current therapies used to manage PD, and because of the marked degree of nigral denervation exhibited by these animals, they rapidly develop dyskinesia in response to repeated L-dopa treatment.[6-9] Once primed with L-dopa to exhibit involuntary movements, MPTP-treated primates exhibit the same dyskinesias when subsequently acutely challenged with L-dopa or dopamine agonist drugs that occur in patients with PD. MPTP-treated primates also exhibit many of the other motor complications associated with

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PD, and repeated treatment can lead to the onset of wearing-off, on-off, freezing episodes, and beginning-of-dose and end-of-dose deterioration.[7,10,11] However, the greatest advantage of the MPTP model is that so far it has been entirely predictive of drug action in humans. In this respect, it is extremely useful in evaluating potential new therapeutic strategies for PD before the initiation of clinical trials.

One novel therapeutic approach to PD is to target nondopaminergic systems within basal ganglia that lie beyond the damaged nigrostriatal pathway.[12,13] This approach has been used to investigate the role of novel neuronal targets within the striatum and other regions of basal ganglia. A number of potential therapeutic agents have been described, including agents acting on glutamate, cannabinoid, opiate, and α2-adrenergic receptors and nicotinic and muscarinic receptors. These offer the potential for antiparkinsonian activity in the absence of some of the major side effects of PD currently encountered during long-term management of the illness. In particular, the identification of adenosine A_{2A} receptors in basal ganglia as a potential means of modifying motor behavior through a nondopaminergic mechanism may have important therapeutic consequences for the future management of PD.[14-18]

A_{2A} receptors and basal ganglia. The current concept of basal ganglia dysfunction, the onset of motor symptoms in PD, and the occurrence of L-dopa–induced dyskinesia center on changes that occur in striatal output pathways.[19] In particular, attention has focused on the indirect striatal output pathway from the striatum to the external segment of the globus pallidus (GPe), which comprises medium spiny GABAergic output neurons that bear D_2 receptors on their cell bodies.[20-23] The projection to GPe connects with another GABAergic pathway projecting to the subthalamic nucleus, which in turn makes contact with glutamatergic neurons projecting to the internal globus pallidus (GPi) and hence to the thalamus and motor cortex. The indirect striatal output pathway appears overactive in PD as a result of removal of inhibitory dopaminergic tone.[23,24] In L-dopa–induced dyskinesia, one concept is that the indirect pathway becomes underactive, although the experimental data available are equivocal.[19] However, pharmacologic manipulation of the indirect output pathway may offer control of the major symptoms of PD while alleviating a common motor side effect, namely dyskinesia.

Three major classes of adenosine receptors exist: A_1, A_2 (including A_{2A} and A_{2B}), and A_3 receptors.[25,26] The A_{2A} subtype appears of potential significance to the management of PD because A_{2A} receptor mRNA is enriched in the striatum compared with other brain regions.[27-31] A number of potent ligands interacting with the A_{2A} receptor have been identified, including the xanthine derivatives KF17837 and KW6002.[32-35] These molecules have nanomolar affinity for the rat striatal A_{2A} receptor and are approximately 70-fold selective for this site over A_1 receptors. Using a range of radioactive ligands selective for the A_{2A} receptor, a high concentration of A_{2A} receptor protein has been demonstrated within the basal ganglia in vitro and in vivo.[36-39] A_{2A} receptors are found in high density in the striatum, although they also occur in the nucleus accumbens and GPe. Within the striatum, A_{2A} receptors are localized to the medium spiny GABAergic neurons of the indirect output pathway, thereby labeling an important target in the pathophysiology of PD.[30]

A_{2A} receptors are not only localized to the cell bodies of the indirect output pathway to GPe but also are present on recurrent collaterals projecting back to the striatum and are found on its terminals in GPe.[3] In contrast, A_{2A} receptors are not present on the direct striatal GABAergic output pathway that innervates GPi and the substantia nigra zona reticulata (SNr).[40] Biochemical and electrophysiologic studies have shown that the A_{2A} receptor suppresses GABAergic transmission and release in the striatum but enhances it in GPe.[41-43] In the striatum, ultrastructural localization studies suggest that A_{2A} receptors can modulate GABAergic transmission at multiple cellular sites.[44] A_{2A} receptors are also present on cholinergic interneurons in the striatum, at least as shown by the presence of mRNA using reverse transcriptase (RT)-PCR, and they stimulate the release of acetylcholine through protein kinase A– and C–mediated mechanisms.[28,45-47] An interaction between A_{2A} and D_2 receptors exists with A_{2A} receptors exerting an opposing effect on D_2 receptor-mediated events that may be mediated through an A_{2A}-D_2 receptor dimerization[48] (see also Fuxe, page S19). The nature of this interaction is disputed with views ranging from a dominant role of D_2 receptors over A_{2A} function, to A_{2A} receptors acting at least partially independently of D_2 receptors, to A_{2A} receptors controlling dopamine pathways.[49-52] There may also be a complex interaction between A_{2A} receptors and glutamate transmission that may involve common signaling pathways.[15,53] Adenosine may play an important role in modulating excitatory input to the striatum.[44]

A_{2A} adenosine antagonists, motor activity, and striatal output pathways. Current dopaminergic drugs used for the management of PD are effective in rodent and primate models of the disorder. Similarly, A_{2A} antagonists exhibit the potential for the management of the motor symptoms of PD. For example, A_{2A} antagonists, including KW6002 and KF17837, increase locomotor activity in MPTP-treated or reserpinized mice and reverse haloperidol-induced catalepsy.[54-56] Importantly, KW6002 and the selective A_{2A} antagonist SCH 58261 potentiate rotational behavior produced by L-dopa or dopamine agonist drugs in the unilateral 6-hydroxydopamine (6-OHDA)–lesioned rat.[57,58] Tolerance to this effect was not observed after repeated SCH 58261 treatment.[59] Although short-term treatment with KW6002 did not

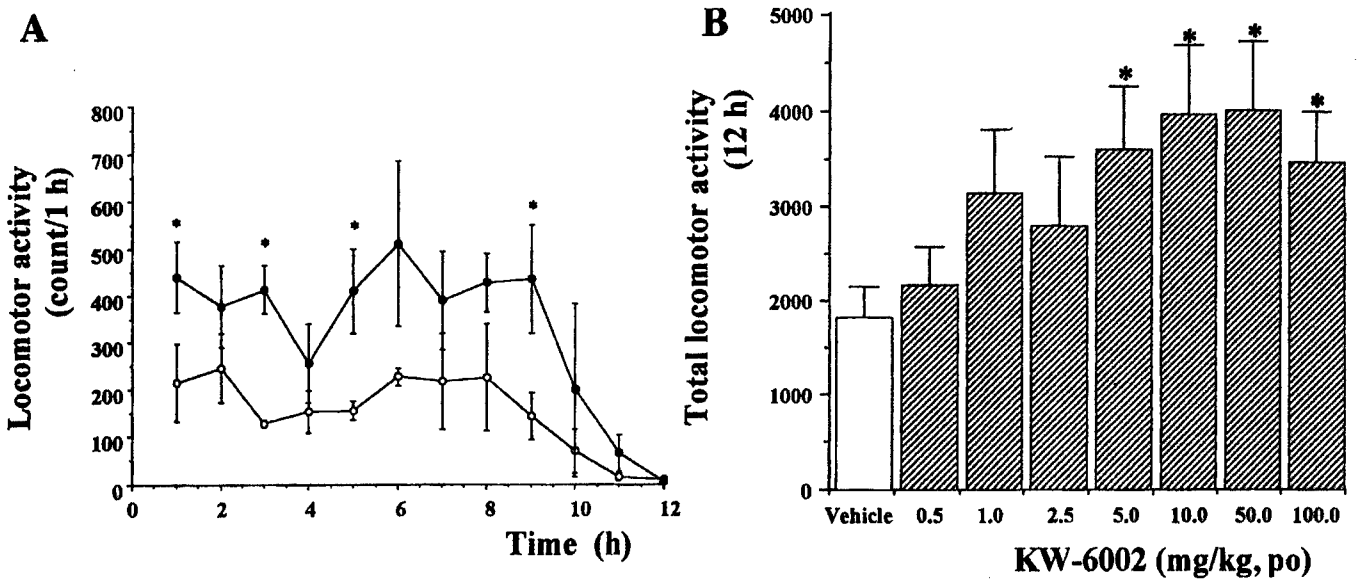

*Figure 1. Effect of the A_{2A} receptor antagonist KW6002 on locomotor activity in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated common marmosets. (A) Time course of the effect of KW6002 (10.0 mg/kg, orally, 0.3% Tween 80, 10% sucrose solution) on locomotor activity. Each point represents the mean locomotor count/1 h (± SEM, n = 4). Open circles show the vehicle treatment group. Closed circles show the KW6002 treatment group. (B) Dose–response effects of KW6002 (0.5 to 100 mg/kg, orally) on locomotor activity. Each column represents the mean locomotor count/12 h (± SEM, n = 4). *p < 0.05 compared with control (vehicle treatment group). Reprinted from Adenosine Receptors and Parkinson's Disease, vol 1, Jenner et al., "Actions of Adenosine Antagonists," pp. 211–227, Copyright 2000, with permission from Elsevier.*

initiate rotational behavior when administered alone, it does reduce motor disability with long-term administration. Another A_{2A} antagonist, CSC, was shown to reverse but not prevent the decrease in duration of rotational behavior observed with long-term administration of L-dopa to 6-OHDA–lesioned rats.[60] A_{2A} antagonists are also effective in rodent models associated with L-dopa–induced dyskinesia. In 6-OHDA–lesioned rats rendered dyskinetic by previous treatment with L-dopa, KW6002 was able to relieve motor disability without eliciting involuntary movements.[61] Although KW6002 produced an additive reduction in motor disability with L-dopa, it did not worsen dyskinesia. The behavioral sensitization to L-dopa seen in 6-OHDA–lesioned wild-type mice seems to depend on the presence of A_{2A} receptors because it does not occur in A_{2A} knockout mice.[62] Neurochemical studies also support actions of A_{2A} antagonists such as KW6002 on basal ganglia function in rodents that are relevant to PD. For example, KW6002 reverses the increase in GABA levels in the GPe caused by 6-OHDA–induced denervation of the striatum in the rat.[63] In addition, KW6002 reverses the 6-OHDA–lesion-induced increase in preproenkephalin A mRNA expression in the indirect output pathway, although it did not do so when coadministered with L-dopa.[15,61] Interestingly, 6-OHDA lesioning decreases striatal adenosine levels and increases A_{2A} receptor mRNA expression.[59] In A_{2A} receptor knockout mice, L-dopa failed to reverse 6-OHDA–induced reduction in striatal dynorphin levels seen in wild-type mice.[62] This suggests that A_{2A} receptors play a role in controlling the effects of L-dopa on the direct striatal output pathway, even though they are not expressed on its cell bodies.

Evaluation in the MPTP-treated primate has provided the clearest indication of potential antiparkinsonian activity of A_{2A} receptor antagonists in humans. We have previously examined the antiparkinsonian activity of KW6002 in the MPTP-treated common marmoset that is known to be responsive to L-dopa and a range of dopamine agonist drugs.[64] In these animals, KW6002 produced a dose-related increase in motor activity after its oral administration (figure 1). The drug was effective with a range of doses, but no further increase in activity was observed above the 10 mg/kg-dose level. The increase in locomotor activity produced by KW6002 differed from that produced by L-dopa or dopamine agonist drugs. KW6002 produced an approximate doubling of basal activity, whereas dopaminergic compounds produced marked hyperactivity indicative more of side effects than of therapeutic profile. The pattern and components of movement were close to that seen in normal marmosets and did not appear driven as occurs with L-dopa and dopamine agonists. The effect of KW6002 was of long duration (>10 hours), exceeding that produced by L-dopa and most dopamine agonist compounds. In addition, KW6002 markedly reduced motor disability in MPTP-treated common marmosets, although not to the same degree seen with L-dopa (figure 2). The effect was again maximal

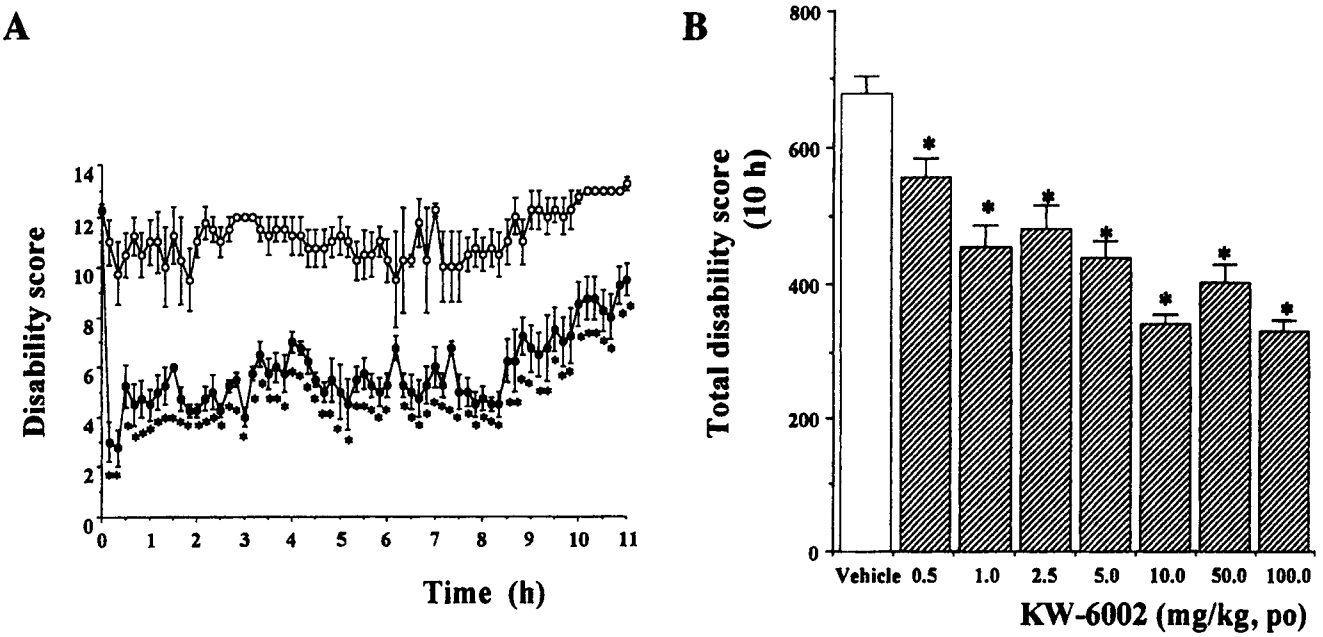

*Figure 2. Effect of KW6002 on motor disability in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated common marmosets. (A) Time course of the effect of KW6002 (10.0 mg/kg, orally) on motor disability. Each point represents the mean disability score/10 min (± SEM, n = 4). Open circles show the vehicle treatment group. Closed circles show the KW6002 treatment group. (B) Dose–response effects of KW6002 (0.5 to 100 mg/kg, orally) on motor disability. Each column represents the mean disability score/10 h (± SEM, n = 4). *p < 0.05 compared with control (vehicle treatment group). Reprinted from Adenosine Receptors and Parkinson's Disease, vol 1, Jenner et al., "Actions of Adenosine Antagonists," pp. 211–227, Copyright 2000, with permission from Elsevier.*

at 10 mg/kg, and when KW6002 was administered at this dose for 28 days, there was no evidence of tolerance in either the increase in motor activity or the reversal of disability (figure 3). Confirmation that the effects of KW6002 are mediated through A_{2A} receptors was shown by the ability of the adenosine A_{2A} agonist APEC to inhibit the improvement in motor function produced by KW6002 in MPTP-treated primates while not itself affecting motor activity.[60,65] These data suggest that long-term administration of KW6002 as monotherapy to patients with PD would lead to sustained antiparkinsonian activity. In addition, KW6002 treatment was not associated with dopaminergic side effects, such as nausea and vomiting, and no other adverse effects were observed.

So far the antiparkinsonian profile of KW6002 is not qualitatively different from that of L-dopa or dopamine agonists in MPTP-treated monkeys and would not represent a major therapeutic advance. However, examination of KW6002 in MPTP-treated primates that had been previously exposed to L-dopa and routinely showed dyskinesia on short-term challenge with L-dopa revealed a significant difference from dopaminergic therapies (figure 4). Therefore, the administration of KW6002 at a maximal antiparkinsonian dose did not provoke involuntary movements in these animals on either short-term challenge or repeated administration for 28 days.[60,64] Subsequent short-term challenge with L-dopa again induced dyskinesia, showing that KW6002 does not alter the priming for involuntary movements but avoids their expression. This finding contrasts with the effects of dopamine agonists, all of which induce dyskinesia identical to that produced by L-dopa in this model. In normal monkeys exhibiting dyskinesia as a result of long-term high-dose L-dopa treatment, the occurrence of involuntary movements was associated with an increase in striatal A_{2A} receptor mRNA, providing one explanation as to why A_{2A} antagonists might not provoke dyskinesia.[66]

A_{2A} antagonists such as KW6002 can increase locomotor activity, reverse motor disability, fail to provoke established dyskinesia, and show no tolerance on repeated administration. Such a profile fulfills many of the criteria required of novel therapeutic strategies for the management of PD.

When used as monotherapy, dopamine agonist drugs are effective in reversing motor symptoms in the early stages of the management of PD, but this effect wanes with time, and L-dopa therapy is inevitably introduced. Subsequently, the effect of L-dopa decreases after long-term treatment and with disease progression. Therefore, it is important to determine whether an adenosine A_{2A} antagonist, such as KW6002, provides additional benefit when used in conjunction with dopaminergic agents. As a consequence, we examined the effects of coadministration of KW6002 with L-dopa and with the D_2 agonist drug quinpirole in MPTP-treated primates.[67] In initial experiments, KW6002 was shown to acutely increase locomotor activity and reverse motor disability as previously demonstrated. However, when motor

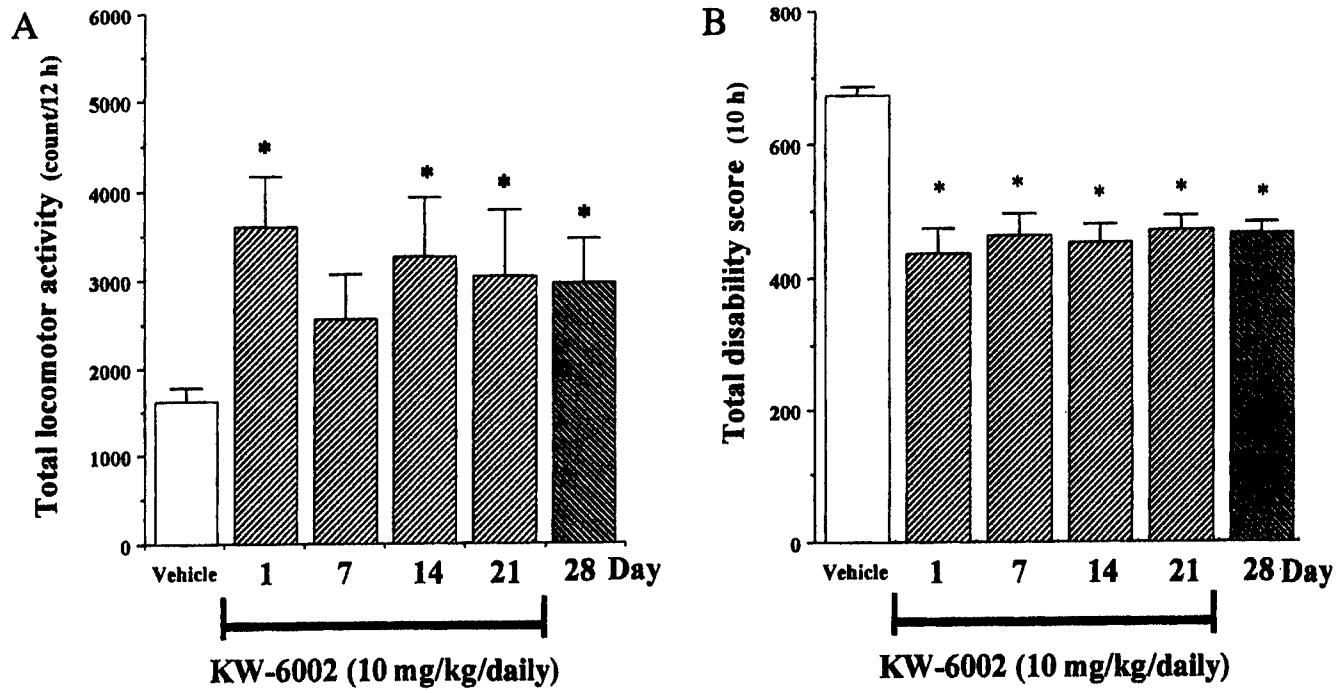

*Figure 3. Effect of long-term administration of KW6002 for 21 days on the (A) locomotor activity and (B) motor disability of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated common marmosets. KW6002 (10 mg/kg, orally) was administered once per day. (A) Each column represents the mean locomotor count/12 h (± SEM, n = 4); (B) each column represents the mean disability score/10 h (± SEM, n = 4). *p < 0.05 compared with control (vehicle treatment group). Reprinted from Adenosine Receptors and Parkinson's Disease, vol 1, Jenner et al., "Actions of Adenosine Antagonists," pp. 211–227, Copyright 2000, with permission from Elsevier.*

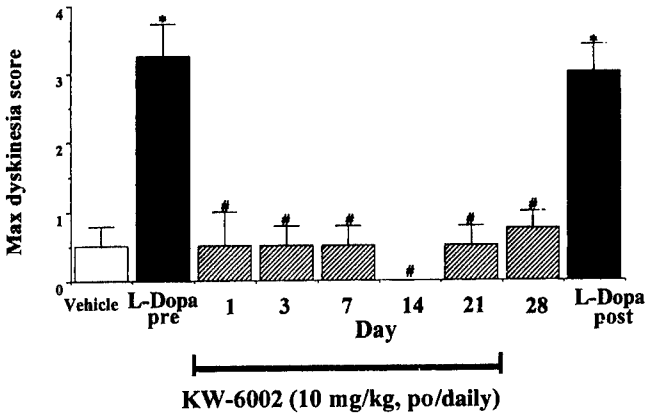

*Figure 4. Effect of KW6002 administered daily for 21 days on dyskinesia in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated common marmosets primed with L-dopa to exhibit dyskinesia. The animals previously received 21 days of long-term L-dopa (10 mg/kg, orally, in 10% sucrose twice daily) plus benserazide (2.5 mg/kg, orally, twice daily) for induction of dyskinesia. Each column represents the mean maximum dyskinesia score for four animals. *p < 0.05 compared with control (vehicle treatment group). #p < 0.05 compared with L-dopa–treated group. Reprinted from Adenosine Receptors and Parkinson's Disease, vol 1, Jenner et al., "Actions of Adenosine Antagonists," pp. 211–227, Copyright 2000, with permission from Elsevier.*

function was examined 24 or 48 hours after KW6002 administration, no residual drug effects were observed. When KW6002 was combined with quinpirole, an additive antiparkinsonian effect was observed immediately after drug administration. However, when quinpirole was readministered 24 or 48 hours after KW6002, there was a potentiation of the effect of quinpirole at a time when KW6002 alone was ineffective. Similarly, when KW6002 was administered in combination with L-dopa, an additive effect was observed immediately after drug treatment. But again, when L-dopa was readministered 24 hours after KW6002 treatment, a similar enhancement of the motor effects of L-dopa was observed, although this did not occur at the 48-hour time point. When KW6002 was administered in combination with L-dopa daily for 5 days to MPTP-treated common marmosets previously exposed to L-dopa and exhibiting dyskinesias, there was no enhancement of the dyskinetic response to L-dopa, and there was even a suggestion of a decreased intensity of involuntary movements.

The results obtained in the MPTP-treated common marmoset are not restricted to one primate species. In other studies carried out in MPTP-treated cynomolgus monkeys, KW6002 also produced an increase in locomotor activity, which was additive with the effects of L-dopa.[68,69] In addition, the effect of KW6002 on parkinsonian disability was not associated with the expression of involuntary movements

in animals primed with L-dopa to establish dyskinesia. Similarly, administration of KW6002 was not associated with an increase in the dyskinetic movements produced by L-dopa.

This finding suggests that in patients with PD, KW6002 will produce an additive antiparkinsonian effect with L-dopa or dopamine agonist drugs without the problem of enhancement of involuntary movements. This profile is consistent with the goal of improved pharmacologic control of PD.

Conclusions. The selective localization of A_{2A} receptors to the indirect striatal output pathway and the manipulation of GABAergic function by A_{2A} antagonists such as KW6002 suggest a potential role in the management of PD. The available data show KW6002 to have properties consistent with use as early monotherapy based on its ability to reverse motor symptoms in MPTP-treated primates. In addition, KW6002 should be effective as adjunct therapy to L-dopa in the middle-to-late stages of PD. The actions of A_{2A} antagonist on the symptoms of PD should not be associated with the expression of dyskinesia when used as monotherapy or with any increase in the intensity of involuntary movements when used in conjunction with L-dopa or dopamine agonist drugs. Other findings suggest that KW6002 may also prevent or delay the priming process that leads to the induction of involuntary movements induced by long-term L-dopa administration in otherwise drug-naïve MPTP-treated primates (see Chase, page S107). Because KW6002 produces additive effects when administered with L-dopa, it should allow a reduction in dosage of L-dopa and hence a reduction in dopaminergic side effects. Importantly, A_{2A} antagonists may also be effective in the management of neuropsychiatric disease, particularly anxiety and depression, because some A_{2A} receptors are localized to limbic areas and the hippocampus and amygdala.[70] KW6002 would then be effective against the motor symptoms of PD and the high incidence of anxiety and depression associated with the disorder. Finally, A_{2A} antagonists may act as neuroprotective agents because they are able to protect the nigrostriatal pathway against 6-OHDA and MPTP toxicity and so may influence the progression of PD (see Popoli et al., page S69).[71-75] To date, the clinical efficacy of A_{2A} receptor antagonists in PD remains to be proven, but early clinical evaluation of KW6002 has shown it to be effective in the control of motor symptoms (see Chase et al., page S107; Kase et al., page S97). Further clinical evaluation is now required to establish the full potential of this class of agents as one of the first nondopaminergic agents for the management of PD.

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Adenosine A_{2A} and dopamine receptor interactions in basal ganglia of dopamine denervated rats

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Abstract—In the unilateral 6-hydroxydopamine–lesioned rat model of Parkinson's disease, blockade of A_{2A} receptors facilitates L-dopa–induced turning behavior by antagonism of A_{2A} transmission, which is increased after dopamine depletion. After long-term intermittent administration of doses that produced the same effect on turning behavior, SCH 58261 (5 mg/kg) + L-dopa (3 mg/kg) induced a stable turning behavior, whereas L-dopa (6 mg/kg) alone induced a sensitized turning behavior. Behavioral studies were correlated to changes in dynorphin and enkephalin mRNAs in the striatum and in glutamic acid decarboxylase 67 (GAD67) mRNA in the striatum, globus pallidus, and substantia nigra. The expression of dynorphin and, to a lesser extent, enkephalin mRNAs was increased in the lesioned striatum of rats that received long-term L-dopa treatment but not in rats that received long-term SCH 58261 + L-dopa treatment. Similarly, GAD67 mRNA was increased in the striatum and globus pallidus by long-term L-dopa administration but not by long-term SCH 58261 + L-dopa administration. GAD67 mRNA was strongly reduced in the lesioned substantia nigra after long-term L-dopa treatment, whereas the reduction of GAD67 mRNA was less marked after SCH 58261 + L-dopa treatment. By increasing L-dopa turning behavior, A_{2A} receptor antagonism allows the utilization of doses of L-dopa that do not produce sensitization of turning behavior, an effect correlated with the dyskinetic potential of dopamine agonist drugs. Moreover, the combination of SCH 58261 + L-dopa produces little or no change in the striatal, pallidal, and nigral expression of markers correlated with dopamine agonist dyskinetic potential.

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Adenosine transmission, via A_{2A} receptors, plays an important role in the modulation of motor behavior.[1-5] Several studies have reported a direct antagonistic dopamine–adenosine interaction on striatopallidal neurons, where A_{2A} receptors are co-expressed with dopamine D_2 receptors.[6-11] Moreover, indirect interactions between A_{2A} and D_1 receptors have been shown to affect behavior and c-fos induction.[12-15]

Studies in animal models of Parkinson's disease (PD) have shown that modulation of adenosine neurotransmission may be of interest for the management of PD. Early studies in the 6-hydroxydopamine (6-OHDA)–lesioned rat model of PD showed that A_1/A_{2A} antagonists, such as caffeine, induced contralateral turning behavior when administered alone and increased the turning behavior induced by dopamine agonists.[16,17] Synthesis of potent and selective adenosine A_{2A} receptor antagonists offered the possibility to test the usefulness of these drugs for the management of PD. Selective compounds such as SCH 58261, KF17837, and KW6002 decreased haloperidol-induced catalepsy and reserpine-induced akinesia in intact animals and potentiated the anticataleptic effect of L-dopa.[18-22] A study by our group showed that blockade of A_{2A} receptors with SCH 58261 increased the contralateral rotation and Fos-like immunoreactivity induced in the striatum and globus pallidus (GP) by a threshold dose of L-dopa.[23,24] Similarly, SCH 58261 potentiated turning behavior induced by D_1 and D_2 dopamine agonists, suggesting that A_{2A} receptors modulate dopamine transmission mediated by D_1 and D_2 receptors.[14,23] These data were confirmed by studies in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated primates, showing that A_{2A} antagonists counteract the motor deficits induced by degeneration of dopaminergic neurons.[25,26]

A recent study by our group reported that overactivity of A_{2A} transmission after dopamine neuron degeneration might contribute to the motor impairments that characterize PD. This suggests that, by eliminating A_{2A} receptor-mediated negative tone, A_{2A} antagonists may be beneficial for the management of PD.[1] Currently, A_{2A} antagonists are among the most promising compounds for the management of PD.

Effect of long-term SCH 58261 treatment on turning behavior. A major problem related to L-dopa management of PD is the development of motor fluctuations and dyskinesias, which limit the long-term use of this drug. We showed that in 6-OHDA–lesioned rats, administration of the A_{2A} receptor antagonist SCH 58261 for 1, 7, or 14 days

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induced a similar enhancement of L-dopa turning behavior, suggesting that tolerance did not develop during this treatment period.[27] Similarly, another A_{2A} antagonist, KW6002, retained its activity during a 21-day treatment period.[28] A recent study conducted in our laboratory in unilaterally 6-OHDA–lesioned rats showed that treatment with L-dopa (6 mg/kg) or with SCH 58261 (5 mg/kg) plus a threshold dose of L-dopa (3 mg/kg), although producing similar contralateral turning after the first administration, resulted in turning behaviors of different intensity during long-term intermittent treatment. Whereas L-dopa (6 mg/kg) alone induced a sensitized turning behavior during the course of treatment, SCH 58261 plus L-dopa (3 mg/kg) produced a stable turning response.[27] In previous studies, the sensitized turning response induced by long-term L-dopa administration was correlated with the dyskinetic potential of this drug;[29] therefore, the lack of sensitization during treatment with SCH 58261 plus L-dopa was indicative of a low dyskinetic potential.

These data are consistent with results obtained in $A_{2A}^{-/-}$ mice, showing that the lack of A_{2A} receptor prevents L-dopa–induced behavioral sensitization in 6-OHDA–lesioned mice.[30]

Effect of long-term SCH 58261 treatment on striatal gene expression. Alterations in the expression of dynorphin, enkephalin, and glutamic acid decarboxylase 67 (GAD67) mRNAs after dopamine denervation have been suggested to reflect an imbalance in the activity state of striatal output neurons, which is believed to underlie motor impairment in PD.[31-33] As for dopaminergic neuron degeneration, L-dopa–induced dyskinesia in experimental models of PD is associated with alterations in striatal mRNA levels, including overexpression of dynorphin and GAD67 mRNA on striatal output neurons.[34-38]

Based on these observations, we evaluated whether the prediction of low dyskinetic potential for SCH 58261 plus L-dopa correlated with long-term changes in basal ganglia activity markers. For this purpose, we compared the effect of long-term intermittent administration of SCH 58261 (5 mg/kg) + L-dopa (3 mg/kg) with L-dopa (6 mg/kg) alone on the striatal mRNA levels for dynorphin, enkephalin, and GAD67.[39] mRNA levels were evaluated by in situ hybridization 3 days after discontinuation of treatment. As reported by previous studies,[31,33] GAD67 and enkephalin mRNA levels were significantly increased, and dynorphin mRNA decreased, in the striatum ipsilateral to the 6-OHDA lesion, suggesting an imbalance in the activity of striatal efferent neurons with the indirect striatopallidal pathway rendered hyperactive over the direct striatonigral pathway in accordance with the current model of PD.

GAD67, dynorphin, and enkephalin mRNA levels were significantly increased in the lesioned striatum by L-dopa (6 mg/kg) treatment as compared with the vehicle. Moreover, an increase in enkephalin mRNA level was observed in the intact striatum after long-term L-dopa (6 mg/kg) treatment.[39] By contrast, long-term treatment with SCH 58261 plus L-dopa (3 mg/kg) did not modify GAD67, dynorphin, and enkephalin mRNAs as compared with the vehicle (figures 1 and 2).

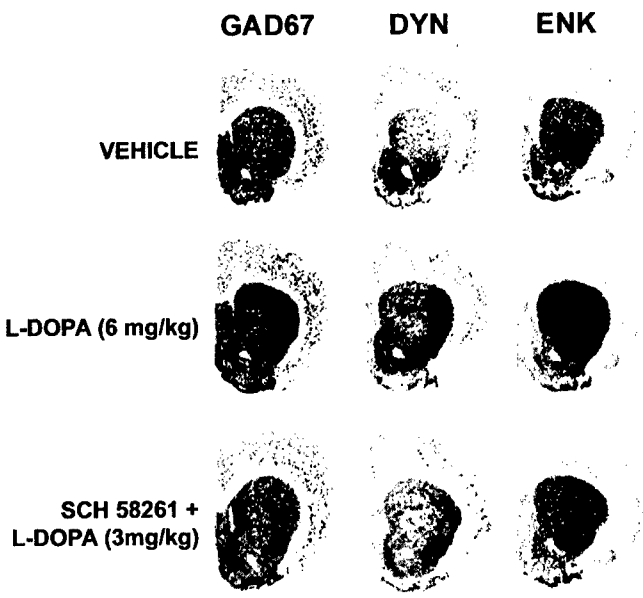

Figure 1. In situ hybridization autoradiograms showing mRNA expression for glutamic acid decarboxylase 67 (GAD67), dynorphin (dyn), and enkephalin (enk) in striata from 6-hydroxydopamine (6-OHDA)–lesioned rats treated long term (19 days, twice a day) with the vehicle, L-dopa (6 mg/kg), or SCH 58261 (5 mg/kg) + L-dopa (3 mg/kg). Rats were killed 3 days after the last injection.

Therefore, long-term intermittent administration of the A_{2A} antagonist SCH 58261 plus L-dopa (3 mg/kg) to 6-OHDA–lesioned rats produced long-term changes in the striatum different from those produced by long-term intermittent L-dopa (6 mg/kg) alone. Modifications in GAD67 and peptides mRNA suggest that long-term L-dopa (6 mg/kg) treatment induces adaptive changes in neuronal activity in dynorphin-containing striatonigral neurons and enkephalin-containing striatopallidal neurons. Adaptive changes in striatal efferent neurons are considered to play an important role in the genesis of L-dopa–induced dyskinesia, and overactivity of striatonigral neurons has been often correlated with dyskinetic movements.[34,36] Our data, by showing an increased activity of striatonigral and striatopallidal neurons, suggest that both neuronal populations may participate in the onset of dyskinesia, although the changes observed in the direct striatonigral pathway are much larger than those observed in the indirect striatopallidal pathway and are specifically observed only in the lesioned striatum.

Long-term administration of SCH 58261 plus L-dopa (3 mg/kg) did not produce modifications in striatal GAD67, dynorphin, and enkephalin mRNAs. Therefore, the absence of a sensitized turning behavioral response during long-term intermittent treatment with SCH 58261 plus L-dopa (3 mg/kg) appears to correlate with the absence of long-term adaptive

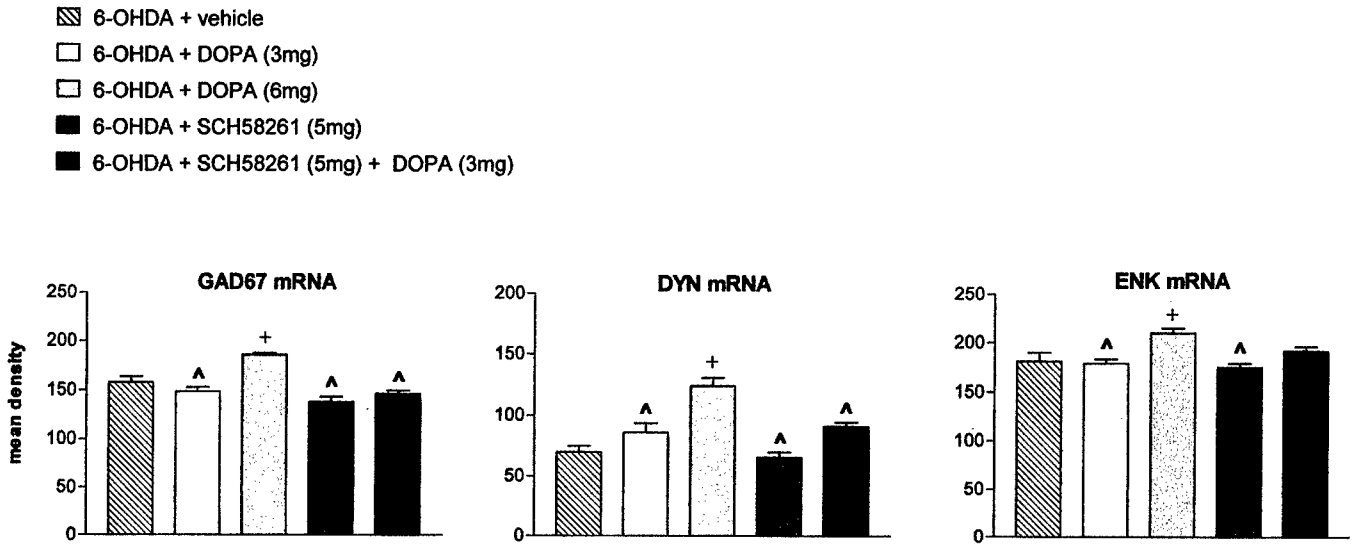

Figure 2. Effect of drug treatments on glutamic acid decarboxylase 67 (GAD67), dynorphin (dyn), and enkephalin (enk) mRNA levels. Mean of gray values (mean ± SEM) from the dorsolateral striatum of 6-hydroxydopamine (6-OHDA)–lesioned rats treated long term (19 days, twice a day) with vehicle, L-dopa (6 mg/kg), or SCH 58261 (5 mg/kg) + L-dopa (3 mg/kg). $^{+}p < 0.05$ *vs 6-OHDA + vehicle;* $^{\wedge}p < 0.05$ *vs L-dopa (6 mg/kg).*

changes in striatal neurons, an effect that may be related to the failure of this treatment to produce dyskinetic effects.

Effect of long-term SCH 58261 treatment on globus pallidus and substantia nigra gene expression. GP and substantia nigra reticulata (SNr) play a crucial role in parkinsonian symptoms and the dyskinetic responses observed after long-term treatment with L-dopa. Therefore, we investigated changes in neuronal activity in these areas after treatment with SCH 58261 and L-dopa using the same experimental paradigm described previously. For this purpose, we evaluated changes at the single-cell level for GAD67 mRNA as a marker of neuronal activity.

In GP and SNr ipsilateral to the 6-OHDA lesion, the number of GAD67 mRNA silver grains per cell was significantly increased compared with the intact side (table).

Treatment with L-dopa (6 mg/kg) significantly enhanced GAD67 mRNA levels in the GP ipsilateral to the lesion as compared with the vehicle (table). In contrast, the lower dose of L-dopa (3 mg/kg), SCH 58261 (5 mg/kg), or SCH 58261 (5 mg/kg) plus low-dose L-dopa (3 mg/kg) failed to modify GAD67 mRNA in the GP as compared with 6-OHDA–lesioned rats treated with the vehicle (table).

Long-term intermittent treatment with L-dopa (6 mg/kg) and, to a lesser extent, with SCH 58261 (5 mg/kg) plus L-dopa (3 mg/kg) reduced the amount of GAD67 mRNA labeling in the SNr ipsilateral to the lesion as compared with the vehicle treatment (table). Low-dose L-dopa (3 mg/kg) or SCH 58261 (5 mg/kg) alone did not produce any change in GAD67

Table *Effect of drug treatment on GAD67 mRNA levels in the globus pallidus and substantia nigra and on turning behaviour after the first and last drug administration. Drugs were chronically injected for 19 days, twice a day. In each area, silver grains above each cell stained with thionin were counted and normalized to the total number of cells analyzed. Data from each group were averaged and expressed as a percentage of the unlesioned + vehicle group.* $^{a}P < 0.01$ *vs. unlesioned + vehicle;* $^{b}p < 0.01$ *vs. 6-OHDA + vehicle.* $^{c}p < 0.05$ *vs. 6-OHDA + vehicle;* $^{d}p < 0.05$ *vs. 1st administration day".*

				Total turns	
Experimental group	n	Globus pallidus, %	Substantia nigra, %	day 1	day 19
Unlesioned + vehicle	4	100.00 ± 12.56	100.00 ± 5.98	0 ± 0	0 ± 0
6-OHDA + vehicle	4	128.02 ± 5.6[a]	118.47 ± 7.64[a]	0 ± 0	0 ± 0
6-OHDA + L-dopa (6 mg/kg)	4	147.10 ± 5.3[ac]	90.63 ± 1.65[b]	359 ± 56	769 ± 123[d]
6-OHDA + L-dopa (3 mg/kg)	4	130.58 ± 9.43[a]	108.51 ± 12.03	23 ± 10	159 ± 79
6-OHDA + SCH	4	130.25 ± 1.58[a]	107.46 ± 1.58	0 ± 0	0 ± 0
6-OHDA + SCH + L-dopa (3 mg/kg)	4	125.23 ± 10.39	104.51 ± 6.54[c]	366 ± 64	492 ± 83

mRNA in the SNr as compared with unlesioned rats and 6-OHDA–lesioned vehicle-treated rats.

The GAD67 isoform of the GABA-synthesizing enzyme is involved in general metabolic activity and is considered an index of GABAergic neuron activity.[40] The observed increase in GAD67 mRNA in the GP and SNr after 6-OHDA lesion, in agreement with previous reports,[41-44] suggests that changes in the activity of afferent neurons to GP and SNr may take place after dopamine denervation, such as an increased excitatory input from the subthalamic nucleus.[45]

Neuronal activity in the GP, which is part of the indirect pathway, was altered by long-term L-dopa (6 mg/kg) treatment as shown by the increase in GAD67 mRNA levels. Recent studies reported that in parkinsonian patients, the electrical activity of the external GP neurons was altered after L-dopa therapy.[46,47] A study by Boraud et al.[48] reported that only dyskinetic doses of L-dopa were able to alter the electrical activity of pallidal neurons in MPTP-lesioned primates. Overactivation of GABAergic GP neurons may in turn inhibit the SNr, which may play a critical role in dyskinetic movements.

Nigral expression of GAD67 mRNA was strongly inhibited by long-term L-dopa (6 mg/kg) administration, an effect consistent with the reported inhibition of GABAergic efferents in primate models of L-dopa–induced dyskinesia.[41,49] Together, these results suggest that long-term L-dopa administration may produce profound modifications in direct and indirect striatal efferent pathways, resulting in an excessive inhibition of the SNr, which would in turn correlate with dyskinetic movements.

SCH 58261 plus L-dopa (3 mg/kg) did not affect GAD67 mRNA in the GP and decreased GAD67 mRNA levels in the SNr to a lesser degree than L-dopa alone (6 mg/kg). Therefore, our results suggest that the degree of SNr inhibition could play an important role in dyskinetic movements. This conclusion is supported by studies reporting that an inhibition of the SNr is produced by L-dopa treatment regardless of the presence of dyskinetic movements, although the inhibition appears to be greatest in the presence of dyskinetic movements.[49-52]

Together, these results suggest that whereas improvement of motor disabilities requires a decrease in neuronal activity in the SNr, which is overactive after dopamine denervation, dyskinetic movements are correlated with changes at different levels of basal ganglia circuitry, such as altered activity of striatal and pallidal neurons, resulting in an excessive inhibition of SNr efferent neurons.

A_{2A} and dopamine receptors produce modifications at the level of transcription factors contained in indirect and direct striatal efferent neurons, which modulate long-term adaptive responses of these neurons.[12,15,23,53] By potentiating the behavioral effects of L-dopa, SCH 58261 allows the administration of smaller doses of L-dopa, which do not produce those adaptive changes in basal ganglia neurons that may cause dyskinesia. Dyskinesia and alterations in gene expression are correlated with the dosage and administration regimen of L-dopa.[34-36] Accordingly, in the present study, low-dose L-dopa (3 mg/kg) alone failed to induce significant changes in gene expression in any brain area.

Conclusions. The present study shows that long-term intermittent treatment with L-dopa (6 mg/kg) results in profound alterations in neuronal activity in different nuclei of the basal ganglia circuitry, including striatum, GP, and SNr. SCH 58261 + L-dopa (3 mg/kg) fails to alter the activity of striatal and pallidal neurons, resulting in a more physiologic inhibition of SNr neurons, which would account for the improvement in motor disabilities and the absence of dyskinetic potential reported for A_{2A} antagonists in the models of PD.[25-27]

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Modulation of GABAergic transmission in the striatopallidal system by adenosine A_{2A} receptors

A potential mechanism for the antiparkinsonian effects of A_{2A} antagonists

Akihisa Mori, PhD; and Tomomi Shindou, MSc

Abstract—The selective localization of adenosine A_{2A} receptors to the striatopallidal system suggested a new therapeutic approach to the management of Parkinson's disease (PD). The results of behavioral studies using A_{2A} receptor-specific agents in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated monkeys highlight the therapeutic potential of A_{2A} antagonists as a novel treatment for PD. However, little is known about the role of A_{2A} receptors in basal ganglia function or their pathophysiologic role in PD. Recently, the authors found that presynaptic A_{2A} receptors modulate GABAergic synaptic transmission in the striatum and globus pallidus (GP), suggesting an A_{2A} receptor-mediated dual modulation of the striatopallidal system. Striatal A_{2A} receptors may increase the excitability of medium spiny neurons (MSNs) by modulating an intrastriatal GABAergic network. In addition, pallidal modulation occurs at striatopallidal MSN terminals located at the GP, enhancing GABA release onto GP projection neurons and directly suppressing their activity. Blockade of these modulatory functions by A_{2A} antagonists could counteract excessive striatopallidal neuronal activity provoked by striatal dopamine depletion in patients with PD, leading to a reversal of parkinsonian motor deficits.

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There is a growing body of evidence suggesting that the A_{2A} receptor is a potential target for novel antiparkinsonian therapy.[1] A_{2A} receptors are primarily located within the caudate-putamen (striatum), nucleus accumbens, and olfactory tubercle. This concentration within the basal ganglia suggests a specific functional role for these receptors in neuronal communication in these areas, in particular, a role in motor behavior. Recent advances in neuroscience together with development of A_{2A} receptor-selective agents have contributed to increased knowledge about adenosine and the A_{2A} receptor. Behavioral studies show that A_{2A} antagonists alleviate the motor dysfunction seen in several parkinsonian animal models (e.g., 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine [MPTP]-treated monkeys) but also reveal distinctive features of A_{2A} antagonists.[2,3]

The striatal GABAergic medium spiny neurons (MSNs), which represent more than 90% of striatal neurons, are the only projection neurons in the striatum. The MSNs receive massive glutamatergic inputs from the cortex and thalamus and send indirect (striatopallidal MSNs) and direct (striatonigral MSNs) GABAergic projections to the major output nuclei of the basal ganglia: the globus pallidus (GP) and the substantia nigra pars reticulata.[4-6] They also receive intrastriatal GABAergic and cholinergic inputs and nigrostriatal dopaminergic modulatory inputs. Importantly, A_{2A} receptors are highly expressed in striatopallidal MSNs but not in striatonigral MSNs.[7]

The striatopallidal indirect pathway is of particular interest in Parkinson's disease (PD) because it becomes overactive in this disease state. The neuronal basis of parkinsonism is thought to be, at least in part, a consequence of an imbalance between the two major output pathways, with the striatonigral neurons being relatively underactive in patients with PD.[8] The specific localization of A_{2A} receptors to the striatopallidal system suggests that these receptors contribute to the generation of parkinsonian symptoms and offers a novel target for PD therapy. However, little is known regarding the physiologic and pathophysiologic function of A_{2A} receptors in this system.

To study how A_{2A} receptors work in the basal ganglia, we used a patch-clamp method in slice preparations to investigate GABAergic synaptic transmission at the single-neuron level. The method enables us to identify the neuronal targets of the A_{2A} receptor-related agents more precisely. These studies revealed dual modulation of GABAergic synaptic transmission in the striatopallidal system by presynaptic A_{2A} receptors.

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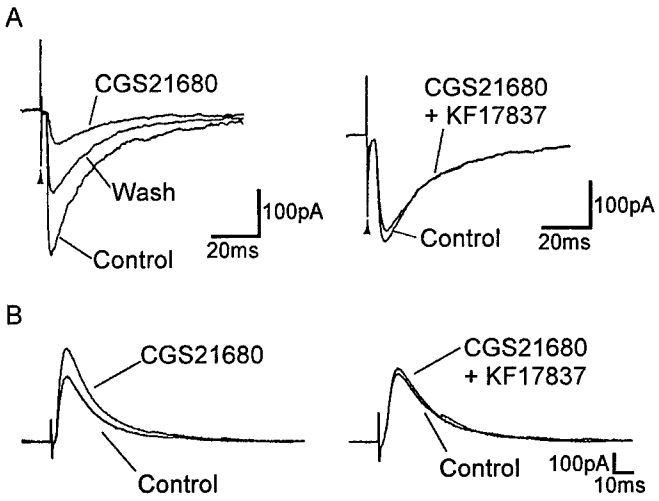

Figure 1. Effects of A_{2A} receptor activation on evoked GABAergic inhibitory postsynaptic currents (IPSCs) onto medium spiny neurons (MSNs) (A) and globus pallidus (GP) neurons (B). The A_{2A} agonist CGS 21680 (1 μmol/L) suppressed evoked IPSCs onto MSNs (A, left) and enhanced evoked IPSCs onto GP neurons (B, left). The A_{2A} antagonist KF17837 (1 and 0.5 μmol/L) blocked CGS 21680-induced phenomena in MSNs (A) and GP neurons (B). (A) is reproduced from Mori et al.[9] and reprinted with permission from Elsevier and (B) is reproduced with permission from Shindou et al.[13]

Suppression of intrastriatal GABAergic transmission by striatal adenosine A_{2A} receptors. To study GABAergic transmission onto MSNs, we adapted the high-resolution whole-cell patch-clamp recording technique to rat striatal slice preparations, directly visualizing these cells.[9] The evoked $GABA_A$ receptor-mediated inhibitory postsynaptic currents (IPSCs) were elicited by focal stimulation under conditions in which excitatory inputs were blocked so that the effects of selective adenosine agents on GABA-mediated inhibition could be observed without complications caused by excitatory transmission. At 1 μmol/L, the A_{2A} receptor-selective agonist CGS 21680 significantly reduced the average amplitude of evoked IPSCs. The suppression of IPSCs by CGS 21680 was concentration dependent and saturated at 1 μmol/L. The A_{2A} receptor-selective antagonist KF17837 at 1 μmol/L completely blocked the inhibitory effect of 1 μmol/L CGS 21680 (figure 1A). In contrast, two distinct A_1 receptor-selective antagonists, DPCPX and KF15372, failed to block the CGS 21680-induced suppression of IPSCs. CGS 21680 also suppressed evoked $GABA_A$ receptor-mediated inhibitory postsynaptic potentials (IPSPs). These results demonstrate the existence of A_{2A} receptor-mediated modulation of striatal GABAergic synaptic transmission onto MSNs.

Analysis of spontaneous miniature synaptic events revealed that the A_{2A} receptor-mediated suppression of GABAergic transmission onto MSNs occurs presynaptically. In the presence of tetrodotoxin to block the propagation of action potentials to the terminals, spontaneous GABA release from presynaptic terminals can be detected as spontaneous miniature IPSCs (mIPSCs). The frequency of mIPSCs correlates with the number of GABA quanta released from presynaptic terminals, whereas mIPSC amplitude correlates with the activity of postsynaptic GABA channels. CGS 21680 decreased the mean mIPSC frequency without changing the mean mIPSC amplitude or the amplitude distributions. These results indicate that CGS 21680 reduced the quantal release of GABA from presynaptic terminals, thus demonstrating that presynaptic, but not postsynaptic, A_{2A} receptors suppress GABAergic synaptic transmission onto MSNs. The electrophysiologic findings are consistent with neurochemical results using striatal synaptosome preparations[10,11] and are reproduced in studies by Chergui et al.[12]

Activation of pallidal adenosine A_{2A} receptors enhances GABAergic synaptic transmission onto GP GABAergic projection neurons. In contrast to the striatum, pallidal A_{2A} receptor activation was found to enhance GABAergic IPSCs onto GP neurons.[13] The enhancement by CGS 21680 (figure 1B) was dose dependent within the range of 0.3 to 3.0 μmol/L and was completely blocked by A_{2A} receptor-selective antagonists KF17837 (see figure 1B) and ZM241385. To investigate whether the A_{2A} receptor-mediated modulation is presynaptic or postsynaptic, paired-pulse facilitation (PPF)[14] and mIPSC analysis were used in GP neurons. The enhancement of evoked IPSCs by CGS 21680 was accompanied by a reduction in the PPF ratio and an increase in mIPSC frequency without affecting mIPSC mean amplitude and amplitude distribution. These observations indicate that pallidal A_{2A} receptor-mediated enhancement of GABAergic synaptic transmission is attributable to an increased probability of presynaptic GABA release and not to increased postsynaptic $GABA_A$ receptor activity.

GP projection neurons have been electrophysiologically[15,16] and immunohistochemically[17,18] identified as GABAergic neurons. The GP neurons give rise to widespread intranuclear axon collaterals and also receive massive GABAergic inputs from the striatum.[19,20] Therefore, the recorded IPSCs in the study were attributable to activation of GABAergic inputs from the striatum or from local axon collaterals of GP neurons. Interestingly, recent immunohistochemical studies indicated that A_{2A} receptors themselves exist in the GP,[21] whereas A_{2A} receptor mRNA is present in striatopallidal MSNs but has not been detected in the GP.[22-24] Thus, IPSCs affected by pallidal A_{2A} receptor activation might be occurring at GABAergic synapses on axon terminals of striatopallidal MSNs (i.e., output of indirect pathway), although further investigation is necessary to demonstrate this definitively.

Striatal and pallidal modulation of GABAergic transmission by A_{2A} receptors: differences and functional implications. *Striatal modulation:*

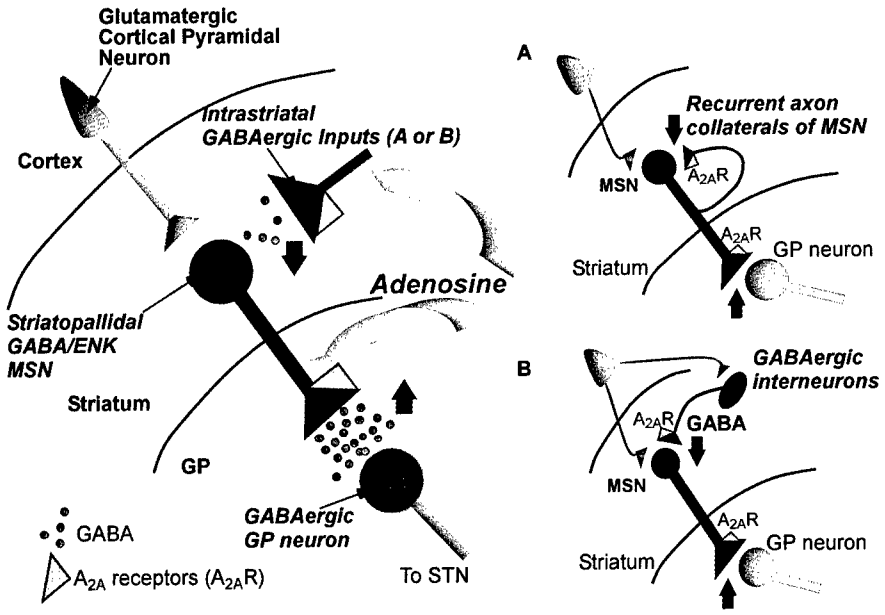

Figure 2. Schematic diagram of the corticostriatal and striatopallidal pathways, representing the A_{2A} receptor-mediated dual modulation of GABAergic synaptic transmission in the striatopallidal system. Adenosine via presynaptic A_{2A} receptors suppresses GABA release from intrastriatal GABAergic inputs to medium spiny neurons (MSNs) and enhances GABA release from striatopallidal MSNs to globus pallidus (GP) neurons. GABAergic inputs to MSNs are divided into two types: (A) from recurrent axon collaterals, and (B) from GABAergic interneurons. ENK = enkephalin; STN = subthalamic nucleus.

A_{2A} receptor-mediated modulation of excitability of MSNs via intrastriatal GABAergic network. In the striatum, GABAergic inputs are the major regulators of MSN excitability.[25] Two possible GABAergic inputs to striatal MSNs are from axon collaterals of the MSNs themselves and from GABAergic interneurons. MSN excitability may be controlled by feedback inhibition from the axon terminals of MSN recurrent collaterals (figure 2A) or by feed-forward inhibition from GABAergic interneurons (figure 2B).[25-27] Recurrent axon collaterals of MSNs could produce mutual inhibition among spiny projection cells, creating a feedback inhibition circuit within the striatum. MSNs containing substance P and enkephalin are distributed uniformly within the striatum, but MSNs with the same projection sites may be aggregated into small clusters of cells.[28-30] The dendrites and axons of individual MSNs are restricted to the respective compartment in which they are located, so that they only receive afferents from cells of the same type.[31] A_{2A} receptor mRNA is highly expressed in striatopallidal MSNs,[7,22] and high levels of A_{2A} receptors on the terminals of striatal axon collaterals have been detected immunohistochemically.[21,32] Therefore, A_{2A} receptor-mediated modulation at axon terminals of recurrent collaterals of striatopallidal MSNs may relieve feedback inhibition and increase the excitability of these projection neurons.

GABAergic interneurons, although comprising less than 10% of total striatal neurons, have been reported to play a major role in regulating the firing activity of spiny projection cells. The main source of intrastriatal $GABA_A$ response is most likely from GABAergic interneurons rather than from MSN collaterals because of the strong burst response in GABAergic interneurons after cortical stimulation.[33] Inputs from GABAergic interneurons onto MSNs should provide a feed-forward inhibition mechanism. If the A_{2A} receptor is expressed on GABAergic interneurons, they could relay A_{2A} receptor-mediated modulation of MSNs. So far, A_{2A} receptor mRNA has not been detected in striatal GABAergic interneurons;[7,22] however, this issue merits further investigation.

Together, activation of A_{2A} receptors on intrastriatal GABAergic terminals could relieve either or both feedback and feed-forward inhibition mechanisms, resulting in an increase in striatopallidal MSN activity. It remains to be identified which GABAergic synapses onto MSNs subserve striatal A_{2A} receptor-mediated modulation.

Based on the current–voltage relationship of an MSN, Kita[33] proposed a model of the electrophysiologic role of GABAergic inputs to MSNs seen as a large sigmoidal curve (figure 3). The response mediated via $GABA_A$ receptor–chloride channel complexes has the reversal potential of chloride near the spike threshold potentials and thus acts mainly to shunt the glutamatergic inputs. Therefore, GABA input plays a significant role in determining the level of the depolarizing stage of MSN, as illustrated by a downward shift of the current–voltage curve (see figure 3, "with $GABA_A$ inputs"). Striatal A_{2A} receptor-mediated relief of either or both feedback and feed-forward inhibition mechanisms could weaken the GABAergic shunting effect on glutamatergic inputs to MSNs, thereby modulating their membranes to shift to depolarizing stage (i.e., A_{2A} receptor-mediated disinhibition in figure 3). The A_{2A} receptor-mediated suppression rate in striatal IPSPs, approximately 20 to 30% at most,[9] could be enough to depolarize an MSN. MSNs do not frequently fire but are active in maintaining subthreshold excitatory potentials. Thus, these cells are not quiet but are busy in determining whether to fire at depolarizing episodes,[34] and the A_{2A} receptor-mediated disinhibitory modulation may directly cause an increase in activity of the striatopallidal indirect pathway.

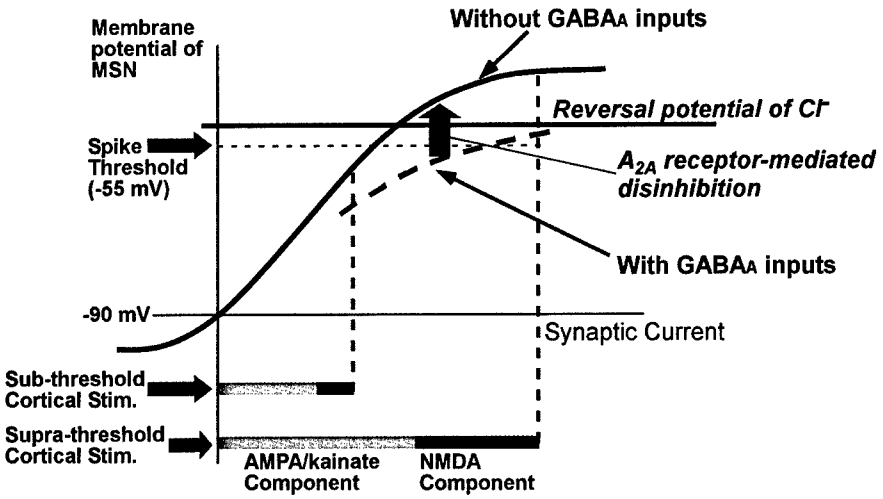

Figure 3. A simplified schematic representation of the relationship between synaptic input and membrane potential change. In the case of subthreshold cortical stimulation, the main synaptic driving force is an α-amino-3-hydroxy-5-methyl-4-isoxazole proprionic acid (AMPA)/kainate response; in the case of suprathreshold stimulation, the NMDA response becomes significant. The $GABA_A$ response via chloride channels has a reversal potential near the spike threshold potentials and acts mainly to shunt the glutamatergic inputs. Striatal A_{2A} receptor-mediated modulation attenuates this shunting effect by suppressing GABA inputs onto medium spiny neurons (MSNs) (i.e., A_{2A} receptor-mediated disinhibition) as illustrated by an upward shift of the current–voltage curve. From Kita,[33] modified with permission. (Copyright 1996 by the Society for Neuroscience).

The intrastriatal GABAergic network plays important roles in the temporal and spatial filtering of various and massive inputs from other striatal interneurons, the cortex, and other parts of the brain.[25,35] Modulation via A_{2A} receptors may play a critical role in tuning MSN excitability and, as a consequence, in regulating information processing through the striatum.

Pallidal modulation: A_{2A} receptor-mediated enhancement of activity of GABAergic nerve endings in the GP. A_{2A} receptor-mediated modulation in the GP serves to directly enhance GABA release from nerve terminals located within the GP. Analysis of mIPSCs has demonstrated that the modulation occurs at synaptic terminals, independent of the propagation of action potentials triggered by excitation of cell bodies to the terminals. mIPSC analysis using agents that regulate the cyclic adenosine monophosphate (cAMP)-dependent pathway revealed that sequential activation of the cAMP-dependent cascade is involved in pallidal A_{2A} receptor-mediated GABA release. This mechanism is calcium independent and is triggered by activation of A_{2A} receptors located at axon terminals.[36] The results have provided evidence of A_{2A} receptor-mediated isolated modulation system at GABAergic nerve endings located at the GP. Pallidal A_{2A} receptor-mediated enhancement of GABA release may directly suppress the excitability of GP projection neurons, resulting in increased neuronal activity in the subthalamic nucleus (STN).

Neurochemical evidence supporting dual A_{2A} receptor-mediated modulations in the striatopallidal system: pallidal GABA levels in vivo. In vivo rat microdialysis studies performed by Ochi et al. have verified the existence of A_{2A} receptor-mediated dual modulation in the striatopallidal system.[37] The studies demonstrated that intrastriatal injection and intrapallidal infusion of the A_{2A} agonist CGS 21680 induced a significant increase in pallidal GABA levels in normal rats. These results suggest that pallidal GABA release is regulated via A_{2A} receptors located in the striatum and GP. The effect of striatal A_{2A} receptor activation on pallidal GABA levels was blocked by tetrodotoxin locally infused into the GP, whereas the effect of local activation of pallidal A_{2A} receptors on pallidal GABA release was not. This result indicates that striatal A_{2A} receptor modulation targets neurons in the striatum projecting to the GP, whereas pallidal A_{2A} receptor modulation acts directly on nerve terminals in the GP.

Implications for the use of A_{2A} receptor antagonists as a novel therapy for PD. Current models of the pathophysiology of PD emphasize an increase in the overall activity in the striatopallidal indirect pathway.[8] Therefore, A_{2A}-mediated dual modulation could contribute to the generation of the parkinsonian state. Neurochemical studies using microdialysis have verified that the dual modulation by A_{2A} receptors occurs in vivo. 6-Hydroxydopamine (6-OHDA)–lesioned rats exhibited significantly increased basal GABA levels in the GP.[37] These results are consistent with the pathophysiologic hypothesis that excessive activation of the indirect pathway occurs in the parkinsonian state.[8] Excessive levels of pallidal GABA in 6-OHDA–lesioned rats were reduced by systemic oral administration of a novel A_{2A} receptor-selective antagonist, KW–6002, at 3 mg/kg, the pharmacologically effective dose of KW–6002 in rodent models.[38-40] This indicates that A_{2A} receptor blockade reverses the increase in pallidal GABA levels in 6-OHDA–lesioned rats and suggests that the A_{2A}-mediated modulation is involved in enhancing the excitability of the striatopallidal indirect pathway in vivo. KW–6002 exhibits antiparkinsonian effects in MPTP-treated monkeys[41-43] and in patients with PD,[44,45] with a therapeutic profile distinct from those of current dopaminomimetic therapies (see also Kase et al., page S97; Chase et al., page S107).

In conclusion, A_{2A} antagonists target the dual

modulation of GABAergic synaptic transmission by A_{2A} receptors in the striatopallidal system to counteract abnormal activity in the indirect pathway in patients with PD. Several important issues require further investigation: 1) pathophysiologic changes in the adenosinergic system (e.g., altered extracellular adenosine concentration) in those with PD; 2) the contribution of A_{2A} receptors localized to other subcellular sites, in particular postsynaptic dendritic receptors;[11] and 3) involvement of striatal A_{2A} receptor-mediated modulation of the GABAergic network may also provide important insights into information processing in the basal ganglia.

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III. A_{2A} receptors in neuroprotection of dopaminergic neurons

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An important observation that emerged from the symposium "A_{2A} Receptors in Neuroprotection of Dopaminergic Neurons" is that A_{2A} adenosine antagonists may behave like therapeutic drugs in Parkinson's disease (PD) through more than one mechanism. Schwarzschild et al.[1] pointed out that an important emerging issue is the possibility that A_{2A} antagonists, besides being useful in the care of patients with PD for acutely controlling symptoms, may also be beneficial in controlling the progression of neuronal degeneration. Although the etiology of PD is still unknown, new advances in molecular neuroscience recently led to the idea that neuronal degeneration may be stopped and that specific neuroprotective strategies are possible. One of these strategies could be a reduction in neurotoxicity in the substantia nigra or even in the striatum. Popoli et al.[2] reported that the A_{2A}-selective antagonist SCH 58261, systemically administered before the administration of the excitotoxin quinolinic acid (QA), prevents the effects of QA on motor activity, EEG changes, lesion size, striatal gliosis, and glutamate outflow. Therefore, the protective effects may be related to the capability of the A_{2A} antagonist to antagonize the striatal increase in glutamate induced by QA. In this regard, it is important to identify the dose-related effects of A_{2A} antagonism on the efflux of glutamate. SCH 58261 administered at intraperitoneal doses >0.01 mg/kg does not influence QA-stimulated glutamate release. A reduction in glutamate outflow is likely protective in neurodegeneration processes as in those induced by in vivo ischemia: SCH 58261 administered soon after ischemia at the same intraperitoneal dose as that used by Popoli et al.[3] reduces striatal glutamate outflow and necrotic damage.[4] A reduction in glutamate outflow after A_{2A} antagonist administration is likely attributable to antagonism of presynaptic striatal A_{2A} receptors. A strong suggestion of the presence of A_{2A} receptors on the corticostriatal glutamatergic terminal arose in the studies of Rosin et al.[5] presented at this meeting. However, in animal models of PD, with the aim of controlling motor disabilities, SCH 58261 is used at higher doses (1 to 5 mg/kg, intraperitoneally) than those that are protective against striatal glutamate outflow. Therefore, the question arises whether regulation of glutamate outflow contributes to the therapeutic potential of A_{2A} antagonists once neuronal degeneration linked to PD has progressed. In the CNS, A_{2A} receptors are mainly localized on the cell bodies of striatal GABA–enkephalin neurons. Antagonism of these receptors may account for regulation of glutamate outflow in different corticobasal ganglia structures. Likely, a reduction in glutamate outflow in the substantia nigra may account for protection against the progressive dopaminergic degeneration.[6] However, it should be considered that reduction in glutamate outflow does not necessarily imply neuroprotection.[7] An increase in striatal glutamate outflow was observed in the 6-hydroxydopamine (6-OHDA) model of PD by intrastriatal administration of SCH 58261, and it may be considered favorable in acutely controlling motor disabilities of PD in relation to disturbed striatal excitatory transmission.[8] The possibility that distinct mechanisms account for neuroprotection and motor-stimulating properties was stressed in the presentation of Schwarzschild et al.[1]

Alberto Ascherio[9] reported on stimulating epidemiologic studies that demonstrate a reduced risk of PD among caffeine consumers. This result is restricted to the male population. Interestingly, recent data[10] report that caffeine intake is associated with a reduced risk of PD in women who never used replacement hormones but with an increased risk in female hormone users and heavy coffee consumers. Schwarzschild et al.[1] discussed the possibility that the reduced risk of PD among caffeine consumers is the result of a neuroprotective effect of caffeine, as supported by the finding that caffeine, at doses comparable with those of typical human exposure, attenuates the loss of striatal dopamine induced in the experimental 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) model of PD. Whereas the neuroprotective effect of caffeine appears related to the block of A_{2A} receptors,[6] Castagnoli et al.[11] reported the capability of (E)-8-styrylxanthinyl–derived A_{2A}-selective antagonists, CSC and KW6002, to protect against MPTP-induced neurotoxicity by an A_{2A} receptor-independent effect. CSC in particular significantly inhibits monoamine oxidase (MAO) B activity in vitro, blocking the conversion of MPTP to the active toxic metabolite 1-methyl-4-phenylpyridinium (MPP^+); this pharmacologic effect was equally potent on MAO B isolated from A_{2A} receptor knockout and wild-type mice.[12] These data again indicate that mul-

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tiple mechanisms may account for the therapeutic potential of A_{2A} antagonists in PD, some related to antagonism of A_{2A} receptors and some to A_{2A} receptor-independent inhibition of the production of toxic metabolites that may be involved in nigrostriatal dopaminergic neuron degeneration. Schwarzschild et al.[1] underlined that the data obtained with A_{2A} antagonists to date are sufficiently compelling to warrant reconsideration of the clinical trial design of these drugs in PD. One issue that should be explored in future studies is whether such mechanisms are neuroprotectively relevant once the disease has progressed and drug therapy is underway.

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Caffeinated clues from epidemiology of Parkinson's disease

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Although there is a growing interest in the genetic determinants of Parkinson's disease (PD), the low concordance for clinical disease among monozygotic twins clearly indicates the etiologic importance of nongenetic factors.[1] On top of the list of potential candidates is cigarette smoking. For many years, researchers have been intrigued by the strong inverse association between cigarette smoking and risk of PD, but despite extensive investigation it remains unclear whether nicotine or some other component of cigarette smoke reduces the risk of PD or whether people predisposed to PD have an early aversion to smoking.[2] More recently, coffee and caffeine consumption have emerged as powerful predictors of risk of PD. Here we will review the epidemiologic evidence supporting this association and discuss the contribution of epidemiologic studies in clarifying the potential biologic mechanisms.

Early case-control studies of coffee and PD. Whereas the inverse association between cigarette smoking and risk of PD has been known for decades, relatively few early studies examined the potential role of coffee or caffeine consumption. Questions on coffee and tea consumption and risk of PD were included in some of the case-control studies on smoking[3,4] or environmental factors[5] and risk of PD, but published results were not adjusted for smoking and thus were difficult to interpret. The first smoking-adjusted analysis of coffee consumption and PD was reported by Jimenez-Jimenez et al.,[6] who studied 128 patients with PD (60 women) attending a movement disorder clinic in Madrid and 256 control subjects attending the emergency room of the same hospital for non-neurologic disorders. The data supported a 30% lower risk of PD among coffee drinkers (coffee drinking was reported as yes/no only) as compared with nondrinkers in men and women, but the results were not significant and received little attention. More detailed analyses of coffee consumption and risk of PD were later conducted in case-control studies in Germany[7] and Sweden.[8] Hellenbrand et al.[9] compared the dietary habits of 342 patients with PD (118 women) recruited from nine German clinics with those of 342 control subjects from the same neighborhood or region. Only patients diagnosed in 1987 or later and aged 65 years or younger were included. The odds ratio (OR) comparing participants in the highest with those in the lowest quartile of coffee intake was 0.27 (95% CI, 0.14 to 0.52), whereas no inverse association was found with tea. The authors also reported a strong inverse association between niacin intake and risk of PD and suggested that the observed association between coffee consumption and risk of PD could be the result of its high niacin content. Niacin is required for the synthesis of the cofactor nicotinamide adenine dinucleotide (NADH) and for the function of glutathione reductase, and the authors speculated that a defective or suboptimal functioning of this and other enzymes requiring NADH might be relevant to the development of PD. However, a significant inverse association between coffee intake and risk of PD persisted after adjusting for niacin in addition to smoking and caloric intake;[9] further, an inverse association between niacin and risk of PD has not been confirmed in prospective studies (see below). In a separate investigation including 113 patients with PD and 263 control subjects, Fall et al.[8] reported a highly significant inverse association between risk of PD and coffee and tea consumption. The multivariate OR comparing drinkers of five or more cups of coffee per day with nondrinkers was 0.14 (95% CI, 0.03 to 0.60). Although there are exceptions,[10] overall the results of case-control investigations favored the existence of an inverse association between coffee or caffeine intake and risk of PD.[11]

The weaknesses of case-control studies for the investigation of the dietary etiology of chronic diseases are well known[12] and include the difficulty of recruiting an appropriate control group, the generally inaccurate assessment of diet during the relevant period that for PD would be several years before the diagnosis, and selective differences in recall of dietary habits between patients and control subjects. Therefore, although the results of case-control studies suggest a potential protective effect of caffeine, recall or selection bias cannot be confidently excluded.

Prospective studies of coffee, caffeine, and PD in men. These problems have been overcome in prospective investigations that took advantage of the follow-up evaluation of large cohorts of persons for many years after they reported their coffee and caffeine consumption. One of these studies, conducted in Honolulu, comprised more than 8,000 Japanese-

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American men who completed a 24-hour diet recall at enrollment (1965 to 1968) and a food frequency questionnaire 6 years later and who were followed for up to 30 years.[13] In this cohort, the age- and smoking-adjusted risk of PD was five times higher among men who reported no coffee consumption at baseline compared with men who reported a daily consumption of 28 ounces of coffee or more. This association was also present among men who never smoked; therefore, it cannot be the result of residual confounding by cigarette smoking. Further, men with higher caffeine consumption at baseline still had a lower risk of PD during the second half of the follow-up period (i.e., more than 15 years later). This last result argues against the possibility that the lower caffeine consumption among men who developed PD resulted from early, unrecognized symptoms of the disease. Analyses adjusted for niacin, alcohol, and other nutrients suggested that caffeine was responsible for this association. However, the size of the study was insufficient to examine the association between caffeine from sources other than coffee and risk of PD among non-coffee drinkers, and no information was collected on consumption of decaffeinated coffee. Therefore, the possibility that compounds other than caffeine contributed to the observed inverse association could not be excluded.

Further, it was important to determine whether this association was also present in men of different ancestry and in women.

Consequently, we have addressed the association between caffeine consumption and risk of PD in two large cohorts: the Health Professionals Follow-up Study (HPFS) and the Nurses' Health Study (NHS) cohorts.[14] These studies included data on caffeinated and decaffeinated coffee and multiple assessments of caffeine consumption during the follow-up period. The HPFS was established in 1986, when 51,529 male health professionals (dentists, optometrists, pharmacists, podiatrists, and veterinarians), aged 40 to 75 years, responded to a mailed questionnaire that included a comprehensive diet survey and questions on disease history and lifestyle. The NHS cohort was established in 1976 when 121,700 women who were registered nurses, aged 30 to 55 years, residing in 11 large states provided detailed information about their medical history and lifestyle practices.[15] Follow-up questionnaires are mailed to participants of both studies every 2 years to update information on potential risk factors for chronic diseases and to ascertain whether major medical events have occurred. The caffeine analyses were based on 157 new cases of physician-diagnosed PD during 10 years of follow-up evaluation in men and on 131 new cases during 16 years of follow-up evaluation in women; a neurologist (85%) or review of the medical records (5%) confirmed most diagnoses, whereas an internist or general physician confirmed the remaining 10%. Intriguingly, we found an inverse association between caffeine intake and risk of PD in men but not in women. Men consuming caffeine from either coffee or noncoffee sources had a lower risk of PD than noncaffeine consumers. Interestingly, a 50% reduction in risk of PD was already observed among men consuming an amount of caffeine corresponding to one cup of coffee per day as compared with men consuming no caffeine. A similar result was found in the Honolulu cohort. Further, in the HPFS cohort, consumption of tea and other caffeinated beverages among men who were not regular coffee drinkers (consumption <1 cup/d) was also inversely associated with risk of PD, whereas no association was found with consumption of decaffeinated coffee. Among women, neither coffee nor caffeine from any source was significantly associated with risk of PD.

The finding that the risk of PD among men consuming caffeine is consistently lower than that of men not consuming caffeine in two large independent cohorts with several years of follow-up evaluation can only be explained if either caffeine consumption reduces the risk of PD or if some common genetic or environmental factor predisposes to caffeine avoidance and PD.[16] The latter alternative is consistent with the proposed existence of a premorbid parkinsonian personality characterized by reduced novelty seeking,[17] or of an underlying preclinical olfactory deficit that prevents the rewarding effects related to smell of coffee or tobacco.[18] This is the same question raised by the results on cigarette smoking, and unfortunately it is difficult to reach a conclusive answer. However, a few further clues can be inferred from epidemiologic studies. At least for cigarette smoking, the underlying common predisposing factor is unlikely to be genetic because an inverse association between cigarette smoking and PD risk has also been observed within monozygotic twin pairs.[19] Further, again for smoking, this predisposing factor must already be present during adolescence because we found in the HPFS cohort that smoking at age 19 years is associated with a significantly lower risk of PD after age 50 years (unpublished data). Similar evidence for caffeine is not available, although the observation that caffeine intake at the beginning of the study predicted risk of PD between 15 and 30 years later in the Honolulu cohort suggests that any common predisposing factor must precede the diagnosis of PD by at least 15 years.[13] To further assess the temporal relationship between caffeine consumption and PD, we have plotted the relative consumption of caffeine among men with PD in the HPFS before and after the diagnosis (figure). An adjusted caffeine intake of 0 mg/d represents an average consumption equal to that of men without PD at the same age and time; error bars indicate the 95% CI. If the lower caffeine intake among men with PD resulted from a progressive aspect of the disease, such as the degeneration of nigrostriatal dopaminergic neurons, then we would expect a decrease over time in relative caffeine intake. In contrast, the figure shows that the adjusted caffeine consumption is relatively stable, indicating that the effect of a hypothetical common predisposing factor

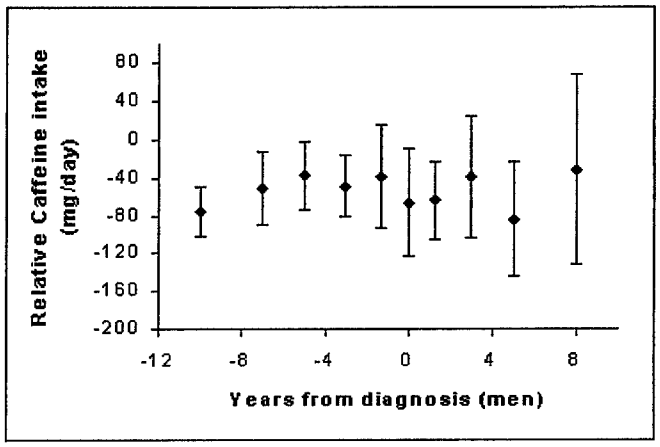

Figure. Age-adjusted difference in caffeine intake between men with Parkinson's disease (PD) and those without PD according to time from diagnosis. Using data from the Health Professionals Follow-up Study, the relative consumption of caffeine among men with PD was plotted against time before and after diagnosis. An adjusted caffeine intake of 0 mg/d represents a consumption equal to the average for men without PD at the same age and time; error bars indicate the 95% CI.

would have already been completely expressed at least 8 years before the diagnosis of PD.

Studies of coffee, caffeine, and PD in women. A further clue to the mechanisms underlying the lower risk of PD among caffeine drinkers as compared with nondrinkers may come from studies in women. The fact that we found no association between coffee or caffeine intake and risk of PD in our large prospective study of women, despite use of repeated and validated measures of consumption during a 16-year follow-up period,[14] is unlikely to be the result of chance. Further, a similar gender difference in the relation of caffeine with PD has also been found in a case-control study that relied on prospectively collected information on coffee consumption.[18] These findings suggest that caffeine may have different effects in men and women, perhaps because of hormonal differences. Caffeine is largely metabolized by the CYP1A2 isoenzyme of the P450 family,[20] which also metabolizes estrogen;[21] by competing for the same enzyme, exogenous estrogen in oral contraceptives[22] or postmenopausal hormones[21] inhibits caffeine metabolism. To address this possibility, we have examined the interaction between use of postmenopausal hormones, caffeine consumption, and risk of PD among participants in the NHS. Overall, use of postmenopausal hormones was not associated with risk of PD. However, we found that among hormone users, women consuming six or more cups of coffee per day had a fourfold higher risk of PD (relative risk [RR], 3.92; 95% CI, 1.49 to 10.34; $p = 0.006$) than women who never drink coffee; in contrast, among women who never used postmenopausal hormones, coffee drinkers had a lower risk of PD than nondrinkers.[23] If caffeine avoidance and increased risk of PD were caused by a premorbid personality or an olfactory deficit occurring more than 15 years before the diagnosis of PD, it would remain unclear how use of postmenopausal hormones would modify this association. Thus, albeit indirectly, this interaction supports a biologic effect of caffeine on risk of PD. Independent confirmation in epidemiologic studies and animal experiments exploring the mechanistic basis of this finding will be important. Meanwhile, this possible interaction should be considered in the planning and interpretation of trials of estrogen supplementation or caffeine use in women with PD, particularly because our results suggest that estrogen supplementation could be harmful among women consuming high amounts of caffeine.

The fact that two of the most common addicting behaviors are inversely associated with risk of PD (results on alcohol are less clear than those on cigarette smoking or caffeine and have not been discussed here) has often been interpreted as supporting an underlying predisposing cause related to resistance to addiction as the most parsimonious explanation. Nevertheless, the strength and consistency of these associations, the lack of a convincing and specific alternative hypothesis, and the evidence of an interaction between caffeine and postmenopausal hormones suggest that nicotine and caffeine could reduce the risk of PD.

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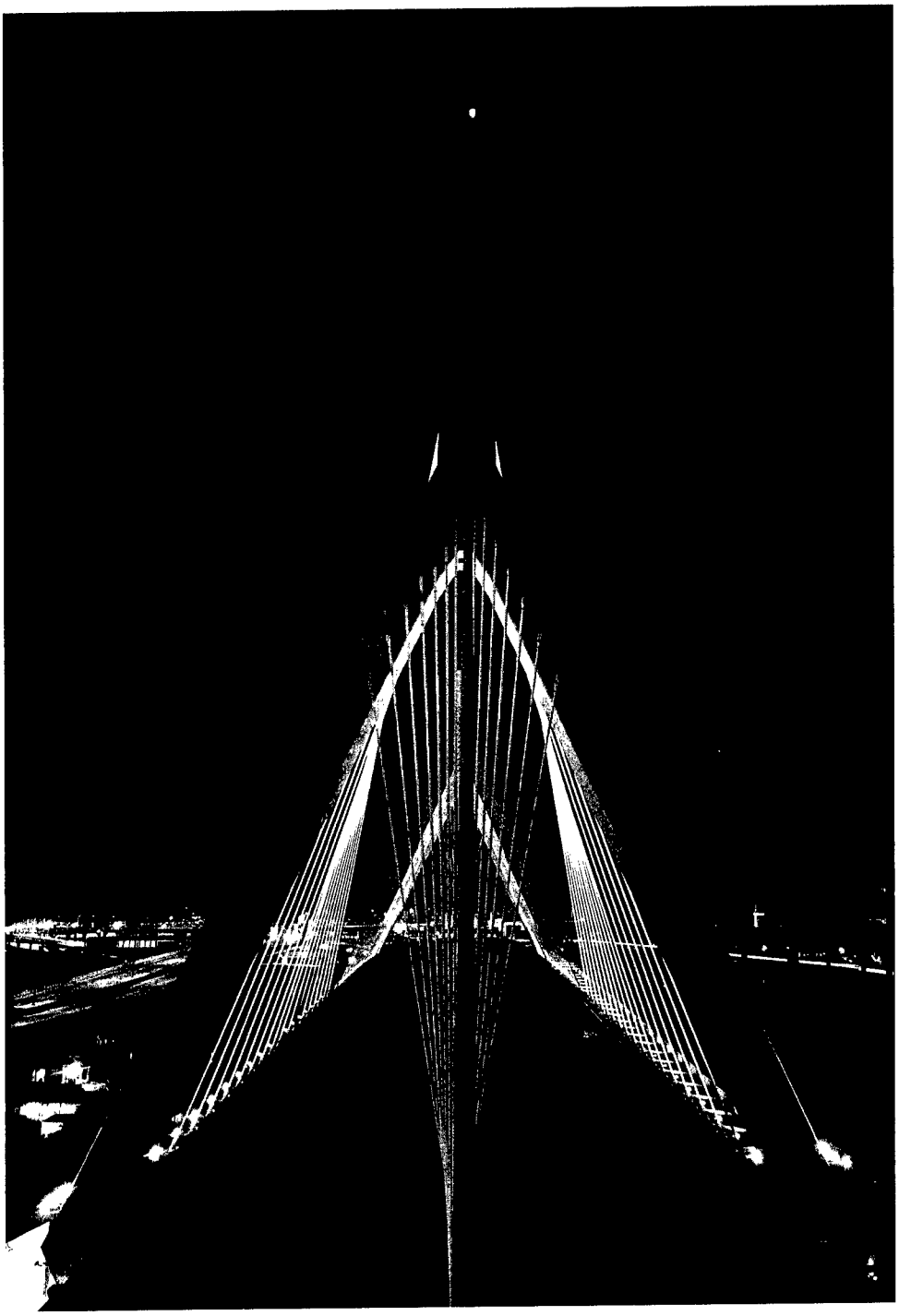

Don Eyles **November 2001**

Neuroprotection by caffeine and more specific A_{2A} receptor antagonists in animal models of Parkinson's disease

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Abstract—A remarkable convergence of epidemiologic and laboratory data has raised the possibility that caffeine reduces the risk of developing Parkinson's disease (PD) by preventing the degeneration of nigrostriatal dopaminergic neurons. The authors review the evidence that caffeine and more specific antagonists of the adenosine A_{2A} receptor protect dopaminergic neurons in several toxin models of PD. Other studies demonstrating protection by A_{2A} receptor inactivation in animal models of stroke, Huntington's disease, and Alzheimer's disease suggest a more global role of A_{2A} receptors in neuronal injury and degeneration. Although the cellular and molecular mechanisms by which A_{2A} receptors contribute to neuronal death are not yet established, several intriguing possibilities have emerged. Now with preliminary clinical data substantiating the antiparkinsonian symptomatic benefit of A_{2A} receptor blockade, the prospects for a complementary neuroprotective benefit have enhanced the therapeutic potential of A_{2A} antagonists in PD.

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Recent epidemiologic studies have established an association between the common consumption of coffee or other caffeinated beverages and a reduced risk of developing Parkinson's disease (PD) later in life.[1] Despite their strength, these epidemiologic investigations are unable to conclusively answer the fundamental question: *Does caffeine help prevent PD, or does PD or its causes help prevent the habitual use of caffeine?* Although this question of causality is difficult to address in humans, animal models can offer useful clues. Here we review the evidence that caffeine and more specific antagonists of the A_{2A} subtype of adenosine receptor are capable of protecting dopaminergic and other neurons from degeneration and death.

Neuroprotection by caffeine in a PD model. Using the well-established 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) model of PD, we have investigated the effect of caffeine on the demise of nigrostriatal dopaminergic neurons.[2-5] Mice exposed to the dopamine neuron-specific toxin MPTP develop biochemical and anatomic lesions of the dopaminergic nigrostriatal system that parallel characteristic features of PD.[6] Caffeine, when administered to mice at doses (5 to 30 mg/kg) comparable with those of typical human exposure, dose dependently reverses the loss of striatal dopamine triggered by MPTP.[2] Caffeine similarly attenuated the toxin-induced loss of dihydroxyphenylacetic acid (DOPAC), dopamine's major CNS metabolite, suggesting that caffeine is not simply altering dopamine metabolism in the remaining nigrostriatal nerve terminals. In addition to these biochemical markers of dopaminergic nigrostriatal function, the density of dopamine transporter (DAT) binding sites was measured as an anatomic marker of nigrostriatal innervation. Again MPTP toxicity was decreased in the presence of caffeine, which significantly attenuated the MPTP-induced loss of striatal DAT (^{3}H-mazindol) binding sites. Caffeine's protective influence on the dopaminergic innervation of the striatum can be directly attributed to its ability to prevent the death of dopaminergic neurons originating in the substantia nigra. Stereologic analysis of nigral dopaminergic (tyrosine hydroxylase-immunoreactive) neurons showed their MPTP-induced loss could be prevented by caffeine pretreatment.[4] The protective effect of caffeine was observed with different MPTP exposure paradigms (single and multiple doses) and in different mouse strains (C57Bl/6 and 129-Steel).[2]

Caffeine (1,3,7-trimethylxanthine) is metabolized by demethylation, initially to the dimethylxanthines theophylline, theobromine, and paraxanthine (1,3-, 3,7- and 1,7- dimethylxanthine, respectively), with the latter predominating as the major dimethyl metabolite of caffeine in humans.[7] Preliminary studies in mice demonstrate that theophylline and paraxanthine, which like caffeine are nonspecific adenosine receptor antagonists at low micromolar concentra-

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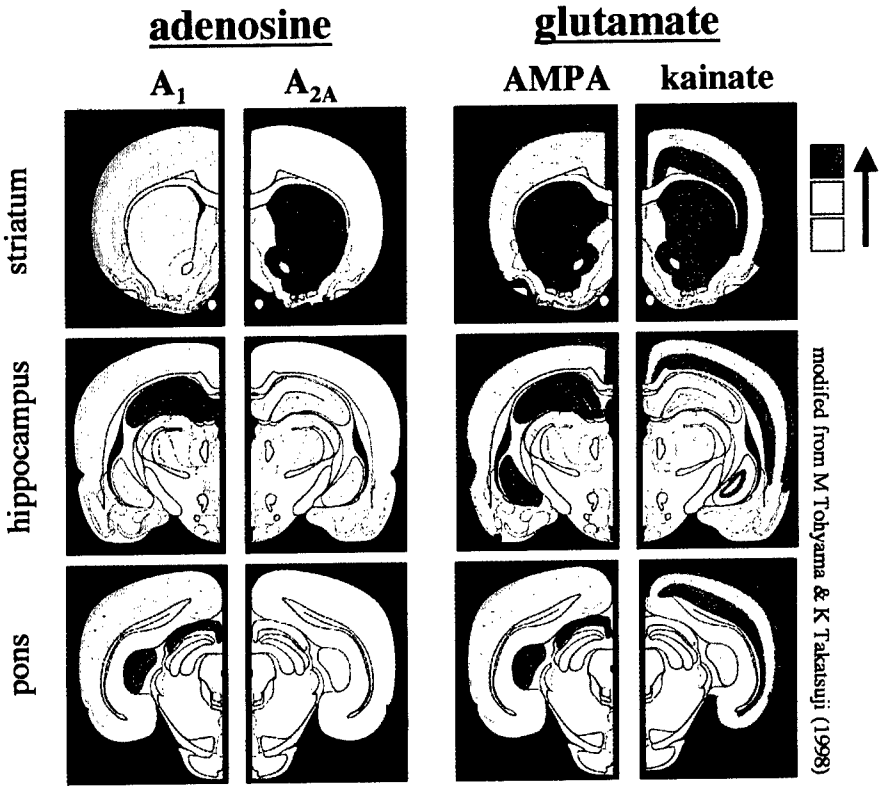

Figure 1. Brain expression patterns for subtypes of receptors for two neurotransmitters known to modulate dopaminergic neuron function. Composite distributions of specific radioligand binding to subtypes of adenosine and glutamate are shown in coronal sections from the rostral, mid, and caudal rat brain (containing striatum, hippocampus, and pons, respectively). Increasing density of radioligand binding to the receptors indicated is color-coded over a spectrum from white to blue to yellow to red. Most receptors that modulate dopaminergic transmission in the striatum are widely distributed throughout the brain, whereas the A_{2A} subtype of adenosine receptor is largely restricted in its expression to the striatum and the underlying olfactory tubercle. (Adapted, with permission, from Tohyama M and Takatsuji K. Atlas of Neuroactive Substances and Their Receptors in the Rat. Oxford: Oxford University Press, 1998.)

tions,[8] can also attenuate MPTP toxicity.[5] In humans, the serum half-life of caffeine (which is typically ingested once to several times per day) is approximately 4 hours.[9,10] Moreover, >80% of caffeine is metabolized to paraxanthine.[7] Therefore, the finding of a protective effect of paraxanthine and caffeine in mice suggests that precise temporal pairing between caffeine and putative dopaminergic neurotoxin exposures in humans would not be critical for caffeine to reduce the risk of developing PD (if caffeine were protective in humans).

Although the typical human pattern of frequent caffeine exposure indirectly supports the plausibility of neuroprotection against PD, it also raises the possibility that tolerance would develop to this protective action of caffeine. Tolerance is a characteristic feature of caffeine's psychomotor stimulant effect, i.e., it decreases after repeated exposure.[11,12] To investigate the possibility that the neuroprotective effect of caffeine is also affected by previous exposure, we assessed the motor stimulant and neuroprotective effects of caffeine in mice treated daily with caffeine or saline for more than 1 week.[3] Repeated daily caffeine administration, under conditions that produced substantial locomotor tolerance, did not attenuate the protective effect of caffeine on MPTP-induced dopaminergic toxicity. Together, these protective effects of caffeine and its metabolites support a causal basis for the inverse relationship between caffeine consumption and the risk of subsequently developing PD.

Neuroprotection by specific A_{2A} antagonists in PD models. The protective effect of caffeine in a mouse model of PD provides a compelling clue to the pathophysiology and the epidemiology of PD. Insight into how caffeine protects dopaminergic neurons may also lead to improved PD therapeutics aimed at slowing the underlying neurodegenerative process. A first step in pursuing this "caffeinated" clue has been the consideration of which of caffeine's known molecular targets may mediate its protective effect. Pharmacologic studies indicate that the CNS effects of caffeine are mediated primarily by its antagonistic actions at the A_1 and A_{2A} subtypes of adenosine receptor.[8] A_{2A} adenosine receptors may be particularly relevant because their expression in the brain is largely restricted to the striatum (figure 1),[13] the major target of the dopaminergic neurons that degenerate in patients with PD. Furthermore, their blockade or inactivation has been known to protect against excitotoxic and ischemic neuronal injury (see below).

Accordingly, we tested relatively specific A_{2A} and A_1 receptor antagonists for their ability to mimic caffeine's attenuation of MPTP toxicity. MPTP-induced nigrostriatal lesions were attenuated by pretreatment with all A_{2A} antagonists tested, including xanthine-based compounds 8-(3-chlorostyryl)caffeine (CSC)[14] and 3,7-dimethyl-1-propargylxanthine (DMPX),[2] KW6002 ((*E*)-1,3-diethyl-8-(3,4-dimethoxystyryl)-7-methyl-3,7-dihydro-1*H*-purine-2,6-dione),[2,15] and those with nonxanthine structures—SCH 58261 (7-(2-phenylethyl)-5-amino-2-(2-furyl)-pyrazolo-[4,3-*e*]-1,2,4-triazolo [1,5-*c*]pyrimidine)[2] and ZM241385 (4-(2-[7-amino-2-[2-furyl][1,2,4]triazolo[2,3-*a*][1,3,5,]triazin-5-yl aminolethyl)phenol) (unpublished observations). The specificity of CSC with respect to its neuroprotective effect in the MPTP model has recently been called

into question with our serendipitous finding that it possesses dual independent actions of high-potency inhibition of monoamine oxidase (MAO) B and antagonism of the A_{2A} receptor.[14,16] Although none of the other aforementioned xanthine- or nonxanthine-based A_{2A} antagonists possess comparable (if any) MAO B activity, the unexpected incomplete specificity of CSC even at low (nanomolar) concentrations highlights the pitfalls of adenosine pharmacology.

To circumvent such pharmacologic limitations and definitively address the question of whether A_{2A} receptor blockade mimics the neuroprotective effect of caffeine, mice lacking functional A_{2A} receptors because of A_{2A} receptor gene disruption (A_{2A} knockout mice)[17,18] were assessed for their susceptibility to MPTP toxicity. MPTP-induced losses of striatal dopamine and DAT were significantly attenuated in A_{2A} knockout mice compared with their wild-type littermates.[2] These complimentary genetic and pharmacologic approaches clearly demonstrate that A_{2A} receptor inactivation, like caffeine, reduces MPTP toxicity. By contrast, multiple concentrations of the A_1 receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine (CPX) showed no evidence of neuroprotection against the dopaminergic toxicity induced by multiple concentrations of MPTP.[2] Recently, the neuroprotective effect of A_{2A} receptor blockade against dopaminergic neuron injury has been extended to another species and model of PD. The A_{2A} antagonist KW6002 was found to prevent nigral dopaminergic neuron loss induced by 6-hydroxydopamine (6-OHDA) in rats[15] and by MPTP toxicity in mice. Together, these data suggest that caffeine can protect against dopaminergic neuron injury and death through its antagonistic action at the adenosine A_{2A} receptor.

A broader neuroprotective role for A_{2A} antagonists. These findings implicate endogenous adenosine acting on the A_{2A} receptor in the pathophysiology of nigrostriatal neuron lesions. However, this role for the A_{2A} receptor clearly extends beyond its targeting of central dopaminergic pathways to other populations of CNS neurons. For example, recent studies have demonstrated that the A_{2A} receptor contributes to the death of striatal medium spiny neurons in rodent models of Huntington's disease (HD). At low doses, the A_{2A} antagonist SCH 58261 attenuates striatal lesions induced by local infusion of the excitotoxin quinolinate.[19] Moreover, findings in A_{2A} receptor knockout mice show that loss of striatal neurons induced by systemically administered 3-nitroproprionic acid (a complex II inhibitor and relatively specific striatal neuron toxin) is markedly reduced in the absence of the A_{2A} receptor or in the presence of the A_{2A} antagonist CSC.[20] Of note, it is the subset of GABAergic striatal output neurons expressing high levels of A_{2A} receptor (i.e., those that project to the lateral globus pallidus) that degenerates earliest in patients with HD,[21] and whose absence may account for the involuntary choreic movements characteristic of this disorder.

In addition to the protection against striatal and nigral neuron loss offered by A_{2A} antagonists, their ability to protect neuronal populations outside the basal ganglia is well documented. For example, local injection of an A_{2A} antagonist can prevent the excitotoxic death of neurons in hippocampal cortex produced by the ionotropic glutamate receptor agonists kainate and quinolinate.[22,23] Wider cortical damage in a variety of ischemic stroke models can also be attenuated by A_{2A} receptor blockers administered at the time of cerebral blood flow disruption.[24-27] Similarly, transient focal ischemia produces substantially less brain damage in the cortex and striatum of adult A_{2A} receptor knockout mice compared with their wild-type littermates.[18] Interestingly, a finding that focal ischemic brain injury in rats is dramatically attenuated by treatment with low doses of caffeine together with ethanol[28,29] has led to a therapeutic trial of this adenosine antagonist–CNS depressant combination in humans with acute stroke.[30] A potential neuroprotective effect of caffeine and more specific A_{2A} antagonists has also been suggested for Alzheimer's disease (AD) based in part on the finding that these drugs can attenuate β-amyloid neurotoxicity in vitro.[31]

However, it should be noted that A_{2A} antagonists are not universally protective. Outside the CNS, the A_{2A} receptor may generally serve to attenuate ischemic and inflammatory tissue damage,[32,33] such that A_{2A} agonists (rather than antagonists) have also emerged as promising therapeutic candidates. For example, A_{2A} agonists can attenuate ischemic cardiac and renal damage, effects that are reversed by A_{2A} receptor blockade.[34-36] Even within the CNS, under some circumstances, A_{2A} receptor stimulation can confer neuroprotection. Administration of an A_{2A} agonist at the time of spinal cord ischemia and reperfusion significantly reduces resultant neuronal damage.[37] The basis for protective vs pathologic effects of A_{2A} receptor activation likely relates to the variety of cellular and molecular couplings of the A_{2A} receptor. Therefore, the safe and effective development of A_{2A} receptor agents will rely on efforts to clarify the mechanisms of their actions.

Mechanisms of protection by A_{2A} antagonist in PD models. How the A_{2A} receptor or its blockade influences the death of dopaminergic neurons remains uncertain (table 1 and figure 2). An intuitive explanation that the high levels of striatal A_{2A} receptors[38] (see figure 1) directly trigger the demise of the dopaminergic neurons innervating the striatum belies the cellular anatomy of the A_{2A} receptor within the basal ganglia. The vast majority of these receptors are expressed on GABAergic striatopallidal output neurons,[39,40] which are postsynaptic to the dopaminergic neurons that degenerate in PD. By contrast, there is little evidence for appreciable expression of A_{2A} receptors on the dopaminergic nigrostriatal neurons themselves.[40-42] Therefore, A_{2A} receptors on nondopaminergic neurons (or even on

Table *How A_{2A} antagonists may protect in PD models*

Mechanism	Pros	Cons
1. Global ↓ glutamate release (↓ direct excitotoxicity)	*A_{2A}Rs generally ↑ glutamate release *Explains protection of multiple neuronal populations	*Relies on presumed low level of A_{2A}Rs on excitatory neurons (or astrocytes)
2. Local ↓ GABA release (↓ indirect excitotoxicity from GPe→STN→SNc)	*Dense striatopallidal A_{2A}Rs *A_{2A}Rs ↑ GPe GABA release *STN→SNc excitotoxicity data	*Does not explain protection at other CNS sites
3. Glial cell modulation (e.g., ↑ glutamate buffering)	*A_{2A} agonists ↓ glut uptake in cultured CNS glia; modulate NOS and other activities	*Relies on presumed low level of A_{2A}Rs on glial cells *Based only on in vitro studies
4. Direct DA neuron protection	*Possible vesicular mechanism	*?A_{2A}Rs on DA neuron *A_{2A} antagonists not protective in cell cultures
5. Altered toxin metabolism		*MPTP metabolites and MAO B unaffected by A_{2A} antagonists

See text for details and figure 2 for schematic representation of mechanisms 1 to 4 and abbreviations.

non-neuronal cells) may indirectly influence the viability of the dopaminergic nigral neurons.

How may the blockade of postsynaptic A_{2A} receptor on GABAergic striatopallidal neurons improve the survival of presynaptic dopaminergic neurons? The shortest path back to the nigrostriatal dopaminergic neurons may be taken by the retrograde trans-synaptic elaboration of a protective factor.[43] Although specific neurotrophic factors in striatal neurons have been hypothesized to maintain the integrity of innervating dopaminergic neurons,[44] there is no evidence that striatal A_{2A} receptor stimulation

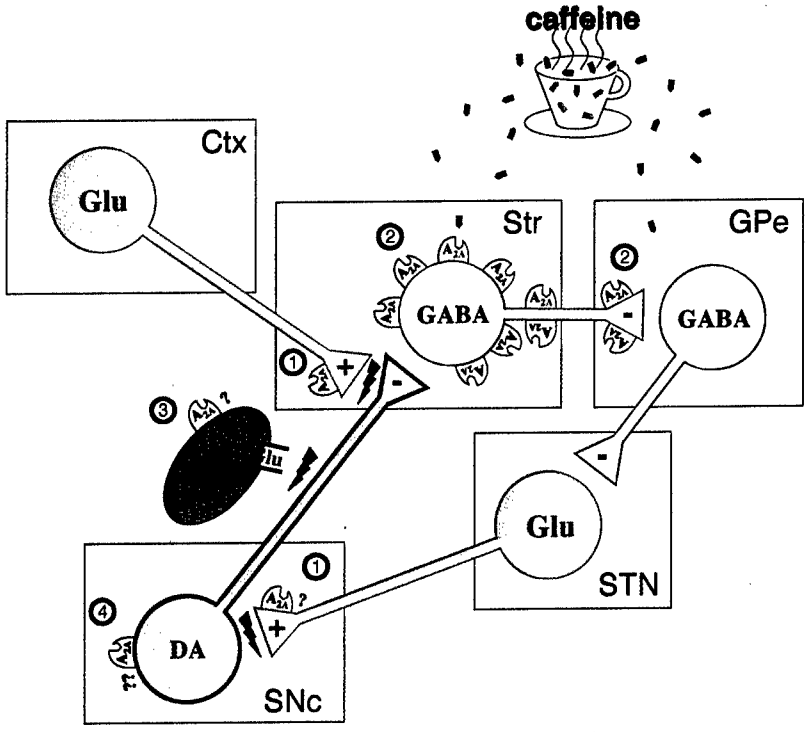

Figure 2. Sites of adenosine A_{2A} receptors whose blockade by caffeine could protect dopaminergic nigrostriatal neurons. As detailed in the text and indicated in this simplified schematic, A_{2A} receptors on the nerve terminals of excitatory glutamatergic (Glu) neurons (1) represent a widely distributed mechanism for enhanced excitotoxicity (lightning symbol) that may converge on dopaminergic (DA) nigrostriatal neurons. Blockade of A_{2A} receptor-facilitated glutamate release may account for reduced excitotoxic injury to cortical and striatal neurons (not shown) and nigral neurons. A_{2A} receptors are densely expressed on GABAergic striatopallidal neurons (2) where their facilitative effect on GABA release in the external globus pallidus (GPe) could indirectly lead to excitotoxic stimulation of dopaminergic nigral neurons via disinhibition of the glutamatergic projection from subthalamic nucleus (STN) to the substantia nigra pars compacta (SNc). Glial cells (e.g., astrocytes) can also express A_{2A} receptors (3) and actively regulate the environment of neurons throughout the CNS. Blockade of these receptors by caffeine may protect neighboring dopaminergic neurons, possibly by activating (disinhibiting) the glial glutamate transporter leading to lower extracellular levels of excitatory amino acids. The unsubstantiated possibility of low levels of A_{2A} receptors on dopaminergic nigrostriatal neurons (4) would allow for a direct protective effect of caffeine on dopaminergic neurons. Question marks (?) reflect the uncertainty over the presence of A_{2A} receptors on certain neuronal or glial cells in vivo. Ctx and Str refer to cortex and striatum, respectively.

inhibits this hypothetical retrograde neurotrophic influence (or conversely that A_{2A} antagonists enhance it).

Perhaps more realistic, even if more circuitous, is the possibility that striatal A_{2A} receptor blockade leads to improved dopaminergic neuron survival through a polysynaptic feedback loop involving the A_{2A} receptor-laden striatopallidal neurons (see figure 2, site 2). A_{2A} receptor stimulation of GABAergic striatopallidal neurons increases extracellular GABA in the globus pallidus.[45] The increased pallidal level of inhibitory transmitter in turn may reduce the activity of the GABAergic projection from the globus pallidus to the subthalamic nucleus (STN), leading to disinhibition of its glutamatergic projections.[46] One of these activated STN outputs projects to the substantia nigra pars compacta, where its enhanced release of glutamate may exert an excitotoxic effect on the dopaminergic nigrostriatal neurons.[47,48] Increased excitatory tone applied to the dopaminergic nigral neurons in combination with their metabolic deficits induced in the MPTP model (and possibly in PD) could contribute to the cumulative injury of dopaminergic neurons.[49] Experimental blockade or reversal of STN excitatory activity has been shown to attenuate the death of dopaminergic nigral neurons induced by 6-OHDA.[50,51] Therefore, striatal A_{2A} receptor stimulation could exacerbate an STN-mediated excitotoxic component of dopaminergic nigral neuron degeneration, and conversely A_{2A} antagonists may modify the circuit to slow the degenerative process.

Although this circuitry model of dopaminergic neuron protection by A_{2A} antagonists incorporates a critical role for the prominent striatal A_{2A} receptor (see figure 1), it does not easily explain their protective effects on nondopaminergic neurons residing at other CNS locations. Because evidence for a broader neuroprotective effect now extends from the hippocampal to frontal cortex and from nigra to striatum, alternative hypotheses that involve A_{2A} receptor modulation of a generalized CNS process have become more compelling. One such mechanism is the well-established facilitation of glutamate release by A_{2A} receptor stimulation (see figure 2, site 1),[52] which has been consistently observed in the cortex, basal ganglia, and brainstem.[53-55] This phenomenon likely involves A_{2A} receptors located on glutamatergic nerve terminals because it can be observed in cortical synaptosomes,[56] in which an indirect effect of striatal A_{2A} receptors is less plausible, and in intact brain. Recent ultrastructural analysis of A_{2A} receptor distribution has strengthened the evidence for its presynaptic location on glutamatergic nerve terminals.[57] Whereas A_{2A} agonists generally enhance release or overflow of glutamate, A_{2A} antagonists have been found to attenuate glutamate release or overflow triggered by depolarization, ischemia, or the glutamate receptor agonist quinolinate in an excitotoxin model of HD.[19,58-61] Therefore, A_{2A} antagonist attenuation of local excitatory amino acid release throughout the CNS may alleviate an excitotoxic component common to most models of neurotoxicity and neurodegeneration. Whether A_{2A} antagonists attenuate MPTP-induced elevations in striatal or nigral neurotransmitters remains to be seen.

Recently, adenosinergic modulation of glial cell function has emerged as another widely distributed CNS mechanism by which A_{2A} antagonists may lessen neuronal cell death (see figure 2, site 3). Stimulation of A_{2A} receptors present on cultured astrocytic glial cells from the cortex or brainstem was found to enhance glutamate efflux, whereas A_{2A} blockade reduced levels of extracellular glutamate.[62,63] Genetic and pharmacologic approaches suggested that A_{2A} receptor regulation of a specific glial glutamate transporter (GLT-1) might account for this effect. Earlier studies suggested that A_{2A} receptors could modulate other glial functions (such as nitric oxide synthase and cyclo-oxygenase activities) that might play an important role in the survival of their neuronal neighbors.[64,65] Therefore, ubiquitous glial elements in the CNS may also host A_{2A} receptor involvement in multiple models of neurodegeneration.

Other candidate mechanisms for dopaminergic neuron protection by A_{2A} antagonists have been suggested that are unique to the toxin models of PD in which the protection has been demonstrated. For example, reduced cyclic adenosine monophosphate (cAMP) in dopaminergic neurons leading to increased vesicular sequestration of 1-methyl-4-phenylpyridinium (MPP^+; the active toxin metabolite of MPTP) has been proposed as an explanation for how A_{2A} antagonists attenuate neurotoxicity in the MPTP model of PD.[15] However, this proposal is primarily based on MPP^+ uptake studies in a pheochromocytoma cell line, relies on the uncertain presence of A_{2A} receptors on dopaminergic neurons, and does not explain A_{2A} antagonist protection of nondopaminergic neurons. Nevertheless, the possibility of a simple direct cellular survival effect of A_{2A} antagonists via a small number of (as yet unsubstantiated) A_{2A} receptors on the dopaminergic neurons themselves (see figure 2, site 4) has not been ruled out. Recent findings of protection by caffeine or more specific A_{2A} antagonists against neuronal and nonneuronal cell death in vitro support this possibility.[31,66]

Another important consideration is the possibility that A_{2A} receptor blockade may protect against MPTP toxicity simply by limiting MPTP access to the CNS or its conversion by MAO B to the active toxin MPP^+. Before it was discovered that CSC possesses potent MAO B inhibitory activity independent of its A_{2A} antagonist properties,[14,16] its attenuation of MPTP metabolism in striatum had suggested that A_{2A} blockade could reduce MPTP toxicity by inhibiting MAO B activity (see Castagnoli et al., page S62).[67] However, caffeine and genetic inactivation of the A_{2A} receptor did not significantly alter striatal MPTP concentration in vivo nor did they substantially alter MAO B activity in vitro.[2,14,16] Moreover,

the A_{2A} antagonist KW6002 did not appreciably alter the brain levels or kinetics of MPP^+ after systemic MPTP administration.[15] Therefore, inhibition of MPTP metabolism or MAO B activity does not explain the neuroprotective of caffeine and more specific antagonists of the A_{2A} receptor in models of PD.

Significance for PD. The demonstration that caffeine and more specific A_{2A} antagonists protect dopaminergic nigrostriatal neurons in multiple animal models of PD has pathophysiologic, epidemiologic, and therapeutic significance for PD.

Understanding the neurobiology of the A_{2A} and other adenosine receptors will provide insight into the role of endogenous adenosine in basal ganglia biology and PD pathophysiology.

Establishing the ability of caffeine to protect dopaminergic neurons in PD models and identifying a plausible mechanism of action greatly strengthen (but do not prove) the hypothesis that a neuroprotective effect of caffeine is the basis for its inverse epidemiologic association with risk of PD.[68]

With A_{2A} antagonists emerging as promising therapeutic candidates based on their motor-enhancing symptomatic effects,[69,70] an additional neuroprotective benefit would considerably enhance their therapeutic potential.

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Monoamine oxidase B inhibition and neuroprotection

Studies on selective adenosine A_{2A} receptor antagonists

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Abstract—The principal therapeutic agents used in the management of Parkinson's disease (PD) enhance nigrostriatal dopaminergic flux through either replenishment of depleted dopamine stores or the action of dopaminergic agonists. Adenosine A_{2A} receptor antagonists (e.g., KW–6002) may provide symptomatic relief in PD and perhaps also may display neuroprotective properties based on studies in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model of nigrostriatal neurodegeneration. A second class of compounds that is neuroprotective in the MPTP model comprises inhibitors of the outer mitochondrial flavoenzyme monoamine oxidase B (MAO B), one of the two forms of MAO that regulate levels of brain neurotransmitter substances, including dopamine. In this article, data are presented that document the overlapping A_{2A} antagonist and MAO B inhibitory properties of several 2-styrylxanthinyl derivatives. A limited structure–activity analysis of these compounds and structurally related analogs is provided. The results raise the possibility that a single structure may offer the combined benefits of two pharmacologic strategies, each with symptomatic and potential neuroprotective benefits, for the management of PD.

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Our interests in monoamine oxidase B (MAO B) were stimulated by the seminal discovery that this flavoenzyme catalyzes the conversion of the parkinsonism-inducing compound 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP, see figure 2 below)[1,2] to a mitochondrial toxin[3-6] that localizes[7,8] in and eventually destroys dopaminergic nigrostriatal neurons. The sequence of events accounting for MPTP's neurodegenerative properties has been detailed in several review articles.[9,10] Because of the importance of this subject to many of the topics addressed in this supplement and to the studies summarized in this article, a brief review of the more salient issues related to MPTP's neurotoxicity and the role played by MAO B is presented.

MAO B inhibitors as potential neuroprotectants. MAO A and MAO B, the two forms of this oxidase, catalyze the α-carbon oxidation of a variety of primary amines.[11] For example, MAO catalyzes the oxidation of dopamine (**1**; this and following numbers in parentheses refer to numbered compounds in figures 1 and 2)[12] to the corresponding iminium species (**2**). Subsequent hydrolysis of **2** leads to aldehyde (**3**) that undergoes further oxidation to the carboxylic acid dihydroxyphenylacetic acid (DOPAC; **4**) (see figure 1).[13]

The principal enzymes responsible for the α-carbon oxidation of xenobiotic cyclic tertiary amines such as MPTP (**5**),[14] however, are members of the cytochrome P450 family of enzymes that are located primarily in the liver.[15] In the case of MPTP, the resulting cyclic iminium metabolite 1-methyl-4-phenyl-2,3-dihydropyridinium ($MPDP^+$; **6**), being cyclic, is in reversible equilibrium with the corresponding aminoaldehyde (**7**).[10,16] $MPDP^+$ is a substrate of a second important hepatic enzyme, aldehyde oxidase,[17] which catalyzes the detoxication of this chemically reactive intermediate to the corresponding stable lactam (**8**).[16] Unexpectedly, MPTP also is an excellent substrate for brain MAO B. In the brain, however, aldehyde oxidase activity, if present,[18] does not compete with a subsequent 2-electron ring oxidation of $MPDP^+$ that generates the pyridinium metabolite 1-methyl-4-phenylpyridinium (MPP^+; **9**).[19,20]

Apparently MAO B in the human brain is located primarily in glial cells[21-23] and not in dopaminergic neurons. Consequently, the MPTP-mediated selective loss of the nigrostriatal dopaminergic cells appears to depend on the active transport of MPP^+ into the terminals of these neurons, a process that is thought to be mediated by the dopamine transporter.[7,8,24] Once within the nerve terminals, MPP^+ is localized within the inner membrane of the mitochondrion,[25,26] where it inhibits complex I of the electron transport chain.[3-6] This, in turn, leads to depletion of adenosine triphosphate (ATP) and ultimate neuronal cell death.[27]

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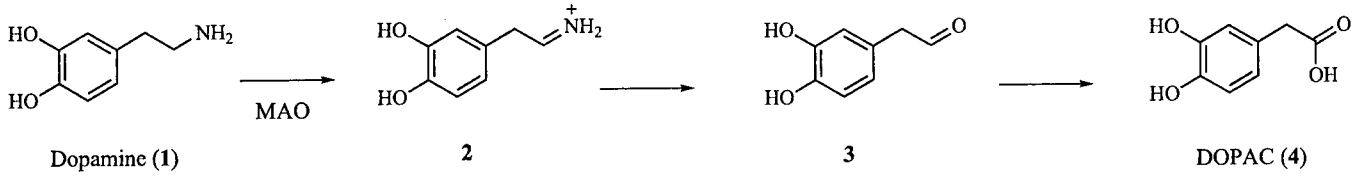


Figure 1. The monoamine oxidase (MAO)-catalyzed oxidation of dopamine (1).

MPTP has proved to be an important agent for studies directed toward understanding the basic mechanisms of neurodegeneration and in particular those molecular events that are responsible for the selective loss of nigrostriatal neurons, a hallmark of idiopathic Parkinson's disease (PD).[28] The MPTP-induced degeneration of nigrostriatal neurons in C57BL/6 mice[29] also is used extensively in studies of potential neuroprotective agents, including A_{2A} antagonists,[30,31] the topic of this supplement. Typically, MPTP is administered either intraperitoneally or subcutaneously to the test animal. After 7 or more days, the striatal dopamine levels are estimated by a liquid chromatography–electrochemical detection (LC-EC) assay.[32,33] The MPTP-induced lesion then can be related to the extent to which striatal dopamine levels have been depleted.[34]

The history of the discovery of MPTP's parkinsonian-inducing properties reads like a detective story. MPTP is a degradation product of street heroin, a chemically unstable analog of meperidine that had been synthesized to bypass the legal authorities.[9,35,36] The possibility that this contaminant might be responsible for the parkinsonism observed in young drug users was established when MPTP was found to cause a parkinsonian syndrome in subhuman primates.[9,37,38] Subsequently, the role played by MAO B in the mediation of the neurotoxicity of MPTP was documented in the monkey and the C57BL/6 mouse model by studies with (*R*)-deprenyl (also known as selegiline), a selective and potent irreversible (mechanism-based) inactivator of MAO B. Pretreatment of susceptible animals with (*R*)-deprenyl prevents the neurodegenerative properties of MPTP.[29,39-41]

Interestingly, this direct relationship between MAO B activity and MPTP neurotoxicity may describe only part of a more complex neuroprotective mechanism. For example, Tatton[42-44] has reported that doses of (*R*)-deprenyl that do not inhibit MAO B still are neuroprotective in the C57BL/6 mouse model. We[45] and others[46] have obtained supporting results showing that (*R*)-deprenyl administration after MPTP has cleared the brain[47] is still neuroprotective. However, the "window of opportunity" is fairly narrow because no protection was observed when (*R*)-deprenyl was administered 180 minutes post-MPTP. Evidence has been presented suggesting that the neuroprotection observed with (*R*)-deprenyl results from suppression of free radical damage caused by the MPP^+-mediated damage to mitochondria.[48]

These data suggest that (*R*)-deprenyl's neuroprotective activity is not mediated exclusively by inhibition of the MAO B-catalyzed oxidation of MPTP. However, this conclusion does not rule out a neuroprotective effect of MAO B inhibition that operates independently of the bioactivation of MPTP. A potentially dramatic link between MAO B inhibition and neuroprotection is the decreased risk of developing PD in tobacco smokers.[49] Elegant PET studies have shown that brain MAO B activity in smokers can be reduced to up to 70% of the corresponding activity observed in nonsmokers.[50] The possible links between smoking, MAO inhibition, and neuroprotection currently are under investigation in our laboratory.

Adenosine A_{2A} receptor antagonists as potential neuroprotectants. Recent studies by Chen et al.[31] suggest that the xanthinyl derivative caffeine also is neuroprotective. This finding is of particular interest

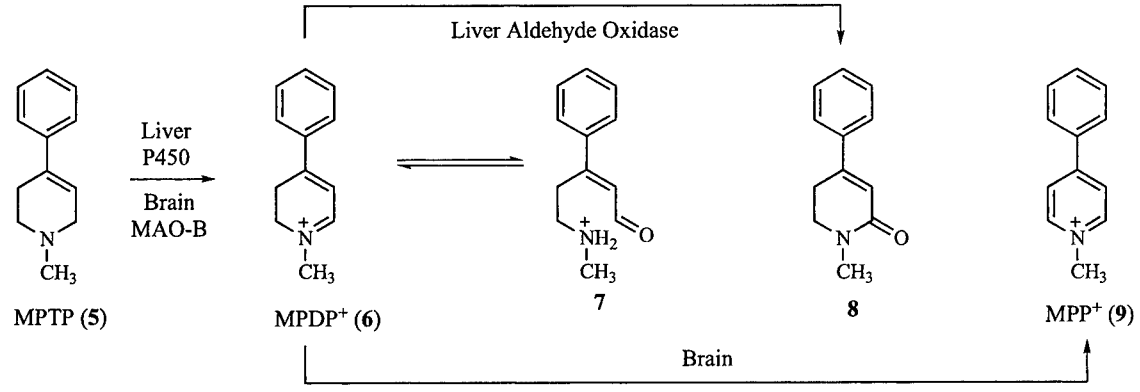
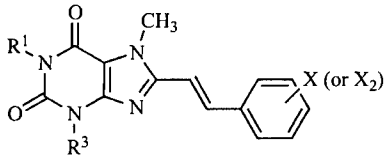


Figure 2. The metabolic fate of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (5).

Table 1 *The K_i values for the inhibition of MAO-B by various (E)-8-styryl-7H-xanthinyl derivatives, including CSC* ***(10)*** *and KW-6002* ***(11)***



Compound	R^1/R^3	X or X_2	K_i value: MAO-B (μM)	K_i value: A_{2A} (nM)
10	methyl	3-chloro	0.1	54*
11	ethyl	3,4-dimethoxy	28	2.2†
12	methyl	3-nitro	0.2	195*
13	methyl	3-fluoro	0.4	83*
14	methyl	3,4-dimethoxy	2.7	197*
15	methyl	H	3.0	94*
16	ethyl	3-chloro	3.0	Not reported
17	ethyl	3,4-methylenedioxy	8.0	6.1‡

The reported K_i values for antagonism of the A_{2A} receptor are also listed.

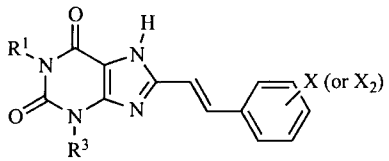
* Jacobson et al., 1993
† Shimada et al., 1997
‡ Suzuki et al., 1996.

MAO-B = monoamine oxidase B.

because there is strong epidemiologic evidence that coffee drinking is inversely related to the development of PD.[51] Furthermore, caffeine is known to increase motility in humans and animals, an effect that presumably is mediated by its antagonistic activity at the A_{2A} receptor.[52] This enhancement of motility caused by caffeine has prompted efforts to develop more potent and more selective xanthinyl-based A_{2A} antagonists as potential antiparkinsonian agents.[53-56] Two important A_{2A} antagonists are the (*E*)-8-(3-chlorostyryl)xanthinyl and (*E*)-8-(3,4-dimethoxystyryl)xanthinyl analogs CSC (**10**)[53,55] and KW–6002 (**11**),[54] respectively. Like caffeine, the A_{2A} antagonist CSC also displays neuroprotective properties in the MPTP mouse model.[57] The observed neuroprotective properties of CSC raised the question of mechanism and led to studies that provided evidence suggesting that CSC inhibited the metabolism of MPTP in the C57BL/6 mouse.[57] Detailed in vitro studies established that CSC is a potent (K_i = 100 nmol/L) and selective competitive inhibitor of MAO B.[57] The reported K_i value for the selective displacement of the preferred A_{2A} ligand ^{3}H-CGS 21680 from the rat striatal A_{2A} receptor for CSC is 54 nmol/L.[55] Consequently, the affinities of CSC for the enzyme and the receptor appear to be comparable. These results argue that the neuroprotection observed in the MPTP mouse model, at least in part, may be caused by the CSC-mediated inhibition of MAO B.

The demonstration that CSC potently inhibits mitochondrial MAO B activity suggested that it might act on MAO B directly, independent of its well-established action on the A_{2A} receptor. However, the standard mitochondrial preparations used to identify MAO B inhibition by CSC likely contain some A_{2A} receptors, leaving open the possibility that CSC

Table 2 *The K_i values for the inhibition of MAO-B by various (E)-8-styryl-7-methylxanthinyl derivatives*



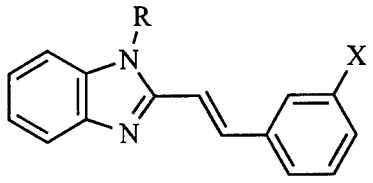
Compound	R^1/R^3	X or X_2	K_i value: MAO-B (μM)	K_i value: A_{2A} (nM)
18	methyl	3-chloro	1.5	Not reported
19	methyl	3-nitro	1.7	438*
20	methyl	3-fluoro	1.9	516*
21	methyl	3,4-dimethoxy	6.0	1100*
22	methyl	H	31.0	291*
23	ethyl	3-chloro	Not determined§	Not reported
24	ethyl	3,4-methylenedioxy	2.5	15‡
25	ethyl	3,4-dimethoxy	63.0	23‡

The reported K_i values for antagonism of the A_{2A} receptor are also listed.

* Jacobson et al., 1993.
‡ Suzuki et al., 1996.
§ The K_i value could not be determined because of limited solubility.

MAO-B = monoamine oxidase B.

Table 3 *The K_i values for the inhibition of MAO-B by (E)-2-styrylbenzimidazolyl derivatives*

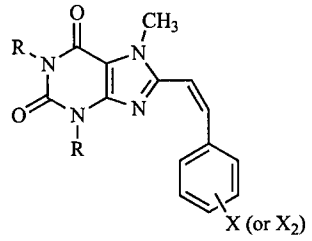


Compound	R	X	K_i values MAO-B
28	H	H	53 μM
29	H	chloro	3.5 μM
30	H	fluoro	5.3 μM
31	methyl	H	17 μM
32	methyl	chloro	1.4 μM
33	methyl	fluoro	2.6 μM

MAO-B = monoamine oxidase B.

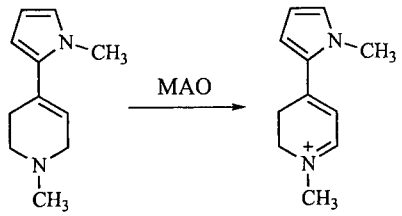
could indirectly inhibit MAO B through its interaction with the A_{2A} receptor. To address this possibility, we took advantage of an A_{2A} receptor knockout mouse model of A_{2A} receptor function. The effects of CSC on MAO B activity of mitochondria prepared from the brains of A_{2A} knockout mice were compared with those of their wild-type littermates.[57] In this experiment, MAO B activity in mitochondrial preparations that were devoid of A_{2A} receptors (i.e., those from knockout mice) were fully inhibited by CSC (with a K_i of approximately 100 nmol/L). These and other data confirmed that the novel MAO B inhibitory action of CSC is independent of its action on A_{2A} receptors.

Initial structure: activity correlations between A_{2A} antagonism and MAO B inhibition. The combination of the neuroprotective and MAO B-inhibiting properties of CSC has prompted us to examine the MAO B-inhibiting properties of other A_{2A} antagonists and related compounds to determine if such dual, independent actions are a common feature of these types of heterocyclic systems. Should this prove to be the case, it may be possible to develop drugs that antagonize the A_{2A} receptor[58] and inhibit MAO B[59-61] and thus have enhanced therapeutic potential for the management of PD.



34: R = C_2H_5; X_2 = 3,4-di-OCH_3
35: R = CH_3; X_2 = 3,4-di-OCH_3
36: R = CH_3; X = H_3
37: R = C_2H_5; X_2 = 3,4-OCH_2O

Figure 3. The cis*-isomer **34** of KW–6002 and related structures.*

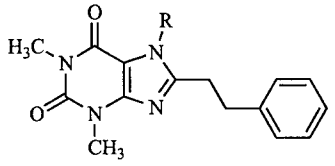


38 **39**
(λ_{max} at 420 nm)

*Figure 4. The monoamine oxidase (MAO)-catalyzed α-carbon oxidation of **38** to the dihydropyridinium species **39**.*

As part of an initial structure activity relationship (SAR) study, several (*E*)-8-styrylxanthinyl analogs (tables 1 and 2; compounds **10** to **25**), most of which are known A_{2A} antagonists, were tested in vitro for their MAO B-inhibiting properties. Of particular interest is KW–6002 (**11**), an A_{2A} antagonist currently undergoing clinical trials for the symptomatic management of PD. Also included in this study are a group of (*E*)-2-styrylbenzimidazolyl analogs (table 3; compounds **28** to **33**) and two compounds in which the styryl double bond has been reduced to give the corresponding 8-phenylethyl analogs **26** and **27** (see figure 5, below). These particular structural types were selected to evaluate the role of the xanthinyl group (compared with the benzimidazolyl group) and the potentially important[62-64] planar features of the styryl group (compared with the 8-phenylethyl group). During the course of our studies, several (*E*)-8-styryl-7-methylxanthinyl derivatives with electron-donating groups on the phenyl ring of the styryl moiety (compounds **11**, **14**, **15**, and **17**) were found to undergo isomerization to the corresponding *cis*-isomers **34** to **37** (figure 3) when exposed to laboratory light.[65,66] A synthetic sample of the KW–6002 *cis*-isomer **34** provided an opportunity to compare the MAO B-inhibiting properties of these geometric isomers.[67]

The MAO B-inhibiting properties of all of the test compounds were examined in a baboon liver mitochondrial preparation, which expresses only MAO B



26: R = H_3
27: R = CH_3

Figure 5. Structures of the 2-(8-phenylethyl)xanthinyl analogs discussed in the text.

activity[68] using 1-methyl-4-(1-methylpyrrol-2-yl)-1,2,3,6-tetrahydropyridine (**38**) as substrate.[69] This is an excellent substrate for this type of study because the MAO B-generated dihydropyridinium metabolite **39** (figure 4) is stable under the assay conditions and has a chromophore with λ_{max} at 420 nm, a wavelength considerably longer than that of the substrate and any of the test compounds.[69,70] By measuring the initial rates of oxidation of **38** to **39** at concentrations of **38** that brackets its K_m value of 61 μmol/L[68] in the presence of varying concentrations of the test inhibitor, it is possible to determine the K_i value for the inhibitor. In all cases, the plots of 1/V vs 1/[S] and of the calculated slopes of these plots against [I] were found to be linear.

The K_i values for inhibition of MAO B by the various test compounds are summarized in tables 1 through 3. Caffeine (not shown) was found to be a weak inhibitor of MAO B, with a K_i value of 6 mmol/L. Conversely, all of the 8-substituted xanthinyl analogs proved to be moderate to potent competitive inhibitors of MAO B. The 7-methyl analogs (**10** to **17**; see table 1) were more potent than the corresponding 7-H analogs (**18** to **25**; see table 2). As expected, CSC (**10**) was an exceptionally potent inhibitor of baboon liver mitochondrial MAO B, with a K_i value of 70 nmol/L. KW–6002 (**11**), which also has been shown to protect against MPTP neurotoxicity in the C57BL/6 mouse[30,31] and 6-hydroxydopamine (6-OHDA) toxicity in the rat,[30] was found to be a moderate MAO B inhibitor, with a K_i value of 28 μmol/L. A comparable K_i value (17 μmol/L) was obtained using a C57BL/6 mouse brain mitochondrial assay and MPTP (**5**) as substrate.[67] The extent to which MAO B inhibition may contribute to the neuroprotective properties of KW–6002 is not clear at this time. Observations by Ikeda et al.[30] argue against such a role because neuroprotective doses of KW–6002 do not alter MPP^+ levels in the striatum when measured 1 to 6 hours after MPTP administration. This finding does not exclude inhibition of MAO as a possible contributor to the neuroprotective mechanism because, as discussed earlier, the neuroprotective properties of the potent MAO B mechanism-based inactivator (*R*)-deprenyl in the MPTP mouse model also appear to involve pathways that are independent of the inhibition of MPP^+ formation.[42-44]

Two other compounds in this series (the 3-nitrostyryl [**12**] and 3-fluorostyryl [**13**] analogs) also proved to be potent inhibitors of MAO B, with K_i values in the nanomolar range (see table 1). Like CSC, these compounds are (*E*)-8-styrylcaffeinyl analogs bearing an electronegative group at the C-3 position of the styryl ring. However, other structural modifications of CSC led to decreased MAO B inhibitory activity. Although all of the (*E*)-2-styrylbenzimidazolyl derivatives (see table 3) exhibited MAO B inhibitory activity, they were considerably less potent than the corresponding xanthinyl derivatives. As with the xanthinyl analogs, the corresponding 1-methyl analogs **31** to **33** consistently were more potent than the 1-H analogs (**28** to **30**). The CSC analog (*E*)-1-methyl-2-(3-chlorostyryl)benzimidazole (**32**) proved to be the most potent inhibitor, with a K_i value of 1.4 μmol/L. Based on these results, we conclude that the xanthinyl moiety is a better system for the development of exceptionally potent MAO B inhibitors. Furthermore, it appears that an electron-withdrawing substituent on the styryl moiety of these series of compounds enhances MAO B-inhibiting activity, suggesting that an electronic effect may contribute to inhibitory activity. The 2-phenylethyl analogs **26** (K_i = 183 μmol/L) and **27** (K_i = 36 μmol/L) were considerably less potent inhibitors of MAO B than the corresponding xanthinyl analogs and benzimidazolyl analogs. Consequently, a planar arrangement of the arenyl groups is probably important for activity. Finally, the photochemically produced *cis*-isomer **34** of KW–6002 was devoid of inhibitory activity.

Conclusions. Primate and rodent data generated in models of parkinsonian motor dysfunction suggest that A_{2A} antagonists may significantly relieve motor disabilities present in PD.[58] The therapeutic potential of compounds such as CSC and KW–6002 may be enhanced according to recent findings that A_{2A} receptor inactivation also confers protection to dopaminergic neurons in models of the neurodegeneration underlying PD (as reviewed elsewhere in this supplement). Our discovery that a subset of A_{2A} antagonists includes compounds with potent and A_{2A} receptor-independent MAO B inhibitor properties raises the possibility that a single structure may offer the combined benefits of two pharmacologic strategies, each with symptomatic and potential neuroprotective benefits for PD. A_{2A} receptor antagonism is currently under investigation in clinical trials designed to test for symptomatic benefit in PD, whereas MAO B inhibition, whose symptomatic benefits have been confirmed recently,[71] is now being assessed for its ability to slow disease progression in PD. Future studies should also take advantage of this apparent molecular coincidence to improve our fundamental understanding of MAO and adenosine receptor biology.

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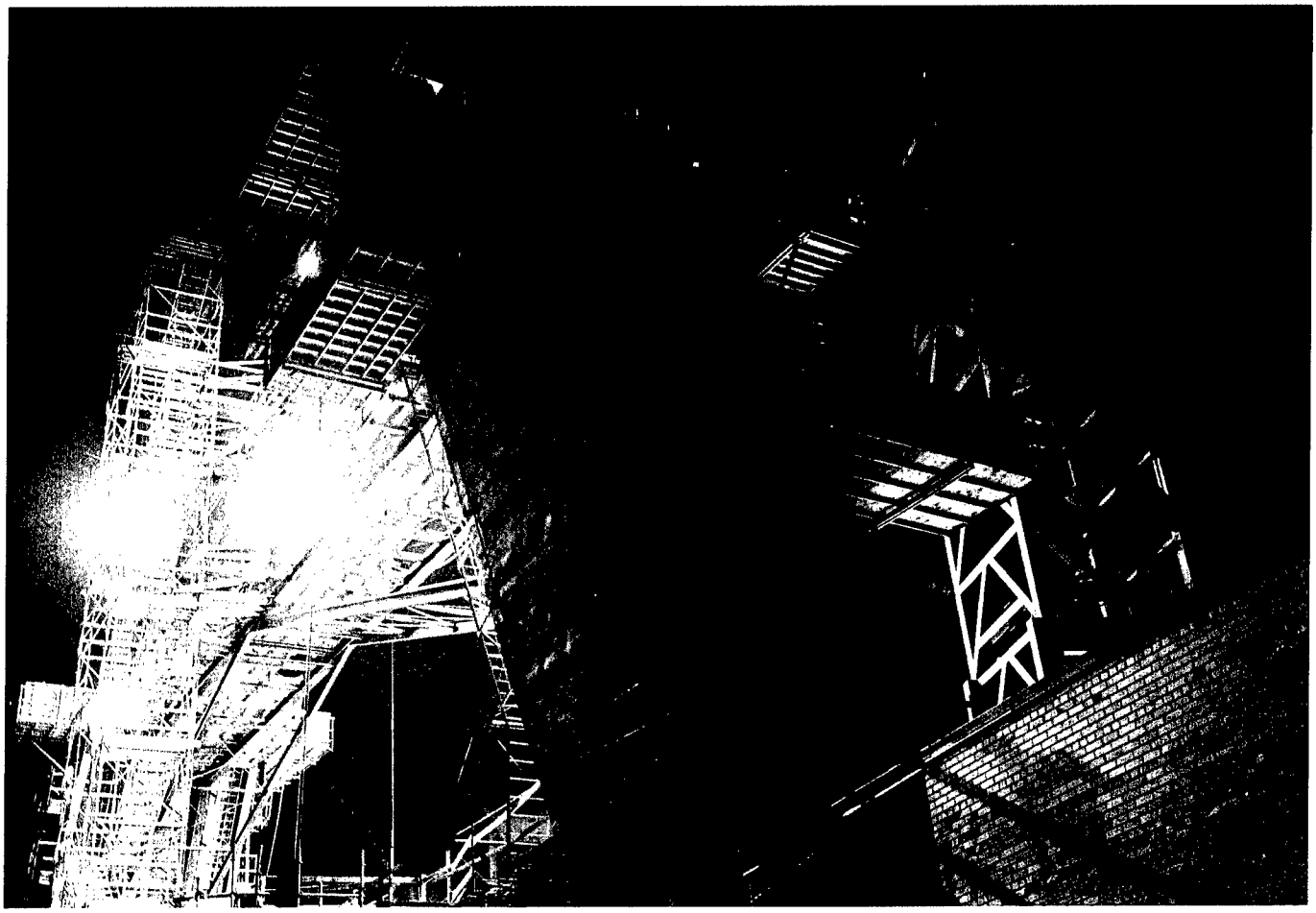

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Modulation of glutamate release and excitotoxicity by adenosine A_{2A} receptors

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Abstract—Because an increased glutamate outflow is thought to play a crucial role in triggering excitotoxic neuronal death, drugs able to regulate glutamate release could be effective for the management of neurodegenerative diseases. In this article, the authors discuss the hypothesis that adenosine A_{2A} receptor antagonists (A_{2A} antagonists) may belong to the aforementioned category. In rats bilaterally lesioned with the excitotoxin quinolinic acid (QA) in the striatum, the A_{2A} antagonist SCH 58261 significantly reduced the motor, EEG, and neuropathologic changes induced by the lesion. Such effects of SCH 58261 occurred only at low doses and were paralleled by an inhibition of QA-stimulated glutamate release. The role played by A_{2A} antagonists in the regulation of glutamate outflow was also confirmed by preliminary results obtained in the model of paired-pulse stimulation in corticostriatal slices. Conversely, based on data obtained in cultured striatal neurons, A_{2A} antagonists appear unable to directly inhibit NMDA effects. In conclusion, A_{2A} antagonists show clear neuroprotective effects in models of brain injury, although their actual therapeutic potential needs to be confirmed in a wider range of doses and in models of neurodegenerative diseases in which presynaptic and postsynaptic effects play different relative roles.

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Excitotoxicity is a common pathogenetic mechanism in neurodegenerative diseases either as a primum movens or a second-line partner triggered by other mechanisms.[1] An abnormal glutamate outflow is thought to play a crucial role in triggering the cellular events leading to excitotoxic neuronal death.[2,3]

Adenosine is an endogenous modulator that regulates many CNS functions. Its effects are mediated by four G protein-coupled receptors (A_1, A_{2A}, A_{2B}, and A_3).[4] A_{2A} receptors (A_{2A}Rs) positively modulate striatal glutamate outflow,[5,6] suggesting that their blockade could represent a suitable approach to the management of neurodegenerative diseases. In agreement with this hypothesis, mice lacking A_{2A}Rs were shown to be more resistant to ischemia- and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced neuronal damage,[7,8] whereas the pharmacologic blockade of A_{2A}Rs prevented the effects elicited by the excitotoxin quinolinic acid (QA) in the rat striatum.[9] Particularly in the latter model, the selective A_{2A} antagonist SCH 58261 (0.01 but not 1 mg/kg intraperitoneally) significantly reduced the effects of QA in terms of exaggerated motor activity (increased motor response to d-amphetamine), EEG changes (reduction in voltage amplitude and altered distribution in the relative EEG power), striatal gliosis, and lesion size (figure 1).

Modulation of excitotoxic injury by A_{2A}Rs

Presynaptic effects. Because QA acts by increasing glutamate outflow and directly stimulating NMDA receptors (NMDARs),[10] in a second set of experiments we aimed to determine whether the neuroprotective effects of SCH 58261 were actually mediated by a presynaptic mechanism (i.e., inhibition of QA-induced glutamate release). In microdialysis experiments in naive rats, striatal perfusion with QA (5 mmol/L through the dialysis probe) enhanced glutamate levels by approximately 500%. SCH 58261, when administered intraperitoneally at the dose of 0.01 mg/kg 20 minutes before QA perfusion, almost completely antagonized QA-stimulated glutamate outflow (figure 2). This is the same dose and administration paradigm of SCH 58261 that was effective in protecting QA-lesioned animals, confirming that the reduction of QA-stimulated glutamate outflow plays a major role in the effects of the drug. Although it has been observed that an increase in extracellular glutamate levels may not be a good index of excitotoxicity,[11] a contribution of increased glutamate outflow in inducing excitotoxic neuronal death is well established. An inhibition of evoked glutamate release has been reported to parallel the neuroprotective effects of some compounds.[9] Therefore, the modulation of glutamate outflow may explain the neuroprotective effects of SCH 58261, although alternative mechanisms (e.g., a possible regulation of excessive microglial cell activation)[12] should also be considered (see Schwarzschild et al., page S55).

To obtain further evidence for the ability of A_{2A} antagonists to modulate neurotransmitter release, experiments were performed using the model of paired-pulse stimulation (PPS) of the electrical response in rat corticostriatal slices. Paired-pulse modification of neurotransmission is attributed to

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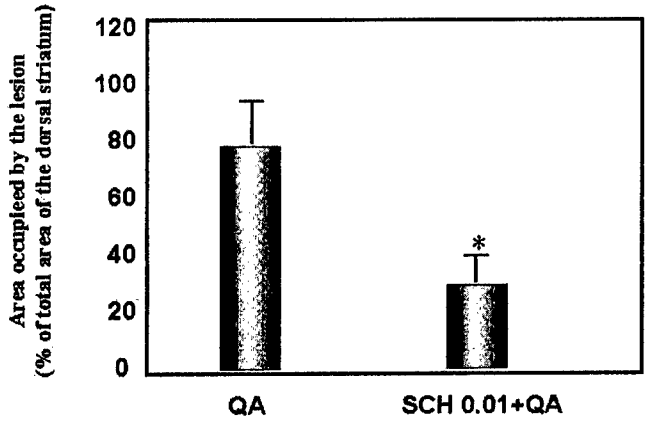

*Figure 1. SCH 58261 exerts neuroprotective effects on quinolinic acid (QA)-induced striatal lesions. Two groups of rats were treated with either SCH 58261 (0.01 mg/kg, intraperitoneally) or vehicle 20 minutes before intrastriatal infusion of QA (300 nmol/1 μL). Four weeks after the lesion, the brains were removed, and serial 20-μm coronal sections were stained with cresyl violet and examined by light microscopy. Images were captured by a color digital camera and analyzed using the Optilab software (Graftek, Mirmande, France). For each animal, the lesion area was measured on the slides showing the largest lesion extension and expressed as a percentage of the total area of the dorsal striatum. *p < 0.05 according to the nonparametric Mann–Whitney U test (see Popoli et al., 2002).*

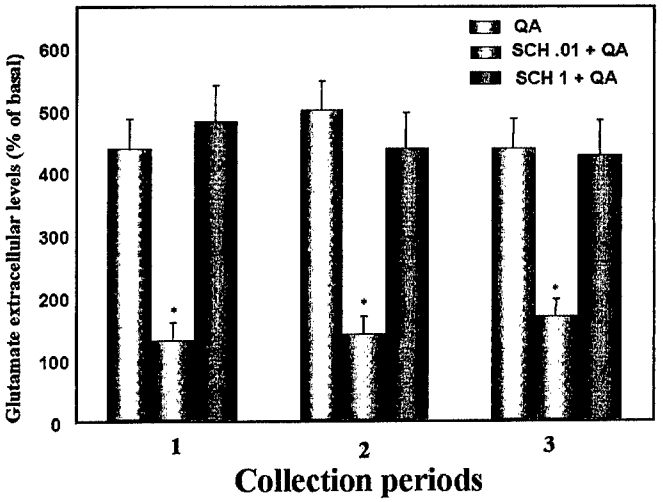

*Figure 2. An inhibition of quinolinic acid (QA)-induced glutamate outflow parallels the neuroprotective effects of SCH 58261. In animals implanted with a concentric dialysis probe in the striatum, the perfusion with QA (5 mmol/L over 30 minutes) induced a marked increase in extracellular glutamate levels, with peak response (from 15 to 30 minutes of the perfusion period) shown here. Such an increase was prevented by SCH 58261 (0.01 mg/kg, intraperitoneally, 20 minutes before QA perfusion). Perfusion rate was 2 μL/min. Samples were collected every 5 minutes (see Popoli et al., 2002). *p < 0.05 vs QA.*

presynaptic changes in release probability.[13] Specifically, manipulations increasing and decreasing neurotransmitter release usually decrease and increase, respectively, the ratio of the second pulse response to the first pulse response (R2/R1). In this model, 100 μmol/L 4-aminopyridine (4-AP), a K^+ channel blocker that increases presynaptic neurotransmitter release, significantly reduces R2/R1 with respect to basal conditions (0.68 ± 0.02 vs 1.30 ± 0.08; $p < 0.05$ according to Student's *t*-test). The coapplication of ZM241385 (100 nmol/L) reduced the effects of 4-AP by 40% ($p < 0.05$ vs 4-AP according to Student's *t*-test). ZM241385 on its own did not influence the R2/R1 ratio. Because in corticostriatal slices the occurrence of paired-pulse facilitation (i.e., the increase in the second vs the first pulse response normally observed in response to a PPS protocol) depends on the integrity of cortical projections,[14] the effects of 4-AP (which exerted a facilitatory influence on neurotransmitter release) and ZM241385 (which inhibited the proreleasing influence of 4-AP) can be ascribed to a modulation of glutamate release. Therefore, these results confirm, in a different model, that adenosine A_{2A} antagonists inhibit glutamate release. The finding that ZM241385 does not affect PPS by itself agrees with the results of previous microdialysis studies showing that SCH 58261 did not influence basal extracellular glutamate levels.[9,15] This suggests that the A_{2A} antagonist-mediated inhibition of glutamate release mainly occurs when glutamate outflow is somehow stimulated, which may represent a great advantage for the clinical application of A_{2A} antagonists.

Postsynaptic effects. To determine whether a postsynaptic component was involved in the neuroprotective effects of SCH 58261 toward QA, experiments were performed on primary striatal cultures. In this preparation, we have found that bath application of QA (900 μmol/L) significantly increased intracellular calcium levels, an effect prevented by the NMDAR antagonist MK-801.[9] Unexpectedly, bath application of SCH 58261 (15 to 200 nmol/L) tended to potentiate QA-induced calcium increase. Therefore, SCH 58261 does not act by reducing this postsynaptic (i.e., NMDAR agonistic) effect of QA. To further explore the apparent inability of A_{2A} antagonists to attenuate postsynaptic NMDAR-dependent effects, additional experiments were performed on striatal neurons. In these experiments, the ability of a different A_{2A} antagonist, ZM241385, to influence NMDA-induced increases in intracellular calcium levels and neuronal cell injury (as measured by lactate dehydrogenase [LDH] release) was tested.

Striatal cells from 17-day-old rat embryos were dissociated, plated, and treated as previously described.[9] Experiments were started 13 to 15 days after plating. Optical fluorimetric recordings with fura 2-acetoxymethyl ester (fura-2AM) were used to evaluate the intracellular calcium concentration ($[Ca^{2+}]_i$). Fura-2AM solution was applied to cells over 60 minutes and then replaced by extracellular solution. The dishes were quickly placed on the microscope stage, and fluorescence changes were measured by a computerized analysis system recording every 6 seconds the ratio between the values of light

intensity at 340- and 380-nm stimulation. NMDA (100 μmol/L) and ZM241385 (10, 50, and 100 nmol/L) were applied directly in the bath. To assess neuronal cell injury, the amount of LDH released from damaged cells in the culture medium was measured by spectrophotometric assay using a cytotoxicity detection kit. By incubating the cultures (striatal neurons obtained as above and plated onto 24-well plates) with 300 μmol/L NMDA over 60 minutes, excitotoxicity was induced, and LDH release was assessed 24 hours thereafter. ZM241385 (10 to 100 nmol/L) was added to the cultures 15 minutes before NMDA.

Bath application of NMDA (100 μmol/L) to cultured striatal neurons significantly increased $[Ca^{2+}]_i$ with respect to basal conditions, an effect that was not influenced by 10 to 100 nmol/L ZM241385. The application of 300 μmol/L NMDA to striatal neurons induced a marked release of LDH (approximately 100% over basal levels). Again, such an effect of NMDA was not significantly affected by 10 to 100 nmol/L ZM241385 (unpublished observations). These results suggest that no direct NMDAR-blocking effects are involved in the neuroprotective effects of A_{2A}R antagonists.

A_{2A}R blockade and neuroprotection. As mentioned previously, although A_{2A} antagonists seem unable to directly inhibit NMDA-dependent effects, this does not represent a true limitation in models of excitotoxicity characterized by a prominent presynaptic component, as in the case of QA toxicity in vivo. QA-induced neuronal death in the rat striatum was almost abolished by the removal of corticostriatal projections.[16] Of course, the real neuroprotective potential of A_{2A} antagonists may be tempered by, or perhaps even replaced by, detrimental postsynaptic effects in neurotoxicity models sustained mainly by direct NMDAR activation.

Another point deserving further investigation is that, at least in the model of striatal lesion by QA, the neuroprotective effects of SCH 58261 only occurred at very low doses. Although unexpected, this finding was in line with the results of previous studies showing the protective effects of very low to low doses of SCH 58261 in models of brain ischemia.[17,18] The most obvious explanation for the aforementioned finding would be that higher doses of SCH 58261 also block adenosine receptors other than A_{2A} (e.g., adenosine A_1 receptors, whose blockade would be detrimental in models of excitotoxicity).[19] Alternatively, the occurrence of peripheral effects after the administration of the higher dose of SCH 58261 can be invoked. Therefore, low doses of SCH 58261 may have protective effects in excitotoxic processes by the inhibition of adenosine A_{2A}R-stimulated glutamate release, whereas higher doses could also block adenosine A_{2A}R-mediated effects on blood pressure,[20] eventually reducing blood flow (and then nutrient supply) to the compromised brain area and further stimulating glutamate release. Finally, because an inflammatory reaction accompanies and contributes to brain injury[21] and because A_{2A}R activation has been reported to reduce proinflammatory events in the brain,[22] it is conceivable that higher doses of A_{2A}R antagonists may neutralize the potentially beneficial effects achieved through the stimulation of A_{2A}Rs by endogenous adenosine.

In conclusion, A_{2A}R antagonists seem to be promising neuroprotective drugs, although their true therapeutic potential needs to be confirmed in a wider range of doses and in models of neurodegenerative diseases in which presynaptic and postsynaptic effects play different relative roles.

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IV. Adenosine A_{2A} receptors in nonlocomotor features of Parkinson's disease

Introduction

Ennio Ongini, PhD

Symptoms beyond motor abnormalities. Many symptoms of Parkinson's disease (PD) are the consequence of motor disability. However, there are clinical features that appear to be independent of motor impairment. For example, depression is a common problem, and patients tend to become passive and show little interest in daily activities. Sleep difficulties are also common in patients with PD, and sleep disruption appears to be related to disease progression. Other symptoms, such as some degree of orthostatic hypotension or constipation, may depend on autonomic dysfunction. Most patients receive treatment with drugs that improve symptoms but also produce side effects; therefore, it is difficult to separate problems associated with the progression of the disease from those caused by medication. For example, when used long term, the most widely used drug therapy, L-dopa, leads to a variety of severe complications, including dyskinesias, "on-off" fluctuations in drug effectiveness, and a number of psychiatric manifestations. Moreover, L-dopa efficacy generally decreases as the disease progresses.

The ideal new drug would arrest or even reverse the progression of the disease without the complications associated with existing medications. With this aim in mind, it is crucial to assess the potential of the emerging A_{2A} receptor blockers with respect to all features of PD, not just those related to the impairment of the motor system. To achieve this, it is important to understand the role of the A_{2A} receptors in neuronal systems beyond those involved in the control of motor function.

A_{2A} receptors mediate many effects in the CNS. The rich distribution of A_{2A} receptors in close association with dopaminergic pathways in the striatum[1] underlies the well-documented effects of A_{2A} receptor antagonists in a variety of motor-impairment models relevant to PD. Modulation by A_{2A} receptors of dopamine D_2 and possibly D_1 receptors in striatal pathways may also underlie L-dopa–induced dyskinesia, as suggested by the observation that dyskinesia induced by L-dopa is attenuated in $A_{2A}^{-/-}$ animals[2] (see also Chen et al., page S74). This indicates that the presence of A_{2A} receptors is required for L-dopa–induced sensitization. Similarly, A_{2A} receptors also play a role in amphetamine-induced behavioral sensitization. These data suggest that the combination of A_{2A} antagonist and L-dopa is less likely to lead to dyskinesia in patients receiving long-term treatment. Clearly, this adds to the potential advantages of A_{2A} receptor antagonists for management of PD.

There are other behavioral effects that appear to depend on such an interaction between A_{2A} and dopamine receptors in the striatum.[3] In laboratory animals, A_{2A} receptor stimulation leads to behavioral responses mimicking those produced by neuroleptic agents that act by blocking D_2 receptors. Therefore, it has been claimed that A_{2A} agonists have potential as antipsychotic agents, although other effects, such as reduction of blood pressure, need to be addressed before this perspective becomes persuasive. Note that A_{2A} antagonists do not produce significant behavioral changes related to psychotomimetic effects (Weiss et al., page S88).

Although to a lesser extent, A_{2A} receptors are also present in other brain areas[1] where they can modulate physiologic and behavioral events not related to locomotion. One important effect is on the sleep–wake continuum. Adenosine appears to be involved in the regulation of sleep states, and A_{2A} receptors have been shown to be involved.[4] Blockade of A_{2A} receptors leads to increased duration of wakefulness, an effect similar to that produced by caffeine.[5] In addition, $A_{2A}^{-/-}$ mice exhibit a reduction in duration of sleep phases in response to pharmacologic agents and sleep deprivation (Urade et al., page S94). It is not known how these data will impact the therapeutic profile in patients. Because altered sleep occurs in patients with PD, this feature of A_{2A} antagonists needs to be considered. This is particularly true if these agents will be used in combination with L-dopa, which is known to alter sleep states.

Also of relevance to the therapeutic perspective are data showing that blockade or genetic inactiva-

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tion of A_{2A} receptors results in behaviors predictive of antidepressant activity as measured in established experimental paradigms[6] (see El Yacoubi et al., page S82). These data suggest that A_{2A} receptor antagonists can benefit patients with PD by enhancing mood in addition to their motor effects. Although the mechanisms underlying this putative antidepressant effect remain largely unknown, initial evidence suggests that interaction with dopaminergic transmission is involved. Clearly, clinical studies are necessary to determine whether antiparkinsonian effects of A_{2A} antagonists are accompanied by some degree of antidepressant activity.

A_{2A} receptors in the periphery. Within the neuroscience community, attention is commonly directed toward receptors located in the brain only. However, adenosine mediates a wide range of physiologic functions in the periphery through interactions with its receptors located on a variety of cells. It is beyond the scope of this commentary to list all known functions of peripheral A_{2A} receptors; however, the following two adenosine-mediated effects are of particular importance to the clinical use of A_{2A} antagonists. First, A_{2A} receptors are involved in vasodilation. Stimulation of A_{2A} receptors produces vasodilation and a decrease in blood pressure.[7] Conversely, high doses of A_{2A} antagonists or genetic inactivation of A_{2A} receptors increases blood pressure.[8,9] Although these effects may not occur at doses effective in PD models, it is important to determine whether interactions occur with L-dopa, which tends to lower blood pressure. The second point regards the distribution of the A_{2A} receptors in blood elements.[10] A_{2A} receptors are present in platelets, lymphocytes, and neutrophils, where they are involved in mediating platelet aggregation and controlling inflammatory reactions. Although toxicologic studies on new drugs will clarify whether some parameters are altered during continuous drug administration, it is important to be aware of possible A_{2A}-mediated effects produced in the periphery.

Concluding remarks. Together, the results of studies focusing on nonlocomotor activities add interesting features to the known potential of A_{2A} receptor antagonists for treatment of patients with PD. Their ability to reduce dyskinesia via the reduction of L-dopa–induced sensitization is of key relevance to the clinical perspective. Likewise, antidepressant properties and lack of psychotomimetic action are certainly desirable additional characteristics for a new antiparkinsonian agent. Effects on sleep should receive attention to define the overall profile of the new A_{2A} antagonists.

Still to be defined by further studies are the mechanisms at the cellular and neuronal network level, beyond those likely to occur through interactions with dopaminergic pathways in the striatum. Moreover, peripheral effects need to be studied further in view of the continuous blockade of the A_{2A} receptors expected with long-term therapy in patients with PD. Finally, all of us eagerly await the outcome of clinical studies.

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Adenosine A_{2A} receptors in neuroadaptation to repeated dopaminergic stimulation

Implications for the treatment of dyskinesias in Parkinson's disease

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Abstract—The A_{2A} receptor has recently attracted considerable interest as a potential target for Parkinson's disease (PD) therapy based on the motor-enhancing and neuroprotective effects of A_{2A} antagonists in animal models of PD. The unique neuronal localization of the adenosine A_{2A} receptor in the basal ganglia and its extensive interactions with dopaminergic and glutamatergic systems led the authors to investigate a potential role of the A_{2A} receptor in the development of behavioral sensitization in response to repeated dopaminergic stimulation. Because dopamine-induced behavioral sensitization shares several neurochemical and behavioral features with dyskinesia, characterizing this novel aspect of A_{2A} receptor function may enhance understanding and management of dyskinesia in PD. Recent studies from several laboratories suggest that the A_{2A} receptor may be an important mediator of maladaptive changes in response to long-term dopamine stimulation. The authors summarize their investigation of the role of A_{2A} receptors in two paradigms of behavioral sensitization elicited by daily treatment with either L-dopa in hemiparkinsonian mice or amphetamine in naïve mice. The results demonstrate that the A_{2A} receptor is required for the development of behavioral sensitization in response to repeated L-dopa treatment in hemiparkinsonian mice and repeated amphetamine administration in normal mice. Together with pharmacologic studies, these results raise the possibility that the maladaptive dyskinetic responses to long-term L-dopa management of PD may be attenuated by A_{2A} receptor blockade. Potential presynaptic, postsynaptic (cellular), and trans-synaptic (network) mechanisms are discussed.

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Debilitating complications associated with long-term L-dopa management of PD prompt a search for alternative therapeutic strategies. Patients with Parkinson's disease (PD) are profoundly depleted of striatal dopamine as a result of degeneration of the nigrostriatal dopaminergic pathway.[1-3] More than 30 years after its introduction as a dopamine-replacement strategy for PD management, L-dopa remains the most effective and most commonly prescribed therapy.[2,3] Despite its deserved title as the "gold standard" against which all other antiparkinsonian drugs are judged, long-term L-dopa therapy is limited by major motor complications. Long-term L-dopa treatment of patients with PD can lead to the development of debilitating abnormal motor responses,[4-6] most commonly consisting of involuntary dystonic and chronic movements termed dyskinesia. In fact, 5 to 10 years after beginning L-dopa treatment, 60 to 80% of patients have developed some form of dyskinesia.[6-8] Animal model and clinical studies suggest that a profound loss of dopaminergic neurons and long-term treatment with L-dopa are necessary for the development of dyskinesia.[9-11] Such shortcomings of L-dopa and related dopaminergic drugs have prompted a search for alternative treatment strategies that provide symptomatic benefits while avoiding the late motor complications associated with the long-term use of L-dopa.

Adenosine A_{2A} receptors emerge as an attractive target for PD therapy. Extensive neuroanatomic, neurochemical, and behavioral studies have led to the development of A_{2A} receptor antagonists for symptomatic relief from the motor deficits of PD. First, the unique, selective distribution of A_{2A} receptors in striatum and the colocalization of A_{2A} receptors with D_2 receptors provide an anatomic basis for

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selective modulation of the "indirect" striatopallidal pathway and thus a major dopaminergic motor pathway (see Rosin et al., page S12). Second, behavioral studies have demonstrated that the nonspecific adenosine antagonists caffeine and theophylline[12-14] and A_{2A}-specific antagonists CSC,[12-16] KW6002,[17-20] and SCH 58261[14,21-23] enhance motor activity in rodent and nonhuman primate models of PD. Third, neurochemical studies have demonstrated that activation of the A_{2A} receptor reduces the binding affinity of D_2 receptors in the striatum and antagonizes the effects of striatal D_2 receptors on acetylcholine and GABA release and on immediate early gene expression.[14,15,24-29] This antagonistic interaction between A_{2A} adenosine and dopamine receptors may be mediated by a direct A_{2A}-D_2 receptor–receptor interaction within the membrane and by an opposing, independent functional antagonism at the levels of postreceptor signaling pathways and neural networks.[29,30] Fourth, acting through the cyclic adenosine monophosphate (cAMP) pathway, A_{2A} receptors can modulate the conductivity of NMDA receptors[31] and affect subunit phosphorylation of NMDA and α-amino-3-hydroxy-5-methyl-4-isoxazole proprionic acid (AMPA) receptors, an important neurochemical change associated with L-dopa–induced dyskinesia (see Chase et al., page S107). Finally, activation of the A_{2A} receptor has been shown to enhance the release of several neurotransmitters in the brain, including dopamine and glutamate,[27,32,33] critical processes involved in dopamine-elicited behavioral sensitization. Together, these features highlight the potential of targeting the A_{2A} receptor in the search for effective new management of PD symptoms and the dyskinesias that complicate traditional PD therapy. The prospects for benefit in humans are further supported by evidence that the A_{2A} receptor remains fully expressed in the basal ganglia of patients with PD.[34]

Genetic inactivation of A_{2A} receptor attenuates L-dopa–induced behavioral sensitization in hemiparkinsonian mice. In 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-lesioned parkinsonian primates, long-term exposure to therapeutic doses of L-dopa reliably elicits purposeless choreic movements, providing an excellent model of L-dopa–induced dyskinesia in PD. In contrast to repeated L-dopa treatment, repeated treatment of MPTP-lesioned monkeys with the A_{2A} antagonist KW6002 consistently corrects their motor deficits without inducing dyskinesias (see Kase et al., page S97; Jenner et al., page S32). These studies have distinguished A_{2A} antagonists from antiparkinsonian agents that directly stimulate dopaminergic transmission. For example, an A_{2A} antagonist has shown minimal potential to induce dyskinesia by itself in L-dopa–naïve and L-dopa–primed parkinsonian monkeys. However, whether A_{2A} receptors can influence the development of sensitized motor responses to repeated L-dopa administration in parkinsonian animals is entirely unknown.

To critically evaluate the involvement of A_{2A} receptors in the maladaptive neuroplasticity underlying L-dopa–induced dyskinesia, we have adapted a rodent "priming" model in which delayed but progressive rotational behavioral sensitization is induced by repeated treatment with L-dopa in unilaterally 6-hydroxydopamine (6-OHDA)–lesioned mice.[35-38] The rotational behavioral sensitization induced by repeated L-dopa treatment in rodents shares several neurochemical features with L-dopa–induced dyskinesia in the MPTP model in primates:[35,36,38,39] 1) dyskinesia and rotational behavioral sensitization are induced only after the animals have been primed with L-dopa; 2) drugs that do not induce dyskinesia in MPTP-treated primates (such as the D_2 agonist bromocriptine) also do not produce rotational behavioral sensitization in rodents; and 3) in the rodent and primate models of PD, similar patterns of altered neuropeptide expression have been observed after long-term L-dopa treatment. Therefore, this rotational behavioral sensitization model in rodents may help to evaluate how the A_{2A} receptor may influence development of L-dopa–induced behavioral sensitization and may help elucidate the neuronal mechanisms underlying L-dopa–induced dyskinesia.

After unilateral intrastriatal injection of 6-OHDA, wild-type and A_{2A} receptor knockout mice were treated daily with low doses of L-dopa (1.8 mg/kg, intraperitoneally) for 3 weeks. The administration of L-dopa produced delayed but progressive contralateral rotational responses (figure 1) and sensitization of grooming[40] behaviors in wild-type mice. In contrast, A_{2A} receptor knockout mice failed to develop statistically significant behavioral sensitization at this dosage (see figure 1). Most strikingly, any enhancement of rotational response to L-dopa in A_{2A} receptor knockout mice returned to baseline (i.e., that on day 1), where it remained for the rest of the experiment.

Because development of L-dopa–induced behavioral sensitization is associated with a progressively shortened onset and duration of action,[41,42] we also compared how the kinetics of L-dopa–induced responses changed during the 3-week course of daily treatments in wild-type and A_{2A} receptor knockout mice. The time to reach the peak contralateral turning response to a single dose of L-dopa (1.8 mg/kg) was progressively shortened in wild-type mice (from 20 to 30 minutes on day 1, to 20 minutes on day 11, to 10 minutes on day 20). In contrast, the time to reach the peak response in A_{2A} receptor knockout animals remained virtually unchanged throughout the treatment (~20 minutes for days 1, 11, and 20).[40] Parallel with attenuated behavioral sensitization in A_{2A} receptor knockout mice, repeated L-dopa treatment reversed the 6-OHDA–induced reduction of striatal dynorphin mRNA in wild-type but not in A_{2A} receptor knockout mice (figure 1B), raising the possibility that the A_{2A} receptor may contribute to L-dopa–induced behavioral sensitization by facilitating

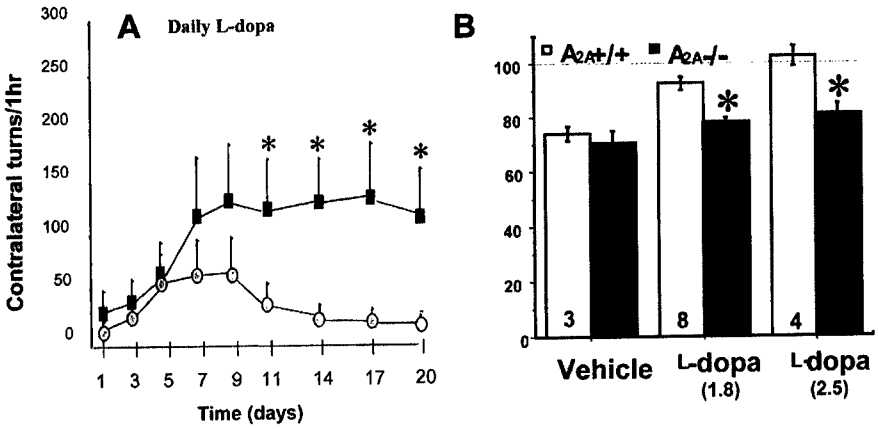

*Figure 1. A_{2A} receptor deficiency attenuates L-dopa–induced behavioral sensitization and striatal dynorphin expression. (A) Wild-type (filled circles) and A_{2A} receptor knockout (unfilled circles) mice were treated with benserazide (2 mg/kg, intraperitoneally) plus L-dopa (1.8 mg/kg, intraperitoneally) once a day for 20 days. Contralateral rotational behavior was evaluated for the 1-hour test period immediately after the administration of L-dopa on the indicated days. Data are expressed as mean ± SEM of the net contralateral rotations (contralateral − ipsilateral turns). *p < 0.01; F(2,22) = 2.92; split-plot analysis of variance followed by Fisher's LSD comparison test compared with the corresponding knockout value; n = 10 wild-type mice and n = 12 knockout mice. (B) The dynorphin mRNA levels were determined by in situ hybridization histochemistry in mice unilaterally lesioned with 6-hydroxydopamine (6-OHDA), followed by daily treatment with L-dopa (1.8 or 2.5 mg/kg) or vehicle for 21 days. Dynorphin mRNA levels (OD) were quantified at the level of midstriatum and expressed as a percentage of the contralateral side (unlesioned striatum). *p < 0.05; Student's t-test compared with the corresponding wild-type group. The numbers inside the bars indicate the animal numbers for each group. Reprinted from Fredduzzi et al[40] with permission. (Copyright 2002 by the Society for Neuroscience).*

adaptations within the dynorphin-expressing striatonigral pathway.

Therefore, the absence of long-term L-dopa–induced sensitization at the behavioral (contralateral rotation and grooming) and cellular (dynorphin mRNA) levels in hemiparkinsonian mice lacking A_{2A} receptors strongly suggests that A_{2A} receptors are required for L-dopa–induced behavioral sensitization. Furthermore, it raises the possibility that the maladaptive dyskinetic responses to long-term L-dopa management of PD may be attenuated by A_{2A} receptor blockade. In line with this notion, Pinna et al.[23] showed that coadministration of the A_{2A} antagonist SCH 58261 with L-dopa (3 mg/kg) stimulated motor activity that did not sensitize after repeated treatments, whereas repeated L-dopa on its own (at 6 mg/kg, a dose that produced the same acute motor response as the A_{2A} antagonist/L-dopa combination) led to clear behavioral sensitization.

More direct pharmacologic evidence has strengthened the possibility that A_{2A} antagonists may help prevent L-dopa–induced dyskinesia. In experiments conducted by Chase et al. (see page S107), coadministration of the A_{2A} antagonist KW6002 with L-dopa or with a dopaminergic agonist blocked the progressive shortening of motor response duration in hemiparkinsonian rats and prevented the development of dyskinesias themselves in parkinsonian monkeys. However, a recent study reported no effect of KW6002 paired with repeated L-dopa doses on the development of dyskinetic responses in hemiparkinsonian rats.[43] Another group similarly found no preventative effect of a different A_{2A} antagonist on the development of shortening motor responses to repeated L-dopa administration in hemiparkinsonian rats,[39] although they did observe a complete reversal of L-dopa response shortening when the antagonist was administered *after* altered L-dopa responses were established. Despite the aforementioned discrepancies, these studies generally support the notion that A_{2A} antagonists may limit or reverse the induction or expression of dyskinesias in PD.

Inactivation of A_{2A} receptors also attenuates behavioral sensitization induced by repeated treatment with amphetamine. The attenuation of L-dopa–induced rotational behavioral sensitization by A_{2A} receptor inactivation raises the question of whether the A_{2A} receptor is required for behavioral sensitization in response to a wide range of dopaminergic stimuli. Similar adaptive neuronal mechanisms (such as supersensitivity of the dopaminergic system) have been suggested to explain L-dopa–induced priming and amphetamine-induced behavioral sensitization,[10,11,45] leading us to propose that A_{2A} receptors may also play an important role in the development of amphetamine-induced behavioral sensitization. To test this hypothesis, we investigated how A_{2A} receptors influence behavioral sensitization induced by the psychostimulant amphetamine and by D_1 and D_2 receptor agonists in a locomotor sensitization paradigm.

Wild-type and A_{2A} receptor knockout mice were treated with amphetamine (2.5 mg/kg, intraperitoneally) daily for 8 days, and the locomotor response to amphetamine was monitored on days 1 and 8 (figure 2). Wild-type mice displayed significantly enhanced locomotor response to amphetamine on day 8 compared with their response to the same dose of amphetamine on day 1 (see figure 2). By contrast, A_{2A} receptor knockout mice did not show any enhancement of amphetamine-induced locomotion on day 8 compared with day 1 (see figure 2). In addition, the locomotor sensitization in wild-type mice and its absence in A_{2A} receptor knockout mice persisted for at least 2 weeks after discontinuation of daily amphetamine injec-

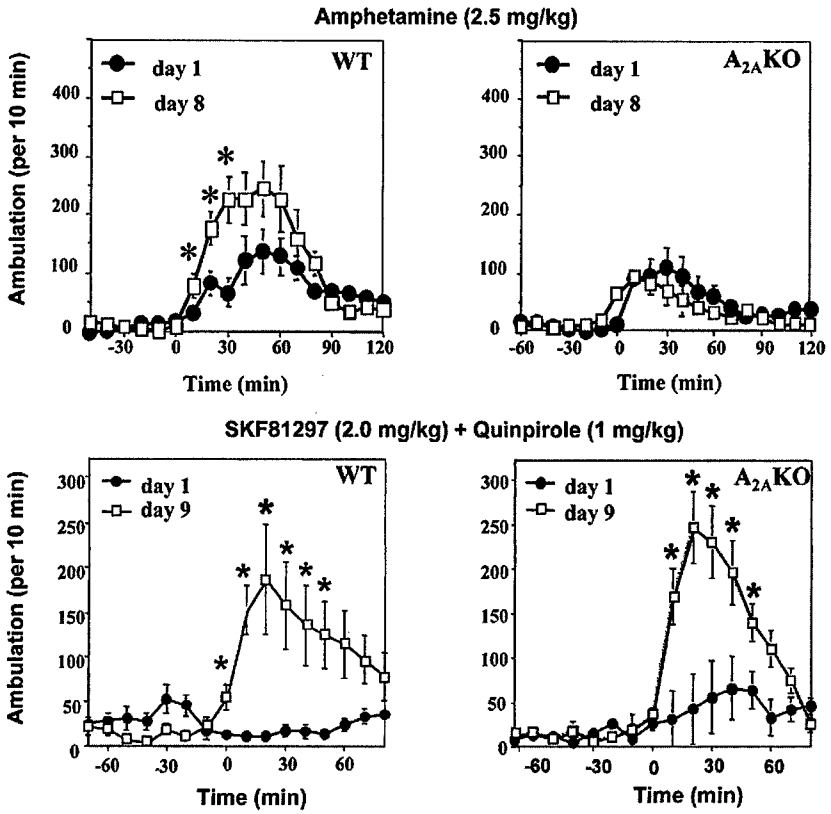

*Figure 2. Selective attenuation of amphetamine-induced behavioral sensitization in mice lacking A_{2A} receptors. Wild-type and A_{2A} receptor knockout mice were treated daily for 8 or 9 days with either amphetamine (2.5 mg/kg) (A) or coadministration of dopamine D_1 agonist SKF81297 (2.0 mg/kg, intraperitoneally) and D_2 agonist quinpirole (1.0 mg/kg, intraperitoneally) (B). Ambulation was recorded for 120 minutes after drug treatment on days 1 and 8. WT = wild-type; KO = A_{2A} receptor knockout. * $p < 0.05$ comparing day 8 with day 1 for wild-type or A_{2A} receptor knockout mice; three-way analysis of variance with repeated treatments for two factors, followed by Tukey's post-hoc test; n = 7 to 8 per group. Daily treatment of amphetamine produced locomotor sensitization in wild-type mice but not in A_{2A} receptor knockout mice. Coadministration of SKF81297 and quinpirole produced enhanced locomotor activity, but the locomotor sensitization was indistinguishable between wild-type and A_{2A} receptor knockout mice. Reprinted from Chen et al.[46] Reproduced with permission.*

tions.[46] Pharmacologic blockade of A_{2A} receptors also blunted the development of amphetamine-induced locomotor sensitization in mice (Bastia et al., unpublished data). Furthermore, the lack of amphetamine-induced locomotor sensitization in A_{2A} receptor knockout mice is the result of neither a nonspecific motor threshold effect nor delayed sensitization and is not attributable to sensitized stereotyped behavior of the type reflected in horizontal fine movements.[46] In correlation with blunted amphetamine-induced locomotor sensitization in A_{2A} receptor knockout mice, daily treatment with amphetamine induced an increase in dynorphin mRNA in wild-type but not in A_{2A} receptor knockout mice.[46]

Consistent with these findings, a withdrawal syndrome that develops after long-term repeated alcohol administration was attenuated in A_{2A} receptor knockout mice and in mice treated with the specific A_{2A} antagonist ZM241385.[47] However, a specific A_{2A} *agonist* has also been shown to attenuate methamphetamine-induced motor sensitization.[48] Although the exact role of the A_{2A} receptor in the development of dopamine-mediated behavioral sensitization remains to be clarified, these studies consistently point to an important role for this receptor in neuroadaptive changes underlying dopamine-mediated behavioral sensitization.

Attenuation of amphetamine-induced and L-dopa–induced (but not D_1 or D_2 agonist-induced) behavioral sensitization suggests a possible presynaptic modulation by A_{2A} receptors. The attenuation of behavioral sensitization in L-dopa–induced rotational sensitization and amphetamine-induced locomotor sensitization suggests that A_{2A} receptors may be required for a broad range of behavioral sensitization induced by repeated dopaminergic stimulation. To further address this issue, we tested the effect of A_{2A} receptor inactivation on locomotor sensitization by D_1 and D_2 receptor agonists. Daily treatment with the D_1 agonist SKF81297 produced identical behavioral sensitization in wild-type and A_{2A} receptor knockout mice.[46] Similarly, the repeated administration of the D_2 agonist quinpirole (albeit using a different treatment paradigm) elicited sensitized rotational behavior in wild-type and A_{2A} receptor knockout mice.[46] Furthermore, coinjection of SKF81297 and quinpirole also resulted in indistinguishable locomotor sensitization in A_{2A} receptor knockout and wild-type mice (see figure 2), suggesting normal D_1 and D_2 receptor responsiveness. These results demonstrated that although A_{2A} receptor inactivation selectively attenuates amphetamine-induced and L-dopa–induced behavioral sensitization, it does not necessarily result in a general, nonspecific loss of neuroadaptive changes to dopaminergic stimulation in the brain.

The apparent selectivity of the attenuation of behavioral sensitization for amphetamine and L-dopa (both of whose actions involve dopamine release at presynaptic terminals) led us to hypothesize that A_{2A} receptor inactivation may prevent behavioral sensitization by impairing a presynaptic mechanism. Early studies show that dopamine accumulation in striatal microdialysates[33,49,50] is generally increased by A_{2A} agonists and decreased by A_{2A} antagonists, suggesting a presynaptic mechanism through which A_{2A} receptor inactivation could prevent psychostimulant-

induced sensitization. In agreement, our preliminary study showed that depolarization-elicited dopamine release was significantly attenuated in striatal synaptosomes from A_{2A} receptor knockout mice.[44] This notion is supported by recent in vivo data demonstrating that basal dopamine efflux in striatum, measured by microdialysis, is significantly lower in A_{2A} receptor knockout mice compared with their wild-type littermates.[51]

In addition to dopamine, glutamate released from nerve terminals in the striatum, nucleus accumbens, and ventral mesencephalon has been strongly implicated in the different phases of sensitization.[52] A_{2A} receptors play a well-established excitatory role in the CNS[27] attributed to their facilitative effect on glutamate release, which has been demonstrated in striatum and cortex (see Popoli, page S69).[53,54] Therefore, alterations in glutamate release caused by A_{2A} receptor inactivation may contribute to the phenotype of attenuated L-dopa–induced and amphetamine-induced sensitization in A_{2A} receptor knockout mice. Moreover, tonic activation of D_1 receptors by psychostimulants has been shown to increase adenosine tone after metabolism of cAMP at the corticostriatal terminals,[55-58] which may facilitate glutamate release via presynaptic A_{2A} receptor stimulation.[27,53,59] We hypothesize that costimulation of dopamine (D_1) receptors and glutamate receptors after increased tone of adenosine in striatum is critical to the establishment of behavioral sensitization (figure 3). Consequently, inactivation of A_{2A} receptors could conceivably affect psychostimulant sensitization by attenuating the release of glutamate or dopamine and by disrupting their synergistic feed-forward loops.

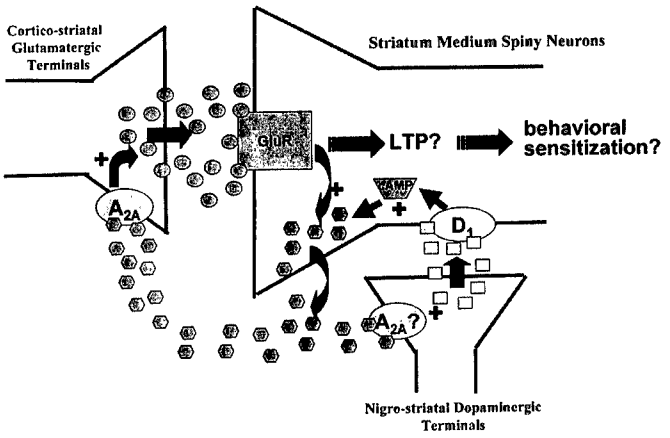

Figure 3. A hypothesis for how A_{2A} receptors facilitate behavioral sensitization—adenosine-dopamine-glutamate feed-forward interactions in long-term potentiation (LTP). A schematic diagram illustrates a possible interplay among adenosine, dopamine, and glutamate systems in the development of LTP and behavioral sensitization. Tonic activation of D_1 receptors by psychostimulants (amphetamine/cocaine) has been shown to increase adenosine tone by increasing metabolism of cyclic adenosine monophosphate (cAMP) at corticostriatal nerve terminals.[55-58] An increased level of extracellular adenosine, acting at A_{2A} receptors, can facilitate glutamate release from corticostriatal nerve terminals.[27,53,59] We hypothesize that the resulting enhanced costimulation of dopamine (D_1) receptors and glutamate receptors on striatal neurons may be critical to the development of LTP, which has been implicated in the behavioral sensitization of L-dopa–induced dyskinesias.[73] Hexagon = adenosine; square = dopamine; oval = glutamate.

Postsynaptic (cellular) and trans-synaptic (network) mechanisms may contribute to modulation of behavioral sensitization by A_{2A} receptors. *Postsynaptic (cellular) mechanisms.* In addition to the aforementioned mechanism, the A_{2A} receptor may modulate L-dopa–induced behavioral sensitization through its cellular-level interaction with the D_2 receptor or glutamate receptors in striatal neurons. Among striatal neurons' multiple intracellular signaling cascades, the cAMP pathway in particular has also been implicated in the basal ganglia plasticity that underlies behavioral sensitization to dopaminergic stimulation.[6,12,45,60,61] The A_{2A} receptor, which is positively coupled to adenylate cyclase and cAMP production through G_s,[29] may influence L-dopa–induced neurochemical and behavioral changes by affecting this pathway (see figure 3). In this regard, a recent study shows that D_2 receptor blockade induces phosphorylation of DARPP-32 protein in wild-type mice but not in A_{2A} receptor knockout mice, indicating a critical role for A_{2A} receptors in striatal cellular signaling involving the cAMP pathway.[62]

Moreover, cellular interaction of adenosine and glutamate receptors could underlie modulation of amphetamine-induced and L-dopa–induced behavioral sensitization by A_{2A} receptors. For example, activation of A_{2A} receptors can also modulate NMDA receptor conductance[31,63] and phosphorylation of NMDA receptors (see Chase et al., page S107). Because NMDA and non-NMDA glutamate receptor antagonists have been shown to interfere with the development of behavioral sensitization to amphetamine[52] and L-dopa,[64] inactivation of A_{2A} receptors could modulate behavioral sensitization by affecting NMDA receptors in striatal neurons. In addition, recent studies have implicated metabotropic glutamate receptor (mGluR) in regulating psychostimulant-induced neurochemical and behavioral effects.[65,66] The reinforcing and locomotor stimulant effect of cocaine was blunted in mice lacking mGluR5.[65] Because several studies have demonstrated a close interaction among A_{2A} receptors, D_2 receptors, and mGluR5 receptors in the basal ganglia,[67-71] A_{2A} receptors may modulate psychostimulant-induced sensitization through interaction with mGluR5 and dopamine receptors.

The molecular interactions among A_{2A} receptors, D_2 receptors, and glutamate receptors may alter cellular mechanisms associated with behavioral sensitization. Interestingly, D'Alcantara et al.[72] recently reported that genetic depletion or pharmacologic

blockade of A_{2A} receptors attenuates long-term potentiation (LTP) in the nucleus accumbens without affecting basal synaptic transmission. Therefore, inactivation of A_{2A} receptors may also modify psychostimulant-induced behavioral sensitization by impairing LTP and "striatal learning" processes.[73]

Trans-synaptic (network-level) mechanisms. Finally, complex interactions between the adenosine receptor and multiple neurotransmitter receptors at a network level need to be considered. Although A_{2A} receptors are almost exclusively colocalized with D_2 receptors in striatopallidal neurons, activation of the D_2 or A_{2A} receptors in striatal slices has been shown to trans-synaptically interact with D_1 receptors to modulate phosphorylation of DARPP-32, an effect that was blocked by tetrodotoxin.[74] These studies illustrate clearly a functional cross-talk between the indirect pathway (coexpressing A_{2A} and D_2 receptors) and the direct pathway (expressing D_1 receptors) at the network level. Understanding such cross-talk between A_{2A} receptors in the indirect pathway and D_1 receptors in the direct pathway may be critical because repeated L-dopa treatment leads to an imbalance between the outputs of the direct and indirect pathways in the basal ganglia. Specifically, L-dopa acts on the direct pathway to produce neurochemical changes underlying behavioral sensitization. Repeated L-dopa administration has been shown reproducibly to increase expression of mRNAs encoding the neuropeptide dynorphin in striatonigral neurons. Increases in dynorphin have been correlated with the development of L-dopa–induced behavioral sensitization,[75-79] indicating overactivity of the (D_1-expressing) direct pathway in L-dopa–sensitized animals. Our finding that long-term L-dopa treatment reverses the 6-OHDA–induced reduction in striatal dynorphin mRNA in wild-type mice but not in A_{2A} receptor knockout mice supports this notion. However, it should be noted that behavioral sensitization to direct D_1 and D_2 agonists (alone or in combination) appears similar in A_{2A} receptor knockout and wild-type littermates, suggesting that significant alterations in D_1 or D_2 receptor-mediated signaling pathways alone are unlikely to be the basis of attenuated sensitization in the A_{2A} receptor knockout mice. Further studies are needed to clarify the cellular mechanism by which the A_{2A} receptor modulates behavioral sensitization induced by amphetamine and L-dopa.

Another network level interaction may involve feedback projection loops from striatum to cortex and back to striatum via glutamatergic subthalamic and GABAergic substantia nigra neurons that indirectly link striatal A_{2A} receptors with excitatory thalamic and corticostriatal neurons. It is possible that this extensive network interaction may be subject to A_{2A} receptor modulation during the development of L-dopa–induced dyskinesia in PD.

Therapeutic implications for PD. The lack of persistent L-dopa–induced behavioral sensitization in A_{2A} receptor knockout mice has implications for the development of A_{2A} antagonists as a potential therapeutic intervention for PD. A_{2A} antagonists are being developed as novel therapeutic agents for PD management based primarily on their well-documented capacity to enhance motor function, and they are now entering clinical phase II trials for patients with PD (see Kase et al., page S97; Chase et al., page S107). The present findings suggest an additional potential benefit of A_{2A} receptor inactivation as early adjunctive therapy with L-dopa for management of PD. Persistent rotational sensitization, grooming sensitization, and increased striatal dynorphin mRNA levels induced by repeated L-dopa administration in hemiparkinsonian mice depend on the presence of the A_{2A} receptor; therefore, A_{2A} antagonists may attenuate the maladaptive dyskinetic responses to long-term L-dopa management of PD. In addition, we have recently demonstrated that A_{2A} antagonists attenuate dopaminergic neurotoxicity in the MPTP model of PD in mice, raising the possibility that A_{2A} antagonists may offer neuroprotective and symptomatic benefits in PD management[80] (see Schwarzschild et al., page S55). Because dopaminergic degeneration is critical for the development of dyskinesia, A_{2A} antagonist-mediated neuroprotection may also contribute to attenuation of L-dopa–induced dyskinesia by slowing dopaminergic degeneration. Together, these multiple potential therapeutic benefits of A_{2A} antagonists (motor enhancement, neuroprotection against dopaminergic toxicity, and prevention of dyskinesia) should greatly encourage their development as a promising treatment for patients with PD.

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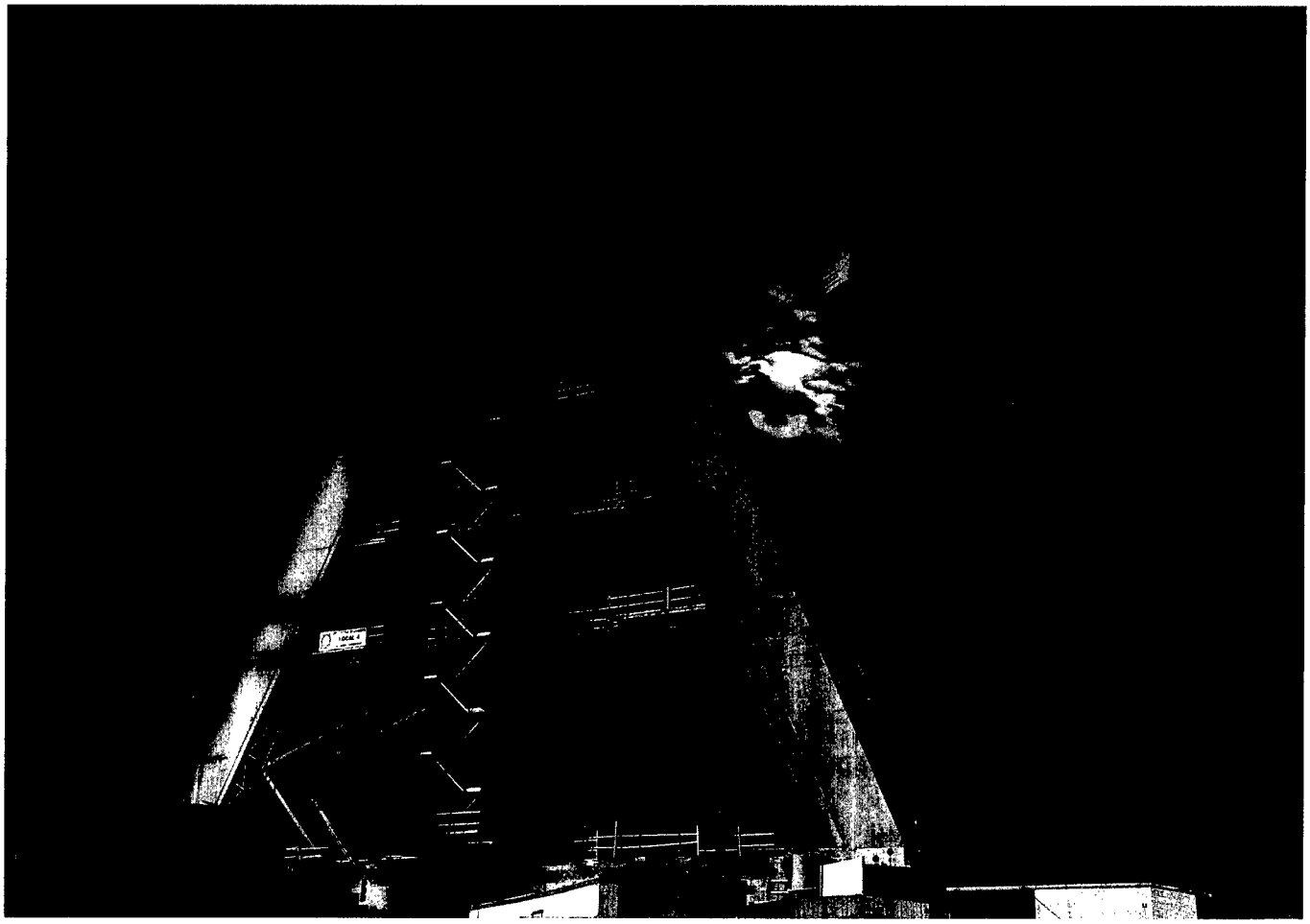

Don Eyles **August 1999**

Adenosine A_{2A} receptors and depression

Malika El Yacoubi, PhD; Jean Costentin, MD, PhD; and Jean-Marie Vaugeois, PhD

Abstract—Adenosine and its analogues have been shown to induce "behavioral despair" in animal models believed to be relevant to depression. Recent data have shown that selective adenosine A_{2A} receptor antagonists (e.g., SCH 58261, ZM241385, and KW6002) or genetic inactivation of the receptor was effective in reversing signs of behavioral despair in the tail suspension and forced swim tests, two screening procedures predictive of antidepressant activity. A_{2A} antagonists were active in the tail suspension test using either mice previously screened for having high immobility scores or mice that were selectively bred for their spontaneous "helplessness" in this test. At stimulant doses, caffeine, a nonselective A_1/A_{2A} receptor antagonist, was effective in the forced swim test. The authors have hypothesized that the antidepressant-like effect of selective A_{2A} antagonists is linked to an interaction with dopaminergic transmission, possibly in the frontal cortex. In support of this idea, administration of the dopamine D_2 receptor antagonist haloperidol prevented antidepressant-like effects elicited by SCH 58261 in the forced swim test (putatively involving cortex), whereas it had no effect on stimulant motor effects of SCH 58261 (putatively linked to ventral striatum). The interaction profile of caffeine with haloperidol differed markedly from that of SCH 58261 in the forced swim and motor activity tests. Therefore, a clear-cut antidepressant-like effect could not be ascribed to caffeine. In conclusion, available data support the proposition that a selective blockade of the adenosine A_{2A} receptor may be an interesting target for the development of effective antidepressant agents.

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Adenosine, a multifaceted neuromodulator. It is well established that, in the CNS, adenosine is a neuromodulator acting through discrete cell-surface receptors.[1] Approximately 10 years ago, the first two adenosine receptors, A_1 and A_{2A}, were identified among putative G protein-coupled receptors. Later, two other receptor types, A_{2B} and A_3, were cloned.[2] Adenosine itself has been established as a potential regulator of complex central functions, such as anxiety states,[3,4] aggressiveness,[5] and sleep.[6]

Caffeine (1,3,7-trimethylxanthine) was recognized more than 20 years ago to act as an antagonist at adenosine receptors.[7] Caffeine is likely to exert its primary action through adenosine receptors because they are the only known sites that bind caffeine at low concentrations.[8] Caffeine has important effects on alertness and is widely consumed by people who need to stay awake.[9] Stimulant effects have been quantified in motor activity studies in rodents.[10,11]

Major depression is one of the most frequent illnesses, and increasing evidence suggests that it has a neurobiologic basis that includes genetic factors. Therefore, major depression may involve several neural systems within the brain, and the dopamine system is a candidate among them.[12,13] Concerning the underlying functional neuroanatomy, it has been suggested that dysfunctions or imbalances at multiple points within limbic cortical-striatal-pallidal-thalamic circuits may be associated with major depressive syndrome.[14]

Adenosine and rodent's blues. Some experimental data suggest that adenosine may be involved in the pathophysiology of mood disorders. First, the activation of central adenosine receptors, via either analogues of adenosine or an increase in endogenous adenosine levels, led to a behavioral state called "learned helplessness," similar to that induced by submitting rats to inescapable shocks.[15,16] Second, in the mouse forced swim test,[17] a preclinical test aimed at screening potential antidepressant agents, adenosine and its synthetic analogue 2-chloroadenosine lengthened the duration of immobility. Dipyridamole, which is known to inhibit adenosine uptake, potentiated the adenosine effect. Conversely, the nonselective adenosine receptor antagonists caffeine and theophylline blocked the nucleoside-induced enhancement of immobility. In the same study, the tricyclic antidepressant agents imipramine and desipramine and the monoamine oxidase (MAO) inhibitor tranylcypromine also reversed adenosine-induced immobility.[18] This prolongation of immobility in animals suggested that adenosine might be involved in the process leading to "behavioral despair."

The adenosine–dopamine connection put forward. Our understanding of adenosine–dopamine interactions in the basal ganglia[19,20] indicates that adenosine modulates dopaminergic functions in dorsal and ventral striatum regions, where the nigrostriatal, the mesostriatal, and the mesolimbic dopaminergic neuronal pathways terminate. Adenosine A_{2A} receptors are predominantly expressed in pallidal-projecting GABAergic enkephalin-containing neurons, which also express dopamine D_2 receptors.[5,21,22] Svenningsson et al.[22] have provided autora-

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diographic evidence for the existence of extrastriatal adenosine A_{2A} receptors throughout the thalamus and cerebral cortex in the human brain. Currently, there is interest in A_{2A} antagonists as therapeutic agents for dopamine-mediated motor disorders such as Parkinson's disease (PD).[23,24] Conversely, increases in striatal dopamine D_2 receptor[25] and dopamine neuronal transporter[26] densities measured by SPECT have been reported in patients with depression as compared with control subjects. These effects may be associated with a reduction in dopaminergic function, reflected in either decreased dopamine release or dopamine receptor upregulation. The dopamine reuptake inhibitor bupropion[27] displays potent antidepressant properties.[28] Moreover, antidepressant effects in patients with depression have also been reported with direct dopamine D_2 agonists piribedil[29] and bromocriptine,[30] which are mainly used to manage PD. Therefore, adenosine receptor antagonists that modulate mesostriatal or mesocorticolimbic dopaminergic neuronal pathways may be therapeutically beneficial for the management of depression.

In addition to the mouse forced swim test,[17] a second test called the automated tail suspension test[31] is commonly used to screen potential antidepressant agents, which are considered effective if one dose can reduce the period of immobility in these tests. The mouse forced swim test does not regularly detect selective serotonin reuptake inhibitors,[32] which are antidepressant agents. Therefore, for investigating antidepressant agents, it is interesting to add the tail suspension test to the mouse forced swim test because selective serotonin reuptake inhibitors are effective in the former.[33] One major drawback of these two tests is that they include a motor component unrelated to the correction of the pathologic disturbance of interest, i.e., low mood. Consequently, it is critical to carry out motor activity tests in parallel to try to differentiate pure motor stimulant effects from the ability to increase escape-directed behavior.[27,33,34]

With this background in mind, it appeared logical to ask whether the blockade or absence of adenosine A_{2A} receptors would influence helplessness in animal models useful to screen potential antidepressant agents. Therefore, the behavior of A_{2A} receptor knockout ($A_{2A}R^{-/-}$) mice[5] as compared with wild-type ($A_{2A}R^{+/+}$) controls has been evaluated in two different screening tests, and the effects of selective A_{2A} antagonists and caffeine on helplessness were also studied. Given their potentially confounding motor effects in these screening procedures, it appeared important to identify whether nonspecific changes in motor activity might be associated with any reversal of the state of despair.

Antidepressant-like behavioral effects in mice lacking the A_{2A} receptor. In the tail suspension test, the duration of immobility was significantly reduced in adenosine $A_{2A}R^{-/-}$ mice as compared with

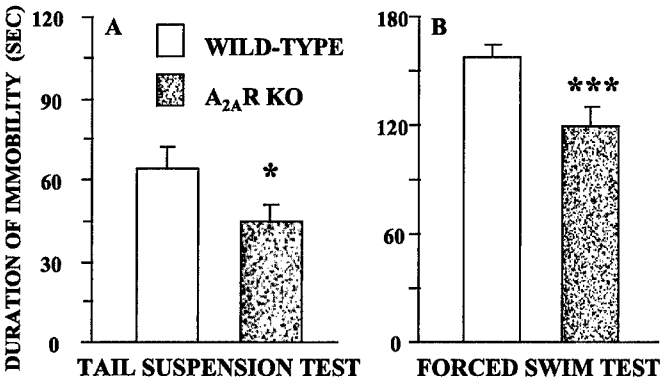

*Figure 1. Immobility times of A_{2A} receptor knockout ($A_{2A}R^{-/-}$) and wild-type mice recorded in the tail suspension or forced swim tests. (A) Duration of immobility in the tail suspension test. Mean ± SEM of data from 29 mice per group. (B) Duration of immobility in the forced swim test. Mean ± SEM of data from 16 mice per group. *$p < 0.05$, ***$p < 0.001$ as compared with wild-type mice by Student's t-test. Reprinted from El Yacoubi et al.[50] Reproduced with permission.*

$A_{2A}R^{+/+}$ animals. In the forced swim test, $A_{2A}R^{-/-}$ animals also behaved differently from their $A_{2A}R^{+/+}$ controls because they showed a strong reduction in the time of immobility (figure 1). Classically, these results may be interpreted as a reduction in helplessness in mice lacking the adenosine A_{2A} receptor. It was previously shown that adenosine $A_{2A}R^{-/-}$ mice displayed a reduced locomotor activity in an open field when compared with $A_{2A}R^{+/+}$ mice.[5,11,35,36] On the contrary, in the forced swim and tail suspension tests, their activities were enhanced as compared with those of $A_{2A}R^{+/+}$ mice, suggesting that the neuronal pathways underlying the two behaviors are at least partly different. Furthermore, it is obvious that antidepressant-induced reduction of immobility cannot be explained by a nonspecific behavioral stimulation because many antidepressant agents tend to decrease motor activity.[33,37] In addition, direct dopamine D_2 receptor agonists, known to usually reduce motor activity when administered in mice,[38] have been shown to increase mobility time in the forced swim test.[39] These encouraging results obtained with adenosine $A_{2A}R^{-/-}$ mice prompted the authors to study the effects of A_{2A} antagonists, nonselective and selective, with the expectation that they also have antidepressant-like properties.

Caffeine does not produce antidepressant-like effects in mice. Antidepressant-like properties of A_{2A} antagonists were initially suggested approximately 10 years ago by Sarges et al.[40] These authors discovered an antagonist compound, CP 66713, with a 25-fold selectivity toward A_{2A} vs A_1 receptor, which was active in the forced swim test. Caffeine, which at low doses causes "positive" subjective effects on mood,[9] is a nonselective A_1 (K_i = 29 μmol/L) and A_{2A} (K_i = 48 μmol/L) antagonist.[41] Functional studies revealed that the main targets for the stimulatory

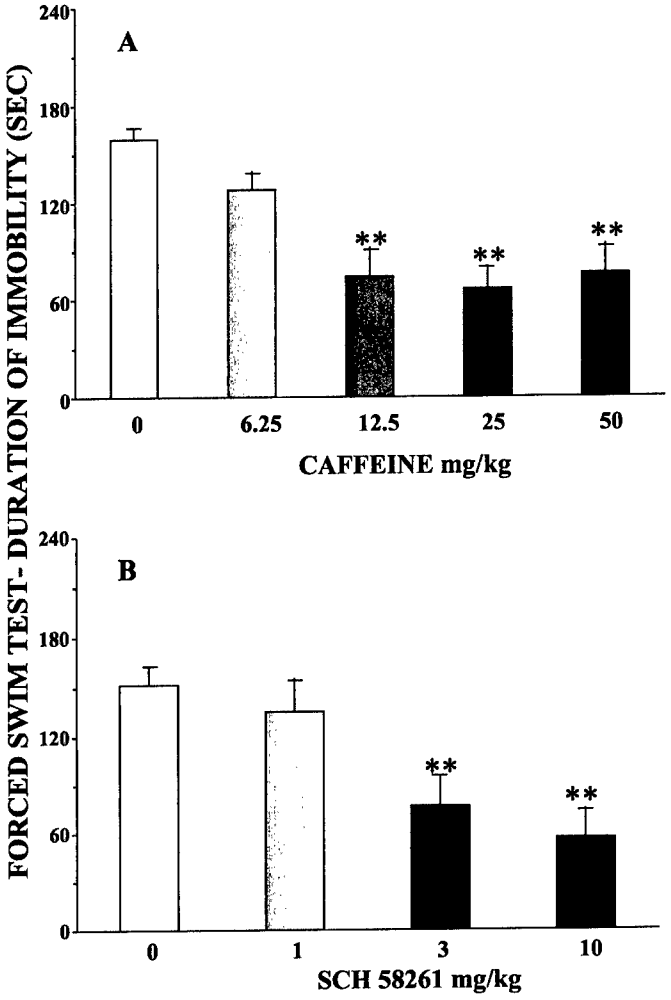

*Figure 2. Effects of caffeine and SCH 58261 in the mouse forced swim test. Testing was for 6 minutes. (A) Caffeine (6.25, 12.5, 25, and 50 mg/kg) or (B) SCH 58261 (1, 3, and 10 mg/kg) were acutely administered intraperitoneally to CD1 mice. The test was performed 30 minutes after treatment. Mean ± SEM of data from 8 to 14 control mice and 8 mice in treated groups. ** p < 0.01, (one-way analysis of variance followed by Student–Newman–Keuls test).*

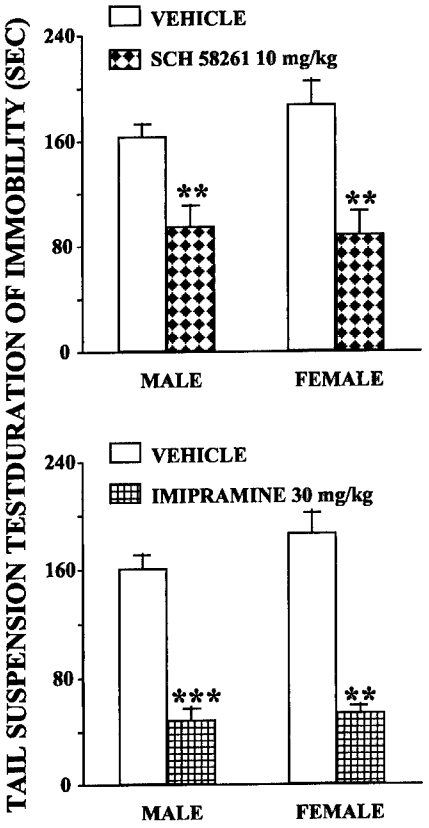

*Figure 3. Effects of SCH 58261 or imipramine in the tail suspension test performed in a novel genetic mouse model of depression. Outbred CD1 mice were used as the foundation population of a line of mice that was selectively bred for its high spontaneous helplessness (immobility scores ≥115 seconds = helpless) in the tail suspension test. Mice of both sexes (seventh generation) were injected intraperitoneally with 10 mg/kg SCH 58261 (upper panel) or 30 mg/kg imipramine (lower panel) 30 minutes before the test. Testing was for 6 minutes. Mean ± SEM of data from 8 to 12 control mice and 9 to 12 mice in treated groups. **p < 0.01, ***p < 0.001 (one-way analysis of variance followed by Student–Newman–Keuls test) as compared with vehicle-injected groups.*

properties of caffeine[7] are the adenosine A_{2A} receptors.[10,11,42] A range of pharmacologically relevant doses of caffeine (6.25 to 50 mg/kg) administered to CD1 mice caused significant [$F(4,45) = 11.47$; $p < 0.001$] anti-immobility effects in the forced swim test (figure 2A), starting at the motor stimulant dose of 12.5 mg/kg.[11] These data are consistent with the results obtained in previous studies in mice.[40,43] However, the fact that caffeine increases motor activity in mice (e.g., figure 4A) may cast doubt on the reliability of the forced swim test to identify this substance as a potential antidepressant. Therefore, to further analyze this effect of caffeine on the forced swim test, an interaction study with the preferential dopamine D_2 receptor antagonist haloperidol[44] was carried out. The aim was to discriminate an escape-directed behavior (i.e., a motivation to engage in active coping attempts to avoid the stressful situation) from a nonspecific locomotor stimulant effect elicited by 25 mg/kg caffeine. Haloperidol depressed baseline and caffeine-stimulated open-field motor activity in a dose-dependent manner (see figure 4A). Two-way analysis of variance (ANOVA) showed that there was no haloperidol × caffeine interaction [$F(2,47) = 2.05$; $p > 0.05$], indicating a parallel evolution of dose–response motor effects after haloperidol administration in vehicle- and caffeine-treated mice. The statistical analysis further showed significant effects of haloperidol and caffeine. By contrast, eticlopride, another preferential dopamine D_2 receptor antagonist, was shown to block the motor stimulant effects of caffeine in rats at doses not active on motor activity by themselves.[45] Finally, the anti-immobility effect of caffeine in the mouse forced swim test was prevented by pretreatment with haloperidol, as indicated by a significant interaction [$F(3,76) = 8.05$; $p < 0.001$] between haloperidol and caffeine treatments (figure 4B). Hence, the motor stimulant and

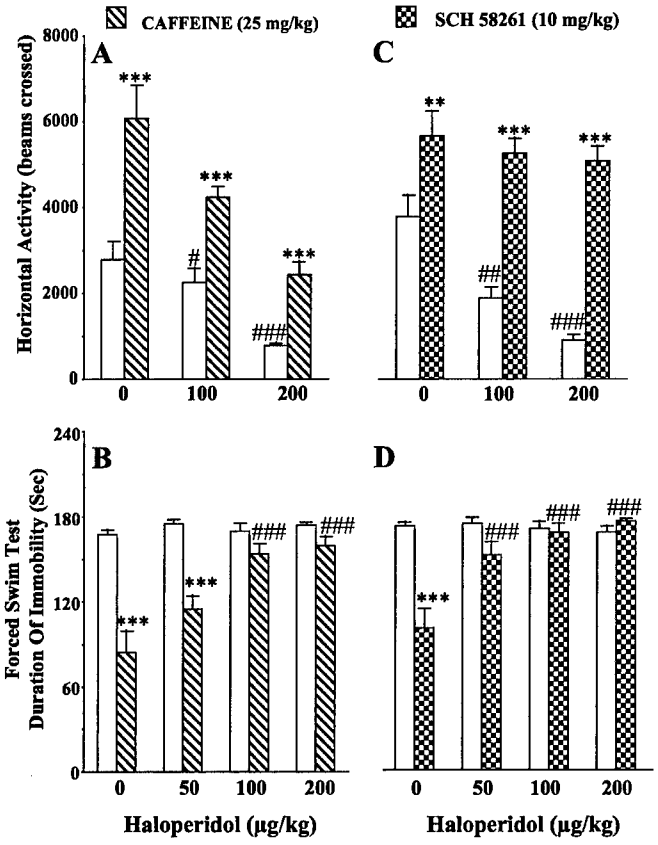

*Figure 4. Effects of haloperidol on stimulation of locomotor activity and anti-immobility response induced by caffeine or SCH 58261. Mice were injected with saline or increasing doses of haloperidol (50, 100, and 200 µg/kg, intraperitoneally). Fifteen minutes later, they were injected intraperitoneally with vehicle or caffeine (25 mg/kg) or SCH 58261 (10 mg/kg). Upper panels, locomotor activity test. Immediately after the second treatment, mice were introduced into the actimeters. The horizontal activity was measured for 45 minutes. Mean ± SEM of data from eight mice per group. Statistics by two-way analysis of variance (ANOVA) followed by post hoc comparisons. (A) Shows no interaction between factors haloperidol and caffeine: $F(2,47) = 2.05$, $p > 0.05$. (C) Reveals an interaction between factors haloperidol and SCH 58261: $F(2,47) = 4.11$, $p = 0.02$. Lower panels, forced swim test. Mice pretreated with haloperidol or saline received vehicle or caffeine or SCH 58261 30 minutes before testing. The duration of immobility was recorded during the last 3 minutes of the 6-minute testing period. Mean ± SEM of data from 14 control mice and 8 to 11 mice in treated groups. Statistics by two-way ANOVA followed by post hoc comparisons. (B) Reveals an interaction between factors haloperidol and caffeine: $F(3,76) = 8.05$, $p < 0.001$. Also, (D) shows an interaction between factors haloperidol and SCH 58261: $F(3,72) = 5.04$, $p < 0.01$. Post hoc comparisons: **$p <$ 0.01, ***$p < 0.001$ as compared with respective caffeine- or SCH 58261-untreated control groups; #$p < 0.05$, ##$p <$ 0.01, ###$p < 0.001$ as compared with respective haloperidol-untreated control groups. Panels C and D reprinted from El Yacoubi et al.[50] Reproduced with permission.*

the anti-immobility effects of caffeine were altered by similar doses of the dopamine D_2 receptor antagonist haloperidol in the present work. These results suggest that caffeine could induce a nonspecific effect in the screening test used here. No genuine antidepressant effects have yet been attributed to caffeine despite the apparent increase it produces in norepinephrine turnover and the downregulation it induces in β-adrenoceptor levels.[46] Our results agree with other studies interpreting the effects of caffeine in the forced swim test as false positive.[43,47] Given the effectiveness of adenosine $A_{2A}R^{-/-}$ mice and of selective A_{2A} antagonists (see below) in the same screening procedures, we suggest that the antagonism at adenosine A_1 receptor elicited by caffeine could have negative effects on the behavioral outcome in the mouse forced swim test.

Selective A_{2A} antagonists are potential antidepressant agents. The effects of three selective A_{2A} antagonists, SCH 58261, ZM241385 and KW6002, have been examined in screening procedures for antidepressant agents. SCH 58261 is a selective adenosine A_{2A} receptor antagonist, with a greater selectivity profile than ZM241385.[48] The xanthine-like derivative KW6002 displays a high affinity for A_{2A} receptor, a moderate A_{2A} vs A_1 selectivity, and is active in experimental models of PD.[49]

These three selective A_{2A} antagonists were shown to be effective in the tail suspension test after short-term treatment using CD1 mice.[50] The A_{2A} antagonists ZM241385 and SCH 58261 also decreased (by 50% for 30 mg/kg ZM241385, and by 68% for 10 mg/kg SCH 58261) the immobility time of animals screened twice as having a "high immobility" (i.e., immobility score ≥115 seconds) in the tail suspension test.[50] Finally, SCH 58261 and the tricyclic antidepressant imipramine increased struggling time in the latter test (figure 3) performed with mice that are selectively bred for their spontaneous helplessness in this test[50]—a genetic mouse model that may be useful to screen potential antidepressant agents.[51]

Further experiments were carried out with the more selective compound SCH 58261, which does not target the adenosine A_{2B} receptor.[48] SCH 58261 was also found to be effective in the mouse forced swim test, with significant [$F(3,31) = 6.83$; $p = 0.001$] effects from 3 mg/kg, intraperitoneally (see figure 2B). Then, an interaction study with haloperidol was also carried out. In this set of two experiments performed in parallel, mice received increasing doses of subcutaneous haloperidol 15 minutes before the administration of an effective dose (10 mg/kg, intraperitoneally) of SCH 58261 and were then assayed in either the locomotor activity (figure 4C) or forced swim (figure 4D) tests. In the locomotor activity test, there was a significant haloperidol × SCH 58261 interaction [$F(2,47) = 4.11$; $p < 0.05$]. As expected, haloperidol by itself induced a decrease in motor activity; however, the stimulant effects of SCH 58261 were not changed by the concomitant presence of haloperidol (see figure

4C). In the forced swim test, the two-way ANOVA also revealed a significant interaction between the two factors [$F(3,72) = 5.04$; $p < 0.01$]. Haloperidol produced no effect over the dose range used (see figure 4D). However, the effects of SCH 58261 in the forced swim test were reversed in the presence of haloperidol (50, 100, and 200 μg/kg, intraperitoneally). It is remarkable to note that the anti-immobility effect elicited by SCH 58261 was prevented by a low dose (0.05 mg/kg) of the dopamine D_2 receptor antagonist, demonstrating a high sensitivity of the goal-directed behavior to haloperidol (see figure 4D). This finding should be put into the context of other studies showing that dopamine D_2 receptor antagonists block anti-immobility effects of antidepressant agents.[34,52] Haloperidol did not counteract SCH 58261-induced stimulant effects when administered in the range of doses used in the present experiment (see figure 4C). It may be pointed out that several previous studies have demonstrated that A_{2A} antagonists are effective in reducing catalepsy induced by high doses of dopamine D_2 receptor antagonists, a screening test for potential antiparkinsonian drugs.[53] Taken together, these results would fit with the hypothesis put forward by Svenningsson et al.[42] that adenosine and dopamine are tonically active at their respective receptors in striatum.

Therefore, dopamine transmission through D_2 receptors is critically involved in the anti-immobility effect elicited by SCH 58261. Dopamine transmission in the frontal cortex and nucleus accumbens has been implicated in the mechanism of action of antidepressant agents.[13,54] One tentative explanation for the dissociation between the two behaviors studied here may reside in the peculiar physiology and pharmacology of dopamine neurons originating in the ventral tegmental area in the midbrain and projecting to the prefrontal cortex. As compared with mesolimbic and nigrostriatal dopamine neurons, mesocortical dopamine neurons have a higher firing rate, and dopamine has a higher turnover rate. Furthermore, mild stressors activate them. In contrast to nigrostriatal dopamine neurons, mesoprefrontal dopamine neurons also may not be well suited for maintaining homeostasis because of the absence or low sensitivity of synthesis- and impulse-regulating autoreceptors.[55] Because of the blockade of synthesis- and impulse-regulating autoreceptors on projections to dorsal and ventral striatum, haloperidol by itself may induce a greater release of dopamine and thus higher synaptic dopamine concentration in these areas, allowing a competition between haloperidol and synaptic dopamine. This could explain, in part, the lack of antagonism of SCH 58261-induced effects in the motor activity test. On the contrary, the dopamine release elicited by haloperidol in the frontal cortex would be much weaker in intensity,[56] allowing the reversal of the anti-immobility effect caused by the selective A_{2A} antagonist. Direct evidence of an increased extracellular dopamine release in the prefrontal cortex of rats, as reported for reference antidepressant agents such as fluoxetine and clomipramine,[54] would lend support to our hypothesis.

The adenosine A_{2A} receptor has been visualized by autoradiography in the prefrontal cortex of the mouse[57] with densities equal to approximately one-tenth of that found in the striatum. The selective adenosine A_{2A} receptor antagonist [^{3}H] SCH 58261 was also found to label the postmortem human prefrontal cortex, with a binding density approximately one-third of that detected in rostral putamen.[22] Numerous neuroanatomic studies have found differences in cerebral blood flow and metabolism in the prefrontal cortex of patients with depression when compared with healthy control subjects.[14] Therefore, it will be of interest to investigate how A_{2A} receptor blockade produces an increase in escape-directed behavior in screening tests. A role of A_{2A} receptors located in the striatum cannot be completely excluded for at least two reasons. First, dopamine transmission in this structure plays an important role in determining the individual flexibility to manage sensory information.[58] Second, dopamine D_2 receptor densities are modified in the striatum of patients with depression relative to control subjects.[25]

Although it is widely accepted that the tests discussed here are useful to screen potential antidepressant agents, selective A_{2A} antagonists should be screened in other preclinical models such as learned helplessness or chronic mild stress, for instance. Ultimately, the question of clinically significant antidepressant action in humans will be addressed when selective A_{2A} antagonists are evaluated in therapeutic trials for pathologic states such as PD[24] if not for depression itself.

In conclusion, these data support the hypothesis that SCH 58261 and other A_{2A} antagonists induce activity in the forced swim and tail suspension tests by a prolongation of escape-directed behavior rather than by a generalized motor stimulant effect. The positive effect is likely mediated by an increase in dopaminergic transmission, possibly in the frontal cortex. Therefore, selective A_{2A} antagonists appear to be attractive targets for drug development as antidepressant agents.

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Potential for antipsychotic and psychotomimetic effects of A_{2A} receptor modulation

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Abstract—The discovery of antagonistic interactions between A_{2A} adenosine receptors and D_2 dopamine receptors in the ventral striatum suggested that A_{2A} receptor activation might modulate the antipsychotic effects of dopamine receptor antagonists and could provide an opportunity for the development of A_{2A} receptor agonists as novel antipsychotic drugs. However, there is limited evidence from preclinical and clinical studies that A_{2A} receptor agonists can exert antipsychotic effects. Furthermore, it remains unclear whether A_{2A} receptor agonists possess a sufficient safety margin or whether their potent hypotensive effects or extrapyramidal side effects would limit their therapeutic utility as antipsychotic agents. The interaction between A_{2A} receptors and D_2 receptors also raises the possibility that A_{2A} receptor antagonists, which hold considerable promise as antiparkinsonian agents, may have dose-limiting psychotomimetic side effects. Preclinical studies using selective A_{2A} receptor antagonists suggest that this class of compound has a low propensity to elicit psychotomimetic side effects or exacerbate those induced by D_2 receptor agonists.

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The discovery of an antagonistic interaction between adenosine A_{2A} and dopamine D_2 receptors in dorsal and ventral regions of the striatum has important implications for a number of psychiatric and neurologic disorders.[1] Although considerable attention has been paid to the potential utility of A_{2A} receptor antagonists for managing movement disorders such as Parkinson's disease (PD),[2,3] there is also evidence for involvement of adenosine in the pathophysiology of schizophrenia and in mediating some of the behavioral effects induced by psychotomimetic drugs.[4,5]

The involvement of neuronal dopamine systems in schizophrenia is well established. Most clinically prescribed antipsychotic agents have high affinity for dopamine D_2 receptors, and the "typical" antipsychotic drug haloperidol is a selective D_2 receptor antagonist.[6-8] "Atypical" antipsychotic agents, such as clozapine and olanzapine, also have high affinity for D_2 receptors[9] and in addition have actions at a number of other receptor systems, most notably 5-HT_2 receptors. Nevertheless, the best predictor of clinical efficacy for typical and atypical antipsychotic agents for managing the positive symptoms of schizophrenia is their occupancy of D_2 receptors.[7,9] Typical antipsychotic agents have a high propensity to induce a range of unpleasant side effects, including hypothermia, photosensitivity, sedation, and muscle spasms. In particular, long-term treatment with typical antipsychotic agents is associated with the development of tardive dyskinesias and other extrapyramidal side effects (EPS) that are thought to be the result of prolonged blockade of striatal dopamine receptors.[10] Atypical antipsychotic agents can be differentiated from typical antipsychotic agents based on having a lower propensity to elicit EPS and in some cases improved efficacy in managing negative symptoms of the disease, such as flattened affect and social withdrawal.[11] It has been proposed that the improved side effect profile of atypical antipsychotic agents is a result of selective blockade of limbic dopamine receptors or blockade of 5-HT_2 receptors.[12,13]

By contrast, drugs that enhance dopaminergic function, either by promoting neurotransmitter release, blocking its reuptake, or through postsynaptic D_2 receptor activation, are psychotomimetic or aggravate existing psychosis.[14-16] Dopaminergic drugs typically used to manage PD, such as the dopamine precursor L-dopa and dopamine D_2 receptor agonists, can lead to psychotomimetic side effects at higher doses. These psychotomimetic side effects limit the usefulness of some antiparkinson drugs in managing symptoms during the later stages of PD and sometimes necessitate the introduction of concurrent antipsychotic drug treatment.[17]

Evidence of antagonistic interactions between adenosine A_{2A} receptor and dopamine D_2 receptor systems within the striatum has been extensively reviewed elsewhere.[1,3,5] The principal lines of evidence supporting this interaction are 1) the discrete colocalization of A_{2A} and D_2 receptors on GABAergic striatopallidal neurons; 2) the observation that stimulation of striatal A_{2A} and D_2 receptors has opposing actions on pallidal GABA release; 3) the ability of

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A_{2A} receptor activation to reduce the affinity of dopamine agonists for the D_2 receptor; 4) the observation that dopamine depletion, dopamine receptor blockade, and A_{2A} receptor activation produce similar patterns of expression of immediate early genes such as c-fos in striatopallidal neurons, whereas activation of D_2 receptors has the opposite effect; and 5) the contrasting effects of A_{2A} receptor and D_2 receptor activation on locomotor activity and catalepsy in animal studies.

Many of the actions of A_{2A} receptor activation mimic the effects of dopamine D_2 receptor blockade, and as such, A_{2A} receptor agonists may provide a novel therapeutic approach for the management of schizophrenia. This hypothesis prompts consideration of whether A_{2A} receptor antagonists, like D_2 receptor agonists, may induce psychoses or may exacerbate the psychotomimetic effects of dopamine agonists used for the management of PD.

Role of A_{2A} receptors in the ventral striatum. The rationale for the use of A_{2A} receptor agonists as novel antipsychotic agents comprises neurochemical evidence of heterogeneous A_{2A}-D_2 receptor interactions in dorsal and ventral regions of the striatum, and behavioral data generated in rodent assays.[4,5,18,19] It is well established that A_{2A} receptors play an important role in regulating the neuronal activity of striatal GABAergic efferents, in particular, the enkephalin-containing neurons of the so-called "indirect" striatopallidal pathway, where A_{2A} and D_2 receptors are colocalized. GABAergic striatopallidal neurons project from dorsal and ventral regions of the striatum and are components of anatomically and functionally differentiated circuitry.[5] The ventral striatum comprises the ventromedial caudate putamen, olfactory tubercle, and nucleus accumbens and receives dopaminergic afferents principally from the ventral tegmental area and glutamatergic input from the medial prefrontal cortex, hippocampus, and amygdala. By contrast, the dorsal striatum comprises the dorsolateral portion of the caudate-putamen, which receives dopaminergic afferents primarily from the substantia nigra pars compacta and glutamatergic input from premotor areas of the neocortex. In addition, the dorsal and ventral regions of the striatum have efferent projections to the substantia nigra pars compacta and ventral tegmental areas, respectively. Therefore, A_{2A} receptors located on striatopallidal GABAergic efferents could potentially regulate the activity of distinct corticobasal ganglia–cortical circuits whose striatal output originates from either dorsal or ventral striatal regions. The importance of this differentiation relates to the functional role of dopamine in the dorsal and ventral striatum. Dopamine receptors in the ventral striatum and nucleus accumbens control initiation of appetitive and aversive instrumental behavior and are thought to mediate the therapeutic effects of antipsychotic agents. By contrast, dopamine receptors in the dorsal striatum play a prominent role in facilitation and maintenance of consummatory behavior and mediate expression of stereotypies and catalepsy induced by D_2 receptor activation or blockade, respectively.[5,9,20-23] Therefore, a drug that preferentially antagonizes ventral D_2 receptor function via an action on GABAergic striatopallidal efferents may have efficacy in managing positive symptoms of schizophrenia with a low liability to elicit EPS.[5]

Preclinical evidence from animal models. The behavioral and neurochemical effects of intracerebral infusion of A_{2A} receptor agonists provide evidence for an atypical antipsychotic profile because more powerful antagonistic A_{2A}-D_2 receptor interactions are apparent within the ventral striatum than within the dorsal striatum.[18,24] It has been recognized for some time that A_{2A} receptor agonists share many of the behavioral properties of dopamine D_2 receptor antagonists in rodents. For example, both classes of compound reduce spontaneous locomotor activity, attenuate the motor effects of the psychostimulants amphetamine and phencyclidine (PCP) and the dopamine receptor agonist apomorphine, and induce catalepsy at higher doses or when infused into the striatum.[4,5,19,25-27] The ability of novel compounds to block amphetamine-, apomorphine-, and PCP-induced motor stimulation is often used as evidence of potential antipsychotic efficacy,[19,28] whereas induction of catalepsy is considered indicative of potential for induction of EPS.[29] The behavioral profile of selective A_{2A} receptor agonists in these assays is consistent with known antipsychotic drugs. However, the literature is unclear as to whether A_{2A} agonists have the same propensity to induce EPS as D_2 receptor antagonists, or possess an atypical antipsychotic profile. Rimondini et al.[4] have reported that CGS 21680 displays a profile similar to clozapine, being more potent at reversing amphetamine- and PCP-induced locomotor effects than inducing catalepsy in rats. Similar findings have been reported in primate studies, in which CGS 21680 was shown to reverse apomorphine-induced stereotypy in dopamine agonist-sensitized *Cebus apella* monkeys at doses that did not elicit EPS.[30] In contrast, studies comparing the potency of CGS 21680 to reduce locomotion and block apomorphine-induced climbing with its ability to induce catalepsy in rodents have concluded that the profile of CGS 21680 is similar to classic D_2 receptor antagonists rather than atypical antipsychotic agents.[19,31] Measures of antipsychotic efficacy and catalepsy can be confounded by nonspecific motor impairment. CGS 21680 potently inhibits locomotor activity when given systemically, but it is not known whether this effect is mediated by central or peripheral A_{2A} receptor activation. Activation of peripheral A_{2A} receptors produces hypotension;[32] a sudden decrease in blood pressure and the associated reflex tachycardia could impair the ability of an animal to maintain a cataleptic posture or respond to a psychostimulant drug.

Table *Effects of adenosine receptor ligands on prepulse inhibition in rats*

Drug	Dose*	Rat strain	Results
Caffeine, nonselective antagonist	10	S-D	No intrinsic effect on PPI.[41]
Theophylline, nonselective antagonist	20–40	S-D	Reduced PPI.[42] Potentiated APO (0.5 mg/kg) disruption of PPI. Effects on APO blocked by A_1 agonist but not by A_{2A} agonist.[42]
KW-6002, A_{2A} antagonist	0.1–30	S-D	No intrinsic effect on PPI.[43] No effect on disruption of PPI induced by peregolide.[43]
CPA, A_1 agonist	0.05–0.5	Wistar	No intrinsic effect on PPI (0.5 mg/kg). Dose dependently reversed deficits in PPI induced by PCP (4 mg/kg s.c.).[52]
CGS-21680, A_{2A} agonist	0.05 μg	S-D	No intrinsic effect on PPI. Reversed deficits in PPI induced by APO (2 mg/kg i.p.).[35]
	0.5	Wistar	No intrinsic effect on PPI. No effect on deficits in PPI induced by APO (1 mg/kg) or amphetamine (5 mg/kg). Reversed deficits in PPI induced by PCP (4 mg/kg s.c.).[36]

*Doses are mg/kg unless otherwise indicated.

S-D = Sprague-Dawley; PPI = prepulse inhibition; APO = apomorphine; PCP = phencyclidine; s.c. = subcutaneous; i.p. = intraperitoneal.

Although systemically administered CGS 21680 has been shown to block the effects of amphetamine and PCP at different doses, the possibility that these apparent antagonistic effects are a consequence of nonspecific rather than antipsychotic actions cannot be entirely excluded. Some of these issues could be resolved by examining the profile of A_{2A} receptor agonists in more complex antipsychotic models that assess attentional processes and do not depend on simple measures of motor stimulation and depression.

It is well established that patients with schizophrenia show impairments in attention and cognition, which are linked to disruptions of sensorimotor gating. Patients with schizophrenia appear to be unable to filter irrelevant or intrusive sensory information and as a consequence may experience sensory inundation or "flooding," which impairs their ability to discriminate and process salient information within their environment.[33] Prepulse inhibition (PPI) of the startle reflex is an operational model of sensorimotor gating that measures attenuation of the startle reflex elicited by a sudden intense stimulus when it is preceded by a stimulus of weaker intensity (a prepulse).[34] Deficits in PPI are observed in patients with schizophrenia and with other conditions in which sensorimotor gating is impaired, such as Huntington's disease, Tourette's syndrome, and obsessive-compulsive disorder. Psychotomimetic drugs such as dopamine receptor agonists, amphetamine, and PCP also disrupt PPI in animals and humans, effects that can be reversed by typical and atypical antipsychotic drugs.[34] PPI is not a model of schizophrenia per se, but rather it is a measure of the sensorimotor gating deficits observed in patients with schizophrenia or induced by drugs, which can be used to assess antipsychotic and psychotomimetic drug effects.

A number of studies have examined the effects of adenosine A_{2A} receptor activation on PPI (table). An early report found that intra-accumbens infusion of the selective A_{2A} receptor agonist CGS 21680 had no intrinsic effect on PPI but blocked the disruption of PPI caused by the dopamine agonist apomorphine.[35] In contrast, a later study that examined the effects of a range of doses of CGS 21680 given systemically observed no effect on apomorphine- or amphetamine-induced disruptions of PPI.[36] However, this study reported that a high dose (0.5 mg/kg) of CGS 21680 attenuated the impairment of PPI caused by the NMDA receptor antagonist PCP. The apparent discrepancy between the effects of CGS 21680 given directly into the nucleus accumbens and administered systemically on apomorphine disruption of PPI is also difficult to reconcile. The brain penetration of CGS 21680 after systemic administration is not well characterized. Although the ability of CGS 21680 to reduce locomotor activity and baseline startle reflex in these studies is interpreted by the authors as evidence of central adenosine receptor activation, these effects may be secondary to peripherally mediated hypotension induced by the drug (see above). The profile of CGS 21680 in the PPI model can be distinguished from that of typical and atypical antipsychotic drugs by the failure of the A_{2A} receptor agonist to block the effects of apomorphine and amphetamine. However, in parallel with atypical antipsychotic drugs such as clozapine and olanzapine, CGS 21680 reverses NMDA receptor antagonist-induced deficits in PPI.[34] It is important to note that reversal of the effects of PCP by CGS 21680 was only apparent at a dose of CGS 21680 that impaired locomotor activity and disrupted the baseline startle reflex. This confounds the interpretation of these results because they may reflect motor impairment induced by CGS 21680 rather than a specific

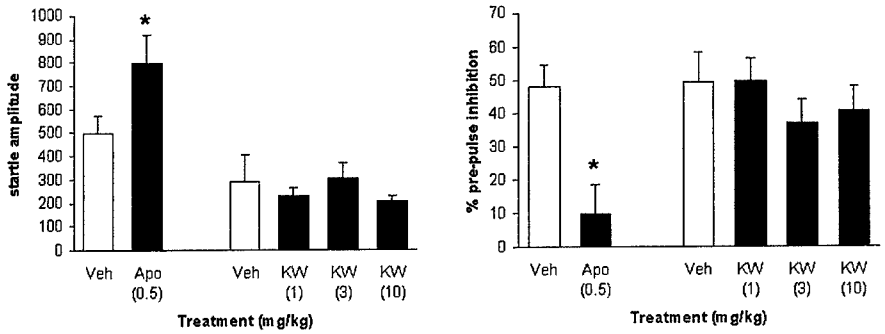

*Figure. Contrasting effects of apomorphine and KW6002 on prepulse inhibition of the acoustic startle reflex in male Sprague–Dawley rats. Apomorphine (0.5 mg/kg) significantly increased startle reactivity and impaired prepulse inhibition. By contrast, KW6002 (1 to 10 mg/kg) had no effect on either parameter. Testing consisted of random presentation of either (A) a startle stimulus (120-dB tone for 40 ms) or (B) a prepulse stimulus (76-dB tone for 20 ms) followed 100 ms later by the startle stimulus. Mean startle amplitudes for 12 startle trials [A] and 12 prepulse trials [B] were determined for each animal, as was percentage prepulse inhibition (%PPI), using the formula (1 − [B]/[A]) × 100. Data were analyzed using one-way analysis of variance. Apomorphine was administered subcutaneously (0.5 mg/kg in 0.01% ascorbate) 20 minutes before testing. KW6002 was administered intraperitoneally (1 to 10 mg/kg in 8% Tween 80) 20 minutes before testing. *$p < 0.05$ vs vehicle treatment.*

antipsychotic-like effect on sensorimotor gating. However, the ability of A_{2A} receptor activation to reverse the behavioral effects of PCP in the PPI model and in locomotor studies[4] may reflect a specific interaction between A_{2A} and NMDA receptors. This interaction is further substantiated by evidence that NMDA receptor antagonists reverse the catalepsy produced by A_{2A} receptor activation.[37] The mechanism by which these two receptor systems interact has not been established, but it may involve effects on neurotransmitter release or receptor signaling within the striatum. Direct infusion of A_{2A} receptor agonists enhances spontaneous outflow of glutamate release in the striatum in young rats but not in older rats.[38,39] It has also been postulated that A_{2A} receptors and NMDA receptors may share a common signaling pathway at the level of the striatum.[40]

A number of studies have also characterized the effects of selective A_{2A} receptor antagonists on PPI. Initial studies using the nonselective adenosine receptor antagonists caffeine and theophylline produced conflicting results. Caffeine had no effect on PPI in a study that demonstrated a robust response to amphetamine.[41] However, a subsequent study found that theophylline (10 mg/kg), at a dose that had no intrinsic effect, potentiated the disruption to PPI caused by a submaximal dose of apomorphine. This effect appeared to be A_1 receptor mediated because it was abolished by coadministration of a selective A_1 agonist but not by a selective A_{2A} agonist.[42] The lack of effect of A_{2A} receptor blockade on PPI has been confirmed in studies using the selective A_{2A} receptor antagonist KW6002. This compound was inactive in PPI across a wide range of doses when given alone (figure) and had no effect on the disruption of PPI induced by the D_2 receptor agonist pergolide.[43]

Clinical studies. There have been no reports to date of the effects of selective A_{2A} receptor agonists or antagonists in patients with schizophrenia. Therefore, clinical reports are limited to studies using nonselective adenosine ligands. It is well established that consumption of the nonselective adenosine antagonist caffeine is high in patients with schizophrenia, and this has prompted a considerable effort to establish whether a causal relationship exists between caffeine intake and schizophrenia.[44] Long-term intake of high doses of caffeine can lead to a condition called caffeinism, which is manifested as increased anxiety, arousal, and increased risk of depression in some patients.[45] Case reports of psychotic symptoms such as paranoia and delusions after excessive caffeine intake are rare but do exist.[46] One small double-blind, placebo-controlled study reported a worsening of psychotic symptoms in patients with schizophrenia after short-term administration of high doses of caffeine, but this negative effect was accompanied by improvements in mood and social involvement.[47] However, a number of other clinical studies have failed to find a significant effect of caffeine intake on symptoms in this patient population.[48,49] The lack of consensus regarding caffeine's effects on patients with schizophrenia suggests caffeine consumption is unlikely to contribute to the manifestation of symptoms of the disorder. Furthermore, psychotropic doses of caffeine are widely consumed in food substances and are generally well tolerated.

Clinical experience of the effects of A_{2A} receptor activation is more limited because of the lack of selective brain-penetrant adenosine receptor agonists. The adenosine reuptake inhibitor dipyridamole, which increases extracellular adenosine concentrations by inhibiting its transport into cells, has been examined in a small trial of 30 patients with schizophrenia.[50] This double-blind study compared patients receiving neuroleptic treatment alone (haloperidol 20 mg/d) with those receiving haloperidol (20 mg/d) plus a dipyridamole supplement (75 mg/d) during an 8-week period. The study found that the combination of dipyridamole and haloperidol was superior to haloperidol alone in reducing positive symptoms of schizophrenia. The combination therapy had no additional benefit over haloperidol alone in managing

negative symptoms of the disorder and therefore would appear to offer no advantage compared with atypical antipsychotic drugs. This study did not examine the tolerability of the combined therapy of dipyridamole and haloperidol for either cardiovascular side effects or EPS. The nonspecific pharmacology of dipyridamole, which combines nonselective adenosine receptor activation with potent inhibition of several phosphodiesterases,[51] also limits the conclusions that can be drawn regarding the mechanism responsible for the effects of dipyridamole in this study.

Conclusions. There is a compelling neuroanatomic and neurochemical rationale for the involvement of adenosine receptors in the etiology of schizophrenia and in the induction of psychotomimetic effects induced by certain classes of psychotropic drugs. Attempts to elucidate the potential for central A_{2A} receptor activation for the management of schizophrenia using animal models have been hampered by the nonspecific side effects and pharmacokinetic properties of the available adenosine ligands. These include motor impairment, hypotension, and poor brain penetration of A_{2A} receptor agonists such as CGS 21680. Peripheral side effects may also factor against the development of centrally acting A_{2A} receptor agonists for managing schizophrenia and related disorders. Irrespective of the limitations imposed by side effects of A_{2A} receptor agonists, the behavioral profile of CGS 21680 systemically injected in the PPI paradigm was not consistent with either D_2 receptor agonists or atypical antipsychotic agents. Furthermore, unlike D_2 receptor agonists, the A_{2A} receptor antagonist KW6002 had no intrinsic effect on PPI and failed to potentiate the effects of D_2 receptor agonist pergolide. Data from PPI studies suggest that A_{2A} receptor antagonists are unlikely to induce psychotomimetic effects when given alone or to exacerbate the psychotomimetic effects of dopamine agonists. These findings are encouraging for the development of A_{2A} receptor antagonists to manage PD either as monotherapy or in combination with dopamine agonists.[52]

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Sleep regulation in adenosine A_{2A} receptor-deficient mice

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Adenosine in sleep regulation. Adenosine is proposed to be an endogenous sleep-promoting substance based on the results of a variety of pharmacologic and behavioral experiments.[1] For example, sleep is induced in rats after administration of metabolically stable adenosine analogues, such as N^6-L-(phenylisopropyl)-adenosine, adenosine-5'-N-ethylcarboxamide, and cyclohexyladenosine,[2,3] which are agonists for adenosine A_1 receptor (A_1R) or A_{2A} receptors ($A_{2A}Rs$). Caffeine is considered to inhibit sleep by acting as an antagonist of adenosine receptor.[4] Adenosine content is increased in the basal forebrain, one of the sleep centers, after sleep deprivation and is proposed to be a sleep substance accumulating in the brain during prolonged wakefulness.[5] Most previous studies on sleep regulation by adenosine have focused on the A_1R-mediated pathway[1,6] because A_1R is widely distributed in the CNS, whereas $A_{2A}R$ is localized mainly in the striatum, nucleus accumbens, and olfactory bulb. However, we found that $A_{2A}R$ is also important in sleep regulation by using several A_1R and $A_{2A}R$ agonists, including N6-cyclopentyladenosine (CPA) and 2-(4-(2-carboxyethyl)phenylethylamino)-adenosine-5'-*N*-ethylcarboxamideadenosine (CGS 21680).[7-12] CGS 21680 is highly selective for the $A_{2A}R$ (K_i = 14 nmol/L), having a much lower affinity for the A_1R (K_i = 2,600 nmol/L), whereas CPA is selective for the A_1R (K_i = 0.6 nmol/L) and has a lower affinity for the $A_{2A}R$ (K_i = 462 nmol/L).[13,14]

Effects of $A_{2A}R$ agonists and antagonists on sleep regulation in rats. We investigated the molecular mechanism of induction of non-REM (NREM) sleep by prostaglandin (PG) D_2, which is also known as a potent endogenous sleep-promoting substance.[15-17] In the course of this study, Satoh et al.[7] found that PGD_2-induced NREM sleep, which is also termed slow-wave sleep, was inhibited by pretreatment of rats with $A_{2A}R$ antagonist KF17837 and also demonstrated that $A_{2A}R$ agonist CGS 21680 induced NREM sleep and REM sleep, which is also termed paradoxical sleep or active sleep. These results taken together indicated that $A_{2A}R$ is involved in the PGD_2-induced sleep and probably also in the regulation of physiologic sleep. Induction of NREM and REM sleep was also observed using other $A_{2A}R$-selective agonists, such as 2-(4-(2-(2-aminoethylaminocarbonyl) ethyl) phenylethylamino)-5'-*N*-ethylcarboxamidoadenosine (APEC) or $A_1/A_{2A}R$-nonselective agonist 5'-*N*-ethylcarboxamidoadenosine (NECA).[8,9] In contrast, A_1R-selective agonists, such as cyclohexyladenosine and CPA, were almost completely inactive for sleep induction.[7-9] The sleep-promoting potency of CGS 21680 varied depending on the infusion location: the most effective site was the subarachnoid space of the rostral basal forebrain.[10] Subarachnoid infusion of CGS 21680 (from 0.02 to 20 pmol/min) to this region for 6 hours during the night (the active period of rats) induced NREM and REM sleep in a dose-dependent manner.[7-9] When CGS 21680 was infused at a flow rate of 20 pmol/min for 36 hours, it induced sleep remarkably during the first night but became ineffective 18 hours after the beginning of infusion.[11] Instead, continued CGS 21680 infusion resulted in a rebound of wakefulness, and rats showed almost complete insomnia during the first and second days after infusion, probably because of desensitization of $A_{2A}R$.[11]

Sleep regulation in $A_{2A}R$ knockout mice. To further explore the involvement of adenosine receptor subtypes in sleep regulation, we compared sleep regulation between wild-type mice and $A_{2A}R$ knockout mice[18] of the inbred C57BL/6 strain. Typical results are shown in figures 1 and 2. When CGS 21680 was infused into the lateral ventricle of wild-type mice at 1 pmol/min for 6 hours (8 PM to 2 AM), it increased the amount of NREM sleep by 160% and REM sleep by 180% as compared with the baseline day (see figure 1A). When the infusion rate was increased to 5 pmol/min, the amount of NREM sleep was increased 230% and REM sleep was increased 220%. Although the binding affinity of CPA for A_1R is higher than that of CGS 21680 for $A_{2A}R$, the administration of CPA to wild-type mice did not alter the amounts of NREM and REM sleep (see figure 1B). These results suggest that activation of $A_{2A}R$, but not of A_1R, is involved in the sleep-promoting effect of adenosine in wild-type mice.

Figure 2 shows the sleep-stage distribution of $A_{2A}R$ knockout mice before and after infusion with

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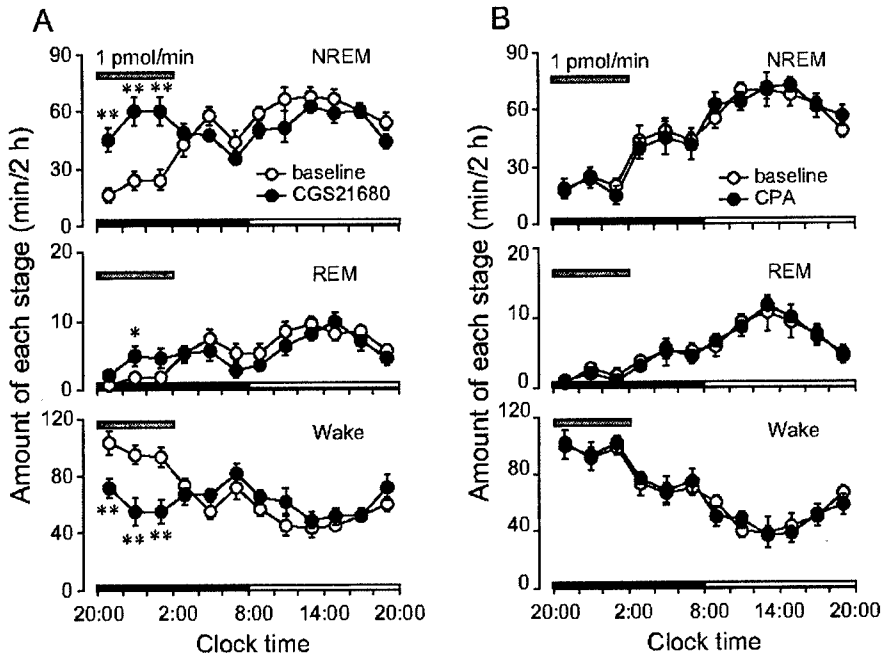

*Figure 1. Sleep-stage distribution produced by CGS 21680 (A) and CPA (B) infusion at a dose of 1 pmol/min into the lateral ventricle of wild-type mice. Male mice (aged 11 to 13 weeks) were maintained in a room at a constant temperature (22 ± 0.5 °C) and constant relative humidity (60 ± 2%) and on a 12:12 light/dark cycle (light on at 8 AM). With pentobarbital anesthesia (50 mg/kg, intraperitoneally), mice were implanted with EEG and EMG electrodes and one stainless-steel cannula for continuous infusion of drugs into the lateral ventricle, as reported previously.[19] After a 10-day recovery period, the mice were placed in experimental cages for adaptation, and the continuous infusion of saline solution into the lateral ventricle was commenced at a speed of 1 μL/h. After an acclimation period of 4 days, sleep-wakefulness states were monitored for 2 days, i.e., baseline and experimental days. CGS 21680 and CPA were administered between 8 PM and 2 AM, indicated by horizontal shaded bars on the experimental day. Open and closed circles stand for the baseline and experimental day profiles, respectively. The EEG/EMG signals were recorded, and the vigilance states were classified by 4-second epochs into NREM, REM, and wakefulness using the "SleepSign" program (Kissei Comtec, Nagano, Japan). Each circle represents 2-hour amounts of NREM, REM, and wakefulness. Values are mean ± SEM (n = 6 in each group). **p < 0.01; *p < 0.05 by paired t-test.*

CGS 21680 or CPA at an infusion rate of 5 pmol/min. During basal conditions, $A_{2A}R$ knockout mice showed clear circadian variations of sleep-stage distribution similar to wild-type mice, with a decrease in sleep during the dark period and an increase during the light period. When CGS 21680 was infused into the lateral ventricle of $A_{2A}R$ knockout mice, the sleep-stage distribution was essentially unchanged (see figure 2A). These results demonstrate that genetic depletion of the $A_{2A}R$ completely abolishes the sleep-promoting effect of CGS 21680, in agreement with the high selectivity of CGS 21680 for $A_{2A}R$. When CPA was administered (5 pmol/min) to $A_{2A}R$ knockout mice, the profile of their sleep-stage distribution

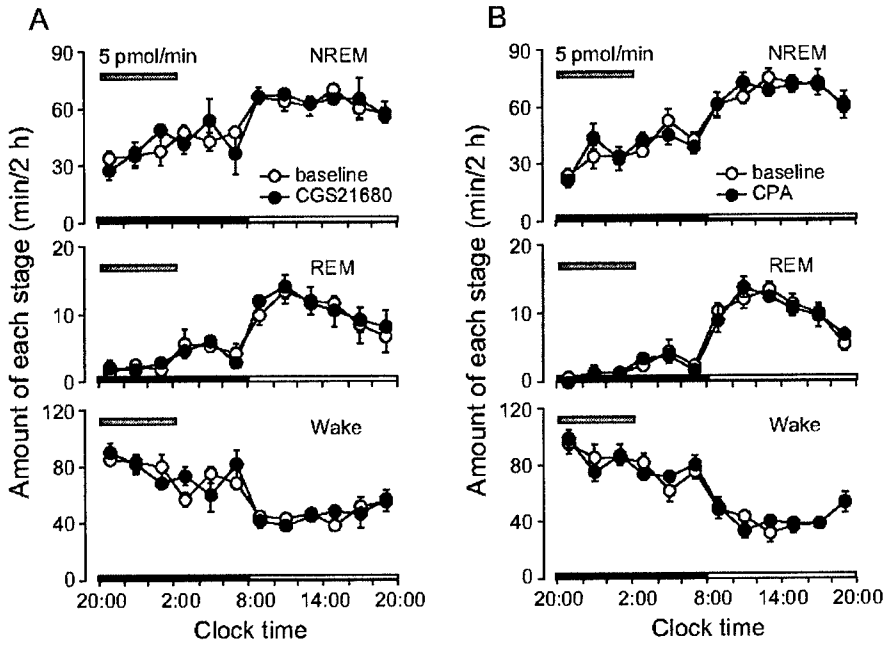

Figure 2. Sleep-stage distribution produced by CGS 21680 (A) and CPA (B) infusion at a dose of 5 pmol/min into the lateral ventricle of $A_{2A}R$ knockout mice. Open and closed circles stand for the baseline and experimental day profiles, respectively. Values are mean ± SEM (n = 3 in CGS 21680 group; n = 6 in CPA group).

was unchanged (see figure 2B). These results suggest that the genetic deficiency of $A_{2A}R$ is not compensated by A_1R in terms of sleep regulation.

PGD_2-induced sleep and rebound sleep after sleep deprivation of $A_{2A}R$ knockout mice. In earlier experiments, when PGD_2 was infused into the lateral ventricle of wild-type mice at a dose of 50 pmol/min for 6 hours during the night, it induced an increase in NREM sleep beginning the first hour after starting the infusion and persisting until 4 hours postinfusion. During the period from 3 hours after starting the infusion to 4 hours after ending the infusion, total NREM sleep time was increased to almost the maximum level observed during the day with normal conditions.[20] The PGD_2 infusion also caused a D type of prostanoid (DP) receptor-dependent increase in the extracellular adenosine concentration in the subarachnoid space of the basal forebrain.[20] To clarify the involvement of $A_{2A}R$ in PGD_2-induced sleep, we infused PGD_2 into the lateral ventricle of $A_{2A}R$ knockout mice at a dose of 50 pmol/min for 6 hours during the night and determined the amounts of NREM and REM sleep. During the 6-hour infusion and continuing for 4 hours postinfusion, PGD_2 induced an increase in NREM sleep in $A_{2A}R$ knockout mice of approximately 40% of the baseline day levels. This increase was significantly less than that seen in PGD_2-infused wild-type mice, in which time spent in NREM sleep increased to 90% of the baseline day level, indicating that the $A_{2A}R$ deficiency attenuates the somnogenic effect of PGD_2 but that PGD_2-induced sleep is partially independent of $A_{2A}R$.

PGD_2 is also involved in the homeostasis of NREM sleep after sleep deprivation because NREM sleep rebound after sleep deprivation was suppressed in PGD synthase knockout mice.[21] We then analyzed the recovery sleep after sleep deprivation of wild-type and $A_{2A}R$ knockout mice for 6 hours. Wild-type mice showed remarkably increased amounts of NREM and REM sleep during the recovery period for 6 hours after sleep deprivation,[21] whereas the $A_{2A}R$ knockout mice did not show such a clear rebound in the amount of NREM sleep, suggesting that $A_{2A}R$ is also essential for NREM sleep rebound after sleep deprivation.

Conclusion and implications for the therapeutic use of A_{2A} antagonists for PD. $A_{2A}R$ is involved in the regulation of NREM and REM sleep because infusion of $A_{2A}R$ agonist CGS 21680 into the lateral ventricle increased the amounts of NREM sleep and REM sleep in wild-type mice but not at all in the $A_{2A}R$ knockout mice. In contrast, A_1R agonist CPA did not affect the sleep profile in either wild-type mice or $A_{2A}R$ knockout mice, suggesting that $A_{2A}R$ is the main adenosine receptor subtype involved in mediating the sleep-promoting effect of adenosine. The amounts of PGD_2-induced NREM sleep and NREM sleep rebound after sleep deprivation were significantly lower in $A_{2A}R$ knockout mice than in wild-type mice, indicating that $A_{2A}R$ is also important for the somnogenic effect of PGD_2 and NREM sleep homeostasis after sleep deprivation. Together, these results support an important role of A_{2A} receptors in sleep regulation and encourage the development of A_{2A} agonists as potential somnogenic drugs and A_{2A} antagonists as potential arousal agents. In addition, these findings highlight the need to carefully monitor the sleep-modifying effects of A_{2A} antagonists as they are evaluated for their therapeutic potential for the management of Parkinson's disease.

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Industry forum: Progress in pursuit of therapeutic A_{2A} antagonists

The adenosine A_{2A} receptor selective antagonist KW6002: Research and development toward a novel nondopaminergic therapy for Parkinson's disease

Hiroshi Kase, PhD

Abstract—Research and development of the adenosine A_{2A} receptor selective antagonist KW6002 have focused on developing a novel nondopaminergic therapy for Parkinson's disease (PD). Salient pharmacologic features of KW6002 were investigated in several animal models of PD. In rodent and primate models, KW6002 provides symptomatic relief from parkinsonian motor deficits without provoking dyskinesia or exacerbating existing dyskinesias. The major target neurons of the A_{2A} receptor antagonist were identified as GABAergic striatopallidal medium spiny neurons. A possible mechanism of A_{2A} receptor antagonist action in PD has been proposed based on the involvement of striatal and pallidal presynaptic A_{2A} receptors in the "dual" modulation of GABAergic synaptic transmission. Experiments with dopamine D_2 receptor knockout mice showed that A_{2A} receptors can function and anti-PD activities of A_{2A} antagonists can occur independent of the dopaminergic system. Clinical studies of KW6002 in patients with advanced PD with L-dopa–related motor complications yielded promising results with regard to motor symptom relief without motor side effects. The development of KW6002 represents the first time that a concept gleaned from A_{2A} biologic research has been applied successfully to "proof of concept" clinical studies. The selective A_{2A} antagonist should provide a novel nondopaminergic approach to PD therapy.

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Since KW6002 was identified as a potent and selective antagonist for adenosine A_{2A} receptors in the early 1990s, research and development of the compound have focused on its potential utility and advantages as a novel nondopaminergic therapy for the management of Parkinson's disease (PD).[1] A number of animal models of PD were used to evaluate its efficacy, side effects, and characteristic features as differentiated from dopaminergic drugs. Physiologic and pathophysiologic functions of A_{2A} receptors were intensively studied in the basal ganglia–thalamocortical circuits in terms of behavioral control to provide basic concepts for the development of A_{2A} antagonists for PD.[1,2]

Biochemical studies. The original adenosine receptor antagonists were xanthines such as caffeine and theophylline,[3] which have little or no selectivity for adenosine receptor subtypes or phosphodiesterases. The xanthine derivative KF17837 [(*E*)-1,3-dipropyl-8-(3,4-dimethoxystyryl)-7-methyl-3,7-dihydo-1*H*-purine-2,6-dione] was synthesized, and its biochemical and pharmacologic properties were identified as the first adenosine A_{2A} "selective" antagonist.[4-6] Subsequently, the diethyl analog KW6002 [(*E*)-1,3-diethyl-8-(3,4-dimethoxystyryl)-7-methyl-3,7-dihydo-1*H*-purine- 2,6-dione] was found to be similar to KF17837 in A_{2A} receptor binding but with a much improved pharmacologic profile in vivo.[1,7] Consequently, KW6002 had high affinity for A_{2A} receptors in rats and humans with K_i values of 2.2 and 12 nmol/L, respectively.[8] The selectivity for A_{2A} receptor over A_1 receptors was 60-fold in rats and 800-fold in humans. It showed little or no affinity (IC_{50} >10 μmol/L) for subtypes of dopamine, noradrenaline, serotonin, and acetylcholine receptors. [^{14}C] KW6002 administered orally was selectively distributed in striatum, nucleus accumbens, olfactory tubercle, and globus pallidus,[9] all brain areas where A_{2A} receptors are enriched.[10,11]

Animal model studies. Studies with animal models of PD revealed nondopaminergic features of KW6002. In rodent models of PD, KW6002 showed anti-PD activities as follows:

1. It prominently attenuated the neuroleptic-induced catalepsy in mice and rats.[12]
2. It antagonized akinesia in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated mice.[12]
3. It potentiated and prolonged the duration of apomorphine-induced turning behavior in 6-hydroxydopamine (6-OHDA)–treated rats.[13]

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In MPTP-treated common marmosets (*Callithrix jacchus*), orally administrated KW6002 showed symptomatic relief of PD as follows:

1. It modestly increased locomotor activity coupled to a significant reversal of motor disability.[14]
2. Abnormal movement such as stereotypy, nausea or vomiting, or other obvious peripheral side effects did not accompany the effect.[14]
3. It potentiated the antiparkinsonian effects of L-dopa without exacerbating its dyskinesia-inducing action.[14]
4. It did not provoke dyskinesia in MPTP-treated marmosets previously exposed to L-dopa, which have dyskinesia in response to all dopaminergic drugs.[14]
5. It did not exhibit tolerance or tachyphylaxis after long-term treatment.[14]
6. It produced additive effects of anti-PD activity when administered in combination with L-dopa or dopamine agonists.[15]

These results with MPTP-treated marmosets were reproduced with MPTP-treated cynomolgus monkeys (*Macaca fascicularis*).[16] In particular, KW6002 led to mild symptomatic improvement in PD symptoms coupled with an inability to provoke established dyskinesias. These studies showed that KW6002 had biochemical and pharmacologic properties distinct from dopaminergic agents such as L-dopa and dopamine agonists.

A_{2A} receptor-mediated mechanisms. Using whole-cell patch-clamp recording in brain slices[17-19] and in vivo microdialysis methods,[20] the major target neurons of A_{2A} receptor-mediated modulation were identified as GABAergic striatopallidal medium spiny neurons (MSNs).[1] These striatal projection neurons may receive A_{2A} receptor-mediated regulation in two distinct modes. The main loci of this adenosine A_{2A} receptor-mediated dual modulation in the striatopallidal system[1,21] are as follows. 1) In the striatum, A_{2A} receptors control excitability of the projection neurons through the intrastriatal GABAergic feedback and feed-forward inhibition network.[17] Major elements regulating the excitability of MSNs in the striatum are GABAergic inputs, which come from axon collaterals of the MSNs themselves and GABAergic interneurons. A_{2A} receptors on the axon terminals of these GABAergic neurons suppress GABA release, resulting in an increase in MSN excitability via relief of intrastriatal GABAergic inhibitory inputs onto the MSNs. This A_{2A} receptor-mediated modulation is further postulated to be involved in information processing within the striatum.[21] 2) In the globus pallidus (GP), A_{2A} receptor activation enhances GABA release from nerve terminals and may suppress excitability of GP projection neurons, which project to the subthalamic nucleus (STN).[18] This enhancement is mediated by a sequentially activated cyclic adenosine monophosphate (cAMP)-dependent cascade, which triggers GABA release by a calcium-independent mechanism.[19]

This "dual modulation" via presynaptic A_{2A} receptors in the striatum and GP is involved in the antiparkinsonian activities of A_{2A} receptor antagonists (figure).[1] In PD and MPTP-treated primates, after destruction of the nigrostriatal dopaminergic pathway, the most relevant alteration is hyperactivity in the striatopallidal pathway. Such hyperactivity is attributed to an imbalance between the direct striatonigral pathway and the indirect striatopallidal pathway and gives rise to the parkinsonian state (see figure, middle).[22,23] Note that A_{2A} receptors are specifically expressed on a subpopulation of MSNs, the striatopallidal MSNs, but not on the striatonigral MSNs.[11,24] Therefore, A_{2A} receptor antagonists selectively block the dual modulation mechanism in the striatopallidal system, leading to suppression of excessive activation in the striatopallidal MSNs (see figure, right). This may shift the striatopallidal/striatonigral neuronal imbalance toward the normal state, resulting in recovery of motor function.

The effect of A_{2A} antagonists is independent of dopamine D_2 receptors,[25] which are colocalized with A_{2A} receptors in striatopallidal MSNs.[26] D_2 receptor knockout mice ($D_2R^{-/-}$) presented a locomotor phenotype with analogies to PD and significantly altered levels of neuropeptide genes expressed in striatal MSNs.[27] No difference in the distribution and level of expression of A_{2A} receptor mRNA and the binding properties of the receptor was found between $D_2R^{-/-}$ and wild-type mice, indicating that the absence of D_2 receptor had no influence on A_{2A} receptor properties.[25] Blockade of A_{2A} receptors with KW6002 reestablished locomotor activity and coordination of movement in $D_2R^{-/-}$ mice and decreased the levels of striatal enkephalin expression to those in normal mice.[25] The results indicate that A_{2A} and D_2 receptors have antagonistic but independent activities in controlling neuronal and motor function in the basal ganglia. Independent functioning of A_{2A} receptors was confirmed by studies using A_{2A} and D_2 receptor knockout mice.[28]

Clinical studies. Based on the foregoing biologic research, we proposed the following clinical development concept for KW6002:

Adenosine A_{2A} receptor selective antagonist for PD therapy.

Effective for monotherapy and combination therapy.

Provide a novel nondopaminergic drug therapy.

A dopamine replacement strategy, using the dopamine precursor L-dopa, has been the most widely used therapy for PD and provides a dramatic benefit to virtually all patients. However, long-term use of L-dopa is often accompanied by motor complications, including involuntary movements associated with the drug's peak effects (peak-dose dyskinesia) and shortening of the therapeutic response (wearing-off). Once established, motor complications are not typi-

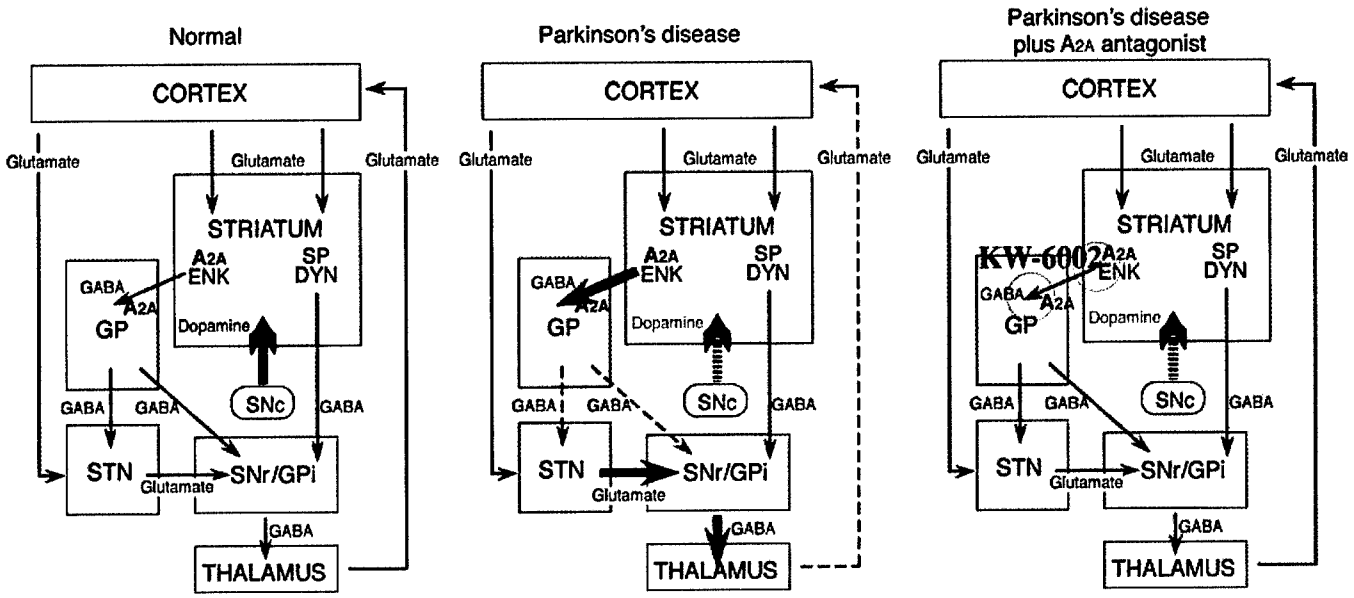

Figure. Schematic of the proposed mechanism of antiparkinsonian activity of A_{2A} receptor antagonists via the dual modulation of striatopallidal medium spiny neurons. (A) Normal condition, (B) Parkinson's disease (PD), and (C) treatment with A_{2A} antagonists in PD. (A) In the normal condition, inhibitory input from the striatonigral direct pathway and disinhibitory input along the striatopallidal indirect pathway are well balanced. At the striatal level, dopamine appears to facilitate transmission along the direct pathway and inhibit transmission along the indirect pathway; these two opposite effects are mediated by dopamine D_1 and D_2 receptors, respectively. Adenosine provides facilitatory control over the indirect pathway via adenosine A_{2A} receptors in the striatum and globus pallidus (GP) by the dual modulation mechanism. (B) Degeneration of nigrostriatal dopaminergic neurons depletes striatal dopamine in PD. The loss of striatal dopamine is thought to disinhibit striatal spiny projection neurons at the origin of the indirect pathway, which leads to a marked suppressed activity of the GP and therefore disinhibition of the subthalamic nucleus (STN). The resulting imbalance between the activity in the direct and indirect pathways leads to the alterations in the internal GP (GPi) and substantia nigra pars reticulata (SNr). Bradykinesia and akinesia observed in PD are postulated to result from increased GABAergic inhibition of thalamic neurons, owing to excessive excitatory drive from the STN to the GPi and SNr. (C) A_{2A} antagonists block the dual modulation of the striatopallidal medium spiny neurons by adenosine, resulting in recovery of GP activity. This relieves excessive excitatory drive from the STN to the GPi and SNr, thereby normalizing the balance between the direct and indirect pathways.

cally controlled with manipulation of L-dopa and other dopaminergic drugs. Features of A_{2A} biology derived from KW6002 studies in animal models and its action mechanism suggested that its clinical development could offer a preferable nondopaminergic alternative for the control of L-dopa–related motor complications.

Two placebo-controlled, double-blind, "proof of concept" studies of KW6002 were conducted in patients with advanced PD and L-dopa–related motor response complications such as wearing-off and peak-dose dyskinesia by the Kyowa 6002-US-001 study group and by Dr. Chase at the National Institutes of Health, both sponsored by Kyowa Pharmaceutical Inc., USA (see Chase et al., page S107).[29,30] Both studies demonstrated the following features of KW6002:

KW6002 was well tolerated.

Off time was significantly reduced compared with placebo.[29]

KW6002 potentiated the antiparkinsonian response at a low dose of infused L-dopa.[30]

Dyskinesia severity was unchanged[29]; or reduced compared with optimal dose of L-dopa.[30]

Studies with KW6002 are ongoing to validate its therapeutic efficacy, safety, advantages, and benefits to patients with PD.

In conclusion, the adenosine A_{2A} receptor selective antagonist KW6002 has been developed as a novel nondopaminergic drug for the management of PD. In particular, therapeutic efficacy and potential advantages of the A_{2A} antagonist have been demonstrated, for the first time, in proof of concept studies for patients with advanced PD and L-dopa motor complications. It may be effective in extending relief for the debilitating symptoms of PD by reducing off time and other complications associated with existing treatments, like dyskinesia. For the mechanism of action of A_{2A} antagonists in PD, involvement of adenosine A_{2A} receptor-mediated dual modulation in the striatopallidal system has been proposed. Adenosinergic function via A_{2A} receptors, which can work independently of the dopaminergic system, may play a critical role in the physiology and pathophysiology of the basal ganglia.

Finally, neuroprotective effects of KW6002 should be noted. Protective effects of KW6002 against dopaminergic neurodegeneration have been demon-

strated in 6-OHDA–lesioned rats and MPTP-treated mice.[31] The results imply that adenosine A_{2A} receptor antagonists not only have an advantage in the symptomatic treatment of patients with PD but also have a therapeutic potential to delay or stop disease progression.

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Discovery of nonxanthine adenosine A_{2A} receptor antagonists for the treatment of Parkinson's disease

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Abstract—During a program to investigate the biochemical basis of side effects associated with the antimalarial drug mefloquine, the authors made the unexpected discovery that the (-)-(*R,S*)-enantiomer of the drug is a potent adenosine A_{2A} receptor antagonist. Although the compound was ineffective in in vivo animal models of central adenosine receptor function, it provided a unique nonxanthine adenosine A_{2A} receptor antagonist lead structure and encouraged the initiation of a medicinal chemistry program to develop novel adenosine A_{2A} antagonists for the management of Parkinson's disease (PD). The authors have synthesized and screened more than 2,000 chemically diverse and novel adenosine A_{2A} antagonists. Early examples from two distinct chemical series are the thieno[3,2-dy]pyrimidine VER-6623 and the purine compounds VER-6947 and VER-7835, which have high affinity at adenosine A_{2A} receptors (K_i values 1.4, 1.1, and 1.7 nmol/L, respectively) and act as competitive antagonists. In particular, VER-6947 and VER-7835 demonstrate potent in vivo activity reversing the locomotor deficit caused by the D_2 receptor antagonist haloperidol, with minimum effective doses comparable with that of KW6002 (0.3 to 1 mg/kg). In conclusion, the authors have discovered potent, selective, and in vivo active nonxanthine adenosine A_{2A} antagonists that have considerable promise as a new therapy for PD.

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From malaria to a new class of A_{2A} antagonists. The endogenous neuromodulator adenosine plays an important role in regulating a number of physiologic functions within the nervous system. In the CNS, adenosine is colocalized with a variety of neurotransmitters in presynaptic terminals from which it is released after depolarization to modulate neuronal activity.[1] Adenosine binds to specific cell surface G protein-coupled receptors, which have been classified into four distinct subtypes: A_1, A_{2A}, A_{2B}, and A_3.[2,3] A_1 receptors are widely distributed throughout the brain, where they regulate neurotransmitter release and neuronal firing by activating potassium channels. In contrast, the distribution of A_{2A} receptors is heterogeneous with high levels of expression in the nucleus accumbens, olfactory tubercle, and striatum, where A_{2A} receptors colocalize with dopamine D_2 receptors.[4,5] When considered with the important role of dopamine in the control of motor activity and in the etiology and management of Parkinson's disease (PD), this observation suggested that adenosine A_{2A} receptors could be a novel target for drugs to manage movement disorders.[4] There is now accumulating preclinical and clinical evidence that A_{2A} antagonists may provide a novel therapy for PD with a lower risk of dyskinesias.[6-8]

Mefloquine (Lariam, Roche Pharmaceuticals, Nutley, NJ; (*R**,*S**)-(±)-α-2-piperidinyl-2,8-bis(trifluoromethyl)-4-quinolinemethanol) is an antimalarial drug chemically related to quinine that is effective against multidrug resistant strains of *Plasmodium falciparum*, the protozoan parasite responsible for malaria. Although generally well tolerated, a number of clinical reports have emerged that suggest that mefloquine is associated with infrequent but severe neuropsychiatric side effects, which include disturbed sleep, heightened anxiety, panic attacks, depression, psychosis, and seizures.[9-14] The mechanism responsible for these effects is not known.

Mefloquine is an asymmetric molecule that is marketed as a racemic mixture consisting of equal parts of (-)-(*R,S*)-mefloquine and (+)-(*S,R*)-mefloquine enantiomers (figure 1).[15] Both enantiomers are reported to possess equal antimalarial potency against *P. falciparum* in vitro[16] and have been shown to penetrate the brain.[15,17] Therefore, either one or both of the enantiomers could account for the adverse CNS effects of the racemate. In an attempt to identify the mechanism responsible for the neuropsychiatric effects of mefloquine, both enantiomers were assessed in a series of receptor binding, enzyme, and functional tests. In addition, mefloquine enantiomers were assessed for overt in vivo behavioral effects in rats after short- and long-term dosing.

Unexpectedly, the results of these studies revealed that the (-)-(*R,S*)-enantiomer of mefloquine is

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Dr. Dourish reported equity or ownership in Vernalis in excess of $10,000/year.

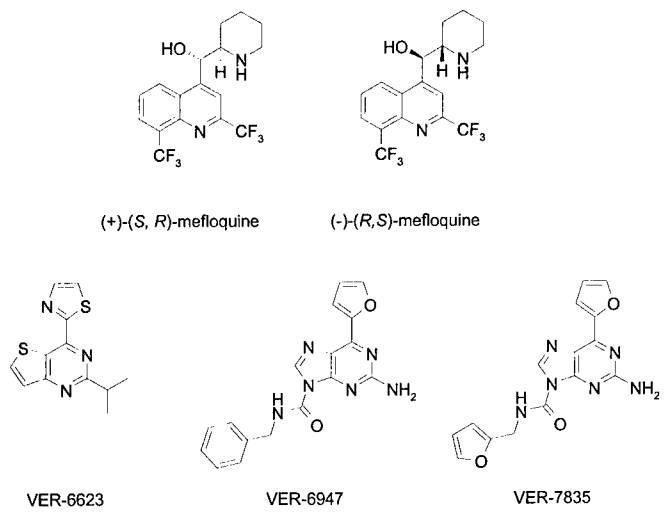

Figure 1. Chemical structures of the (+)-(S,R) and (-)-(R,S)-enantiomers of mefloquine and the Vernalis A_{2A} receptor antagonists VER-6623, VER-6947, and VER-7835.

a potent and moderately selective A_{2A} antagonist. In an initial examination of the effects of mefloquine on 81 receptors and enzymes, the only significant interaction identified was displacement of high-affinity binding to bovine striatal adenosine receptors. In subsequent binding studies using membranes from cells expressing human adenosine receptor subtypes, (-)-(*R,S*)-mefloquine was found to possess high affinity for A_1 and A_{2A} receptors, with K_i values of 255 and 61 nmol/L, respectively, and limited affinity for either the A_{2B} or A_3 receptor subtypes (K_i >6 μmol/L). The binding profile of racemic mefloquine was comparable with that of (-)-(*R,S*)-mefloquine, whereas (+)-(*S,R*)-mefloquine was found to have low affinity for adenosine receptors (table). Functional activity was determined in cells by assessing Ca^{2+} mobilization using a fluorescence imaging plate reader (FLIPR).[18] Because activation of adenosine receptors does not produce a Ca^{2+} signal, cell lines that coexpress adenosine receptors and the promiscuous G protein $G\alpha_{16}$ were employed for use in FLIPR. In FLIPR studies, (-)-(*R,S*)-mefloquine and racemic mefloquine were found to be competitive antagonists. Schild analysis revealed pA_2 values at the A_{2A} receptor of 6.96 for (-)-(*R,S*)-mefloquine and 6.61 for racemic mefloquine, whereas at the A_1 receptor, these compounds were found to possess pA_2 values of 6.40 and 6.21, respectively.

However, (-)-(*R,S*)-mefloquine was ineffective in in vivo animal models, and intracerebral microdialysis studies revealed that this was probably because of the poor ability of the compound (which is highly lipophilic) to partition to the extracellular compartment and bind to receptors on the cell surface. Nevertheless, this discovery provided a unique nonxanthine A_{2A} antagonist lead structure; therefore, we initiated a medicinal chemistry program to develop novel A_{2A} antagonists for the management of PD.

Table *Comparison of the binding affinities of ZM-241385, SCH-58261, and KW-6002 with mefloquine and its enantiomers and novel compounds arising from the Vernalis A_{2A} receptor antagonist program*

Receptor	hA_{2A}	hA_1	hA_{2B}	hA_3
Radioligand	[^{3}H]-CGS21680	[^{3}H]-DPCPX	[^{3}H]-ZM241385	[^{125}I]-ABMECA
Ligand Concentration	20 nmol/L	4 nmol/L	20 nmol/L	0.05 nmol/L
Compound				
ZM-241385	1.56 ± 0.10	774 ± 68.3 (496)	75.3 ± 4.17 (48)	743 ± 43.8 (476)
SCH-58261	4.98 ± 1.29	725 ± 525 (145)	1,115 ± 186 (224)	1,205 ± 313 (242)
KW-6002	35.9 ± 4.76	2,830 ± 663 (79)	1,801 ± 451 (50)	> 3,000 (>84)
racemic mefloquine	104 ± 19	675 ± 167 (6)	8,219 ± 4,079 (79)	6,377 ± 1,794 (61)
(+)-(*S,R*)-mefloquine	6,553 ± 1,122	14,044 ± 3,533 (2)	26,995 ± 14,625 (4)	5,883 ± 1,618 (1)
(−)-(*R,S*)-mefloquine	61 ± 6	255 ± 19 (4)	7,072 ± 3,409 (116)	6,941 ± 2,159 (114)
VER-6409	2.3 ± 0.0	366 ± 40 (156)	1,560 ± 78 (665)	1,759 ± 32 (750)
VER-6440	3.6 ± 0.3	448 ± 55 (123)	1,831 ± 307 (503)	1,741 ± 323 (478)
VER-6489	1.3 ± 0.6	303 ± 33 (227)	1,032 ± 55 (773)	2,083 ± 10 (1,560)
VER-6623	1.4 ± 0.3	208 ± 15 (151)	865 ± 150 (629)	476 ± 47 (346)
VER-6947	1.1 ± 0.2	17 ± 3 (15)	112 ± 22 (100)	1,472 ± 142 (1,307)
VER-7130	1.2 ± 0.3	70 ± 3 (57)	202 ± 10 (164)	2,375 ± 38 (1,925)
VER-7146	2.2 ± 0.0	231 ± 26 (104)	2,156 ± 428 (975)	1,958 ± 236 (886)
VER-7448	3.2 ± 0.4	518 ± 24 (164)	2,408 ± 21 (764)	1,584 ± 245 (503)
VER-7835	1.7 ± 0.2	170 ± 24 (99)	141 ± 55 (81)	1,931 ± 151 (1,116)
VER-8177	3.8 ± 0.6	2,299 ± 31 (598)	2,313 ± 27 (602)	2,883 ± 1 (750)

Assays used human receptors stably expressed in cell lines. Values represent mean K_1 ± SEM of at least three values. For A_1, A_{2B}, and A_3 receptors, the numbers in parentheses are selectivity ratios for the A_{2A} receptor.

Discovery of novel nonxanthine A_{2A} antagonists. *Chemistry strategy.* The initial aims of the medicinal chemistry program were to increase selectivity for the A_{2A} receptor and to discover novel compounds with improved physicochemical properties and in vivo activity.

The design of novel ligands was facilitated by the construction of receptor homology models based on the x-ray crystal structure of bovine rhodopsin for all four adenosine receptor subtypes.[19] A large number (>2,000) of chemically diverse novel compounds were synthesized using an iterative medicinal chemistry approach and by the construction of small compound libraries by parallel synthesis.

Initial modification of close analogues of the lead structure, including an investigation of the impact of stereochemical changes, removal of either or both chiral centers, and simplification of the chiral side chain, led to compounds with improved physicochemical properties and in particular with reduced lipophilicity.[20,21] Replacement of the highly lipophilic trifluoromethyl substituents with a range of other groups to reduce lipophilicity was also investigated.

Modifications to the core ring system led to the discovery of a series of novel thieno[3,2-dy]pyrimidines with significantly improved affinity for the A_{2A} receptor.[22] Many compounds in this series show good selectivity, and several are active in vivo. Further chemistry led to the discovery of a second series of thieno[3,2-dy]pyrimidines, many of which are potent and highly selective with improved in vivo activity.[23]

To further optimize physicochemical properties, we synthesized a range of compounds incorporating additional modifications to the core ring structure. This approach led to the discovery of a series of novel purines with improved "drug-like" properties.[24] This series contains many potent and selective compounds, including a number, such as VER-6947, that show potent in vivo activity.

Biologic screening program. Radioligand binding assays were carried out for all four human adenosine receptor subtypes. A_1 receptors were labeled using [^{3}H]-DPCPX;[25] A_{2A} receptors were labeled using [^{3}H]-CGS 21680;[26] A_{2B} receptors were labeled using[^{3}H]-ZM241385;[27] and A_3 receptors were labeled using [^{125}I]-AB-MECA.[28] Nonspecific binding was defined with 10 μmol/L CHA, CGS 21680, ZM241385, and IB-MECA, respectively. Novel compounds were tested in duplicate at 11 concentrations between 10^{-11} and 10^{-5} mol/L, and inhibition curves were fitted by nonlinear regression (Prism, GraphPad Software Inc., San Diego, CA). K_i values were calculated by the method of Cheng and Prusoff .[29] All values are the mean of at three separate values. Confirmation of functional antagonism of compounds was determined in cells by assessing Ca^{2+} mobilization using FLIPR as described previously for studies with mefloquine.

In vivo efficacy of compounds was determined using a range of models in rodents and primates. Initial in vivo screening was conducted in mice and rats by assessing the ability of novel compounds to reverse locomotor deficits induced by the dopamine receptor antagonist haloperidol. Subsequently, active compounds were assessed for antiparkinsonian efficacy in rats unilaterally lesioned with 6-hydroxydopamine (6-OHDA).

In vitro profile of novel A_{2A} antagonists. A large number (>2,000) of chemically diverse and novel A_{2A} antagonists have been synthesized and screened. The ligand-binding profiles of some early examples of these molecules are illustrated in the table. All of the compounds are potent A_{2A} receptor ligands with K_i values of <4 nmol/L and have receptor affinities similar to or greater than the reference standards, ZM241385, SCH 58261, and KW6002. A number of the compounds are also highly selective for A_{2A} receptors and are >100-fold less potent in displacing binding to A_1, A_{2B}, and A_3 receptors. For example, VER-6623 has a K_i value of 1.4 nmol/L at A_{2A} receptors and is 100-fold, 600-fold, and 300-fold selective over A_1, A_{2B} and A_3 receptors, respectively. This selectivity profile compares favorably with competitor compounds, particularly KW6002, which has a K_i of 31 nmol/L at A_{2A} receptors and is 79-fold, 50-fold, and 84-fold selective over A_1, A_{2B}, and A_3 receptors, respectively.

In FLIPR studies, all Vernalis compounds listed in the table acted as competitive antagonists, producing dose-related shifts to the right in the dose–response curve of the A_{2A} agonist CGS 21680 (10 μmol/L) without depressing its maximal response, and exhibited no appreciable agonist activity in the assay when tested alone (EC_{50} values ⩾10 μmol/L). Schild analysis was used to calculate pA_2 values of 8.76 for VER-6623, 9.57 for VER-6947, and 9.32 for VER-7835 that compare favorably with KW6002, which has a pA_2 of 8.36 at A_{2A} receptors.

In vivo profile of novel A_{2A} antagonists. A temporary form of parkinsonism can be induced in animals and humans by using agents that block central dopaminergic neurotransmission, such as through blockade of dopamine receptors by dopamine receptor antagonists (e.g., haloperidol) or by depleting dopamine storage or inhibiting dopamine release (e.g., by reserpine). Animals treated with haloperidol or reserpine show an impairment of movement, which can range from a mild reduction in locomotor activity to a state of catalepsy, whereas poisoning with these agents leads to parkinsonism in humans[30]. These effects mirror some of the motor impairments observed in patients with PD, which are also attributable to loss of striatal dopamine. The validity of these models has been established by experiments that show that reference antiparkinsonian agents, such as L-dopa, reverse the loss of movement caused by dopamine receptor antagonists and reserpine. Recent studies have also demonstrated the ability of A_{2A} antagonists to reverse haloperidol- and reserpine-induced catalepsy.[31,32]

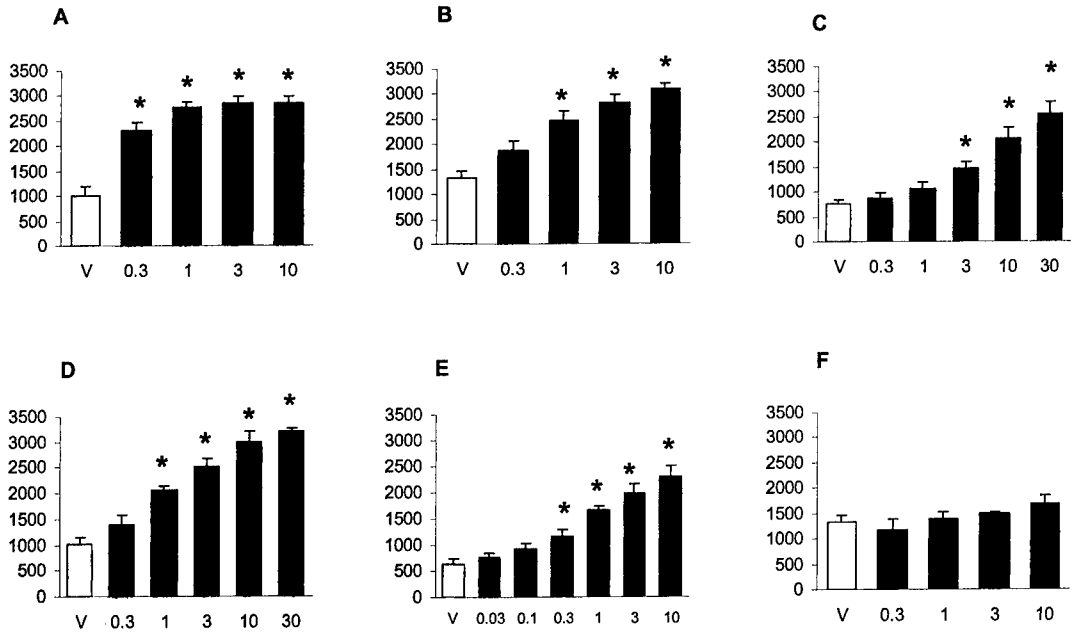

*Figure 2. Reversal of locomotor motor deficits in mice treated with the D_2 receptor antagonist haloperidol (0.2 mg/kg, intraperitoneally) by the A_{2A} receptor antagonists (A) KW6002, (B) CGS 15943, (C) SCH 58261, (D) VER-6947, and (E) VER-7835 but not by the A_1 receptor antagonist (F) DPCPX. All doses are expressed as mg/kg, intraperitoneally. Behavioral scores represent time (s) spent active during a 60-minute test session. *$p < 0.05$ vs vehicle treatment group.*

The primary screen used to assess the in vivo A_{2A} antagonist properties of novel compounds was reversal of the reduction in locomotor activity induced by the dopamine D_2 receptor antagonist haloperidol in mice. This assay provides a relatively high-throughput pharmacodynamic model to rapidly assess bioavailability, duration of action, and brain penetration of novel A_{2A} antagonists. In addition, the assay requires only small quantities of material for testing (~10 mg), thus permitting assessment of compounds originating from small-scale parallel synthesis chemistry.

To validate the model as a screen for the identification of A_{2A} antagonist activity in vivo, experiments were carried out with nonselective and selective adenosine receptor antagonists. The selective A_{2A} antagonists SCH 58261 (0.3 to 30 mg/kg, intraperitoneally) and KW6002 (0.3 to 10 mg/kg, intraperitoneally), the nonselective A_1/A_{2A} antagonist CGS 15943 (0.3 to 10 mg/kg, intraperitoneally), and the A_1 selective antagonist DPCPX (0.3 to 10 mg/kg, intraperitoneally) were assessed for their ability to reverse the motor depressant effects of haloperidol (0.2 mg/kg, intraperitoneally) in outbred albino mice during a 60-minute test session. Haloperidol-induced hypolocomotion was dose-dependently reversed by selective and nonselective A_{2A} antagonists, with a rank order of potency of KW6002 > CGS 15943 > SCH 58261. The A_1 selective antagonist DPCPX was ineffective in the assay (figure 2). The screening of novel compounds from our medicinal chemistry program identified a number of compounds that had potent A_{2A} antagonist effects in the haloperidol hypolocomotion model. All of the Vernalis compounds listed in the table, with the exception of VER-8177, displayed moderate to good in vivo efficacy in the assay. In particular, VER-6947 and VER-7835 dose-dependently reversed the decrease in locomotion induced by haloperidol and had potencies similar to KW6002, with minimum effective doses in the range of 0.3 to 1 mg/kg (see figure 2).

To assess the potential therapeutic efficacy of novel compounds in a rodent model of PD, the 6-OHDA rotation assay was used.[33] Rats were lesioned unilaterally by injection of the catecholamine neurotoxin 6-OHDA into the medial forebrain bundle. After a recovery period of 2 weeks, animals were administered the dopamine agonist apomorphine (0.3 mg/kg, subcutaneously), and the number of rotations made in an ipsilateral and contralateral direction was recorded using an automated device during a 60-minute test session. Apomorphine testing was conducted at weekly intervals for at least 3 weeks, and those animals that displayed at least 100 contralateral rotations during the test session were selected for further adenosine receptor antagonist studies. The A_{2A} antagonists KW6002 and VER-6947 enhanced the contralateral rotation response to a subthreshold dose of L-dopa (figure 3). Interestingly, unlike dopamine agonists or L-dopa, both adenosine receptor antagonists tested produced small increases in ipsilateral rotation, with the effects of KW6002 reaching statistical significance. Ipsilateral rotation in this model can be indicative of a presynaptic effect on dopamine release and is observed, for example, after administration of amphetamine.[33] However, unlike amphetamine, which produces little or no contralateral rotation, the effects of KW6002 and VER-6947 on contralateral rotations were approximately

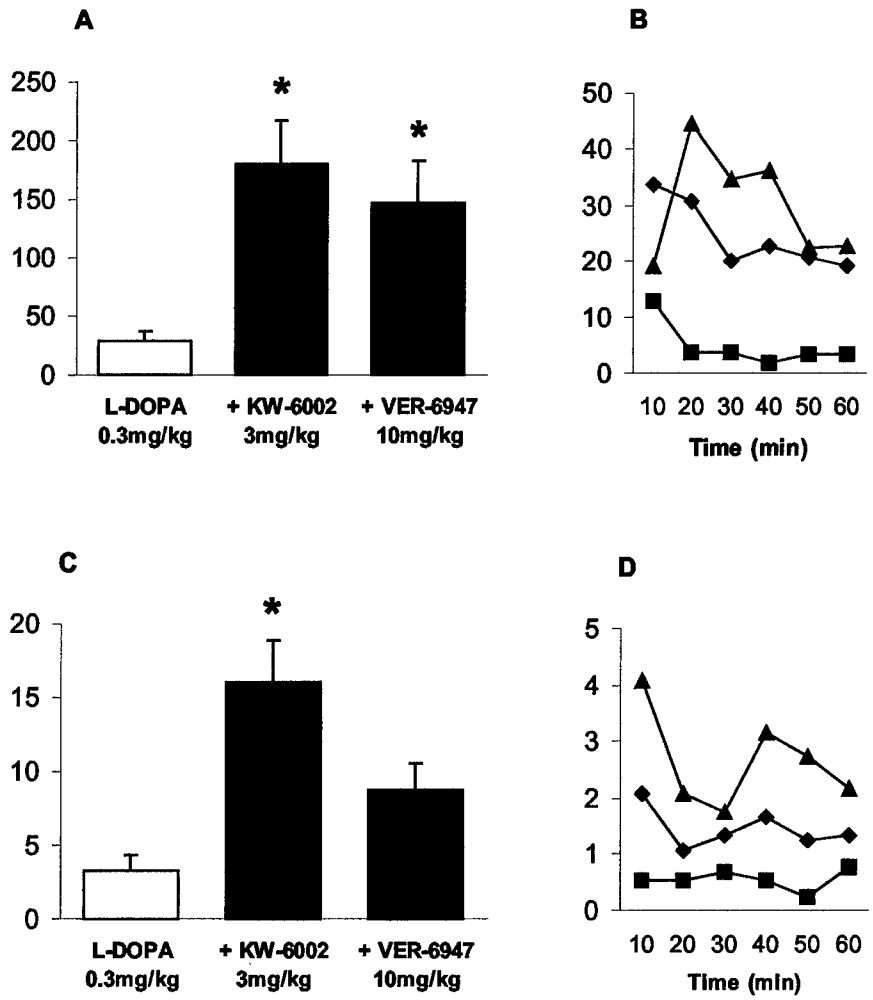

*Figure 3. Effects of L-dopa alone (■) and given in combination with the adenosine receptor antagonists KW6002 (▲) and VER-6947 (♦) on turning behavior in rats unilaterally lesioned with the neurotoxin 6-hydroxydopamine. (A) Total contralateral rotation scores, (B) time course for contralateral rotations, (C) total ipsilateral rotation scores, and (D) time course for ipsilateral rotation scores. For total scores, *p < 0.05 vs L-dopa–treated group.*

an order of magnitude greater than their corresponding effects on ipsilateral rotation. Furthermore, the effects of both adenosine receptor antagonists on ipsilateral rotation were considerably smaller in magnitude than those induced by amphetamine in our hands (data not shown). Although it is possible to interpret these data as evidence of a direct effect of A_{2A} receptor blockade on dopamine release, the profile of A_{2A} antagonists in this model is clearly different from that of amphetamine. Because other investigators using this model have not commonly reported A_{2A} antagonist-induced ipsilateral rotations in 6-OHDA–lesioned animals, the robustness of these findings will depend on replication in subsequent studies. The fact that these findings are not more widely observed suggests that A_{2A} antagonist-induced ipsilateral rotations depend on subtle methodologic factors in this assay, such as previous exposure to dopamine agonists and the degree of dopamine receptor sensitization induced before behavioral testing.

Summary and conclusions. In summary, the discovery that the (-)-(*R,S*)-enantiomer of the antimalarial mefloquine is a potent and moderately selective adenosine A_{2A} antagonist provided a unique nonxanthine lead structure for a medicinal chemistry program to develop novel A_{2A} antagonists. We have established and validated a comprehensive in vitro and in vivo screening program and synthesized and screened more than 2,000 chemically diverse and novel adenosine A_{2A} antagonists. VER-6623, VER-6947, and VER-7835 are early examples of potent, selective adenosine A_{2A} antagonists that have been identified from this program. VER-6947 and VER-7835 have good in vivo activity, with minimum effective doses of 0.3 to 1 mg/kg in a mouse haloperidol hypolocomotion model. Furthermore, equipotent doses of VER-6947 and KW6002 were shown to induce comparable increases in contralateral rotations in rats unilaterally lesioned with 6-OHDA.

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Translating A_{2A} antagonist KW6002 from animal models to parkinsonian patients

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Abstract—Improving the translation of novel findings from basic laboratory research to better therapies for neurologic disease constitutes a major challenge for the neurosciences. This brief review of aspects of the development of an adenosine A_{2A} antagonist for use in the management of Parkinson's disease (PD) illustrates approaches to some of the relevant issues. Adenosine A_{2A} receptors, highly expressed on striatal medium spiny neurons, signal via kinases whose aberrant activation has been linked to the appearance of parkinsonian signs after dopaminergic denervation and to the motor response complications produced by dopaminomimetic therapy. To assess the ability of A_{2A} receptor blockade to normalize certain of these kinases and thus benefit motor dysfunction, the palliative and prophylactic effects of the selective antagonist KW6002 were first evaluated in rodent and primate models. In hemiparkinsonian rats, KW6002 reversed the intermittent L-dopa treatment-induced, protein kinase A-mediated hyperphosphorylation of striatal α-amino-3-hydroxy-5-methyl-4-isoxazole proprionic acid receptor GluR1 S845 residues and the concomitant shortening in motor response duration. In 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-lesioned monkeys, coadministration of KW6002 with daily apomorphine injections acted prophylactically to prevent dyskinesia onset. These and related preclinical observations guided the design of a limited, randomized, controlled, proof-of-concept study of the A_{2A} antagonist in patients with moderately advanced PD. Although KW6002 alone or in combination with a steady-state IV infusion of optimal-dose L-dopa had no effect on parkinsonian severity, the drug potentiated the antiparkinsonian response to low-dose L-dopa with fewer dyskinesias than produced by optimal-dose L-dopa alone. KW6002 also safely prolonged the efficacy half-time of L-dopa. The results suggest that drugs capable of selectively blocking adenosine A_{2A} receptors could confer therapeutic benefit to L-dopa–treated parkinsonian patients and warrant further evaluation in phase II studies. They also illustrate a strategy for successfully bridging a novel approach to PD therapy from an evolving research concept to pivotal clinical trials.

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Progress in basic neuroscience research has accelerated dramatically during the past decade as a result of the increasing support accorded this field. But translation of the findings of these laboratory efforts to the benefit of patients with neurologic disease has not always kept pace. The sluggish transfer of novel therapeutic strategies from bench to bedside may be a key contributor to the dry developmental pipelines currently plaguing much of the pharmaceutical industry.[1] Despite a threefold increase in investment, total new drug applications approved by the Food and Drug Administration have fallen to the lowest levels in 10 years. The decrease in new molecular entities and priority drug approvals is even steeper. Although a number of factors contribute to this slowdown, the problem can be reduced by a tighter focus on essential preclinical assessments, based on heuristic mechanistic hypotheses and validated animal model results, and the rapid use of small-scale, proof-of-concept clinical studies. Additionally, laboratory observations not only help identify interventions with a relatively high probability of success but also clinical findings can be equally important in directing further laboratory efforts. Our recent studies of a selective adenosine receptor antagonist illustrate some of these principles and emphasize the critical need for a symbiotic relationship between basic and clinical research.

In the movement disorders field, inadequacies surrounding the use of currently available treatments for patients with middle- and late-stage Parkinson's disease (PD) have long motivated the search for alternative therapeutic approaches. Recently, these novel strategies have been increasingly based on the hypothesis that motor dysfunction in PD results, in part, because of reactive changes occurring in striatal medium spiny neurons as a consequence of the nonphysiologic stimulation of their dopaminergic receptors.[2] Medium spiny neurons, the preponderant nerve cell in the corpus striatum, receive axonal terminals from the degenerating nigrostriatal dopamine (DA) system and glutamatergic projections from the cerebral cortex.[3] In turn, they project to the output nuclei of the basal ganglia and thus are well situated to regulate information flow into and through these structures.[4] In rodent models of PD, spiny neuron function appears to change as dopaminergic innervation decreases.[5,6] Additional spiny neuron changes occur when their denervated DA receptors are subjected to the intermittent high-

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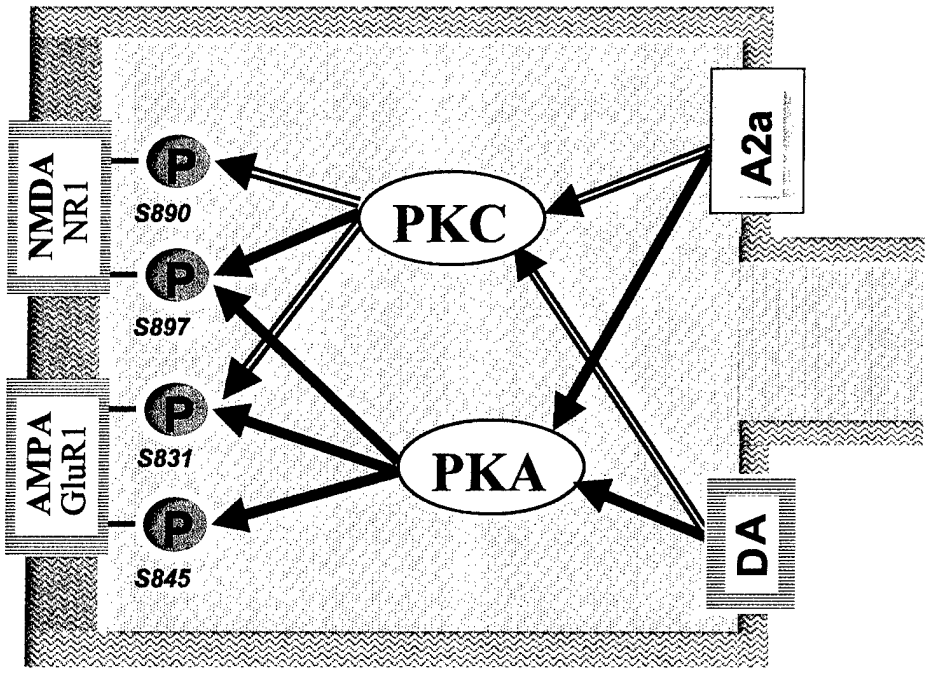

Figure. Schematic representation of dendritic spine of striatal medium spiny neuron to illustrate signaling pathways possibly contributing to the regulation of ionotropic glutamatergic receptors by dopamine (DA) and A_{2A} receptors. DA receptors can signal via cyclic adenosine monophosphate (cAMP) and Ca^{2+}-mediated cascades (via protein kinase A [PKA] and protein kinase C [PKC]/ CaMKII, respectively) to modify the phosphorylation state of nearby α-amino-5-hydroxy-3-methyl-4-isoxazolyl propionate (AMPA) and NMDA receptors. By this means, the aberrant activation of certain of these spiny neuron pathways, first because of DA receptor denervation and subsequently because of their intermittent stimulation, may affect the synaptic strength of corticostriatal input. As a result, spiny neuron output may change in ways that favor the clinical appearance of parkinsonism and motor response complications. Adenosine A_{2A} receptors also appear able to signal through PKC and PKA. Accordingly, A_{2A} antagonists may be able to attenuate the activation of these kinases by the nonphysiologic stimulation of dopaminergic receptors and thus inhibit the hyperphosphorylation of glutamatergic receptor subunits. Representative serine phosphorylation sites on AMPA, GluR1, and NMDA NR1 subunits are labeled. Filled lines indicate direct pathways supported by earlier experimental data; open lines indicate indirect pathways suggested by our recent studies.

intensity stimulation associated with standard dopaminomimetic regimens.[6,7] Soon the animals manifest alterations in motor response to dopaminergic agents, mimicking those that appear in patients with later-stage PD.[8] DA receptor denervation and intermittent stimulation appear to influence signaling kinases and phosphatases within spiny neurons that regulate the phosphorylation state and thus the synaptic efficacy of coexpressed ionotropic glutamatergic receptors.[5,6] Resultant changes in cortical glutamatergic input then modify striatal GABAergic output in ways that compromise motor function.

Pharmacologic agents that inhibit pathologic events in spiny neurons occurring as a consequence of the nonphysiologic stimulation of their DA receptors could have the potential for ameliorating associated motor dysfunction. Recent observations suggest that drugs capable of interacting with striatal adenosinergic receptors may act in this manner. The A_{2A} subtype of adenosine receptor is abundantly expressed on medium spiny neurons.[9,10] A_{2A} receptors signal, in part, through activation of protein kinase C (PKC) and protein kinase A (PKA) (figure).[11-14] Serine and threonine kinases appear to be aberrantly activated in rat models of parkinsonism and motor complications[15,16] and are capable of modulating the phosphorylation state of ionotropic glutamatergic receptors.[17,18] Conceivably, A_{2A} receptor blockade may alleviate motor abnormalities in patients with PD by attenuating the hyperphosphorylation of striatal ionotropic glutamatergic receptors of the α-amino-5-hydroxy-3-methyl-4-isoxazolyl propionate (AMPA) and NMDA subtypes associated with naturally occurring dopaminergic denervation and iatrogenic intermittent stimulation.

Recent observations in rodent and primate models of PD[19-24] have provided increasing support for this possibility. A selective A_{2A} receptor antagonist, KW6002,[25] for example, has been reported to have antiparkinsonian activity when given alone and to potentiate the antiparkinsonian response to coadministered L-dopa without exacerbating dyskinesias in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-lesioned nonhuman primates.[23-25] Moreover, coadministration of the A_{2A} antagonist SCH 58261 or genetic knock out of the A_{2A} receptor has been shown to attenuate L-dopa–induced rotational behavioral sensitization in hemiparkinsonian rodents.[22,26] Therefore, it is conceivable that A_{2A} receptor blockade by KW6002 may be beneficial in PD by mitigating changes in the phosphorylation state of certain striatal glutamate receptor subunits. Moreover, assuming KW6002 acts in this manner, it should be effective when administered either palliatively or prophylactically. These possibilities are readily testable in validated animal models and, if positive, would provide a cogent basis for initiating studies of A_{2A} antagonist therapy in patients with PD. Related efforts by the drug manufacturer are reviewed elsewhere in this supplement (see Kase, page S97).

KW6002 effects on motor function in parkinsonian rats. Our initial explorations of the foregoing considerations were conducted in male Sprague–Dawley rats that evidenced hemiparkinsonism after unilateral 6-hydroxydopamine (6-OHDA) nigrostriatal system lesioning.[27] Systemic administration of low-dose KW6002 (1 mg/kg, orally; Castagnoli et al., personal observation) given alone did not produce rotational behavior in lesioned animals that had been treated only briefly (3 days) with L-dopa; however, high-dose KW6002 (5 and 10 mg/kg) did increase the number and duration of contralateral rotations ($p < 0.01$ compared with 0- and 1-mg/kg KW6002 groups).

Like patients with PD, parkinsonian rats manifest the progressive shortening in the duration of their motor response to intermittent L-dopa treatment that gives rise to response fluctuations of the wearing-off type.[8] In our studies, twice-daily L-dopa treatment for 22 days attenuated the duration of the response to L-dopa ($p < 0.001$). On day 23 of L-dopa treatment, the administration of KW6002 (1 mg/kg and higher), but not of the vehicle, normalized the motor response shortening induced by long-term L-dopa therapy ($p < 0.05$). Moreover, coadministration of KW6002 (1.0 mg/kg), but not the vehicle, acted prophylactically to block the progressive reduction in response duration occurring in rats receiving long-term L-dopa treatment. Accordingly, animals given KW6002 together with L-dopa had a substantially longer response to L-dopa than those receiving the vehicle with L-dopa after 21 days of coadministration ($p < 0.01$).

Therefore, the prophylactic and palliative administration of KW6002 appeared to ameliorate the shortened motor response to L-dopa associated with the long-term intermittent stimulation produced by dopamine precursor treatment. In 6-OHDA–lesioned rats, a relatively low dose of KW6002 was sufficient to prevent or reverse the shortened response duration. Similarly, a recent report has shown that the A_{2A} receptor antagonist 8-(3-chlorostryryl) caffeine reverses L-dopa–induced motor alterations in parkinsonian rats.[28] Unlike typical dopaminomimetic agents, the same KW6002 dose failed to elicit an antiparkinsonian response (i.e., induce contralateral turning) when given alone. As previously reported,[23] KW6002 monotherapy did show antiparkinsonian activity at higher doses. Clearly, the effects of A_{2A} receptor blockade differ from those typically associated with dopamine agonists or antagonists.

KW6002 effects on P-GluR1–immunopositive neurons in rat striatum. The twice-daily administration of L-dopa to hemiparkinsonian rats enhanced the phosphorylation of striatal AMPA receptor GluR1 subunits at serine residue S845.[27] By L-dopa treatment day 21, P-GluR1 immunoreactivity appeared far more pronounced on the side ipsilateral to the lesion than on the contralateral side. KW6002 treatment, but not vehicle treatment, before short-term L-dopa administration decreased the increased serine phosphorylation of GluR1 subunits produced by L-dopa therapy. Attenuation of the increased S845 phosphorylation of striatal GluR1 subunits by KW6002 occurred at a dose (1 mg/kg) that was effective in preventing or reversing the shortened motor response to L-dopa. These findings were corroborated by immunohistochemical analysis indicating that hemiparkinsonian rats treated twice daily with L-dopa for 21 days followed by a single L-dopa challenge 30 minutes before they were killed evidenced an increase in striatal P-GluR1 immunoreactivity. The number of S845 P-GluR1–positive neurons in the dorsolateral striatum ipsilateral to the 6-OHDA lesion was higher than on the intact side in these animals ($p < 0.05$). An increase in P-GluR1–expressing striatal cells did not occur in animals that received 21 days of twice-daily L-dopa plus KW6002 treatment followed by L-dopa challenge.

Results from these rodent model studies appear consistent with our hypothesis that the nonphysiologic stimulation of DA receptors on striatal spiny neurons activates signaling kinases capable of modulating the phosphorylation state and thus the synaptic efficacy of coexpressed ionotropic glutamatergic receptors (see figure). More specifically, the enhanced phosphorylation of glutamatergic receptor subunits in rat striatal neurons in association with the response alterations produced by L-dopa may involve the activation of PKA signaling cascades.[16] Adenosine A_{2A} receptors are highly expressed on the dendrites of medium spiny neurons[9,10] where they could activate PKA.[13,14] Therefore, it is possible that A_{2A} antagonists, by inhibiting this kinase, may attenuate the aberrant hyperphosphorylation of these corticostriatal glutamatergic receptors. These results are consistent with the recent demonstration of the NMDA-mediated inward current by A_{2A} receptors.[29,30] Under such circumstances, prophylactic and palliative effects on L-dopa–associated response complications would be anticipated. Moreover, the apparent participation of AMPA subtype glutamate receptor sensitization in the pathogenesis of L-dopa–induced motor response alterations is consistent with earlier observations indicating the beneficial effects of AMPA receptor antagonists on motor dysfunction in parkinsonian rodents and nonhuman primates.[31,32] Based on these considerations, it was decided to proceed with studies in the phenomenologically more informative primate model of PD.

KW6002 effects on motor function in parkinsonian monkeys. Adult male cynomolgus (*Macaca fascicularis*) monkeys were rendered parkinsonian by the subcutaneous administration of MPTP and later given KW6002 monotherapy.[27] At a dose of 90 mg/kg, parkinsonian severity decreased ($p < 0.01$), but no dyskinesias appeared. Daily injections of apomorphine begun 2 days after initiation of placebo or KW6002 treatment completely suppressed parkinsonian signs and induced abnormal involuntary move-

ments in placebo-treated monkeys within 7 to 10 days. Once induced, dyskinesia intensity peaked within 2 to 3 days and thereafter could be stably reproduced by the same apomorphine dose. In contrast, KW6002 plus apomorphine-treated animals did not evidence any dyskinesias. After 19 days, KW6002 (and placebo) treatment was withdrawn, whereas daily apomorphine injections were continued. Within 10 to 12 days, animals previously treated with KW6002 manifested dyskinesias for the first time. Once again, dyskinesia severity peaked within 2 or 3 days and remained stable when elicited by the same dose of apomorphine. In both groups, the eventual severity of dyskinesias was similar.

In parkinsonian monkeys, KW6002 monotherapy has an amelioratory effect on parkinsonian signs and can potentiate the antiparkinsonian action of dopaminomimetic agents without exacerbating associated dyskinesias.[23,24] We have further found that KW6002 acts prophylactically to prevent onset of DA agonist-induced dyskinesias. This prevention of motor complication onset appears to be a true protective effect because the time to the appearance of apomorphine-induced dyskinesias after KW6002 withdrawal approximated the time to dyskinesia onset when the DA agonist was given alone.

KW6002 effects on motor function in PD patients. Taken together, the results of the preclinical studies reviewed here indicate that selective A_{2A} receptor blockade should benefit motor dysfunction in patients with PD, possibly as a result of limiting the hyperphosphorylation of striatal glutamatergic receptor subunits that attend dopaminergic denervation and subsequent intermittent stimulation. To expeditiously evaluate this possibility, we proceeded to design and conduct a proof-of-concept clinical study of KW6002. The drug, which had been found by its manufacturer (Kyowa Pharmaceutical Inc., Princeton, NJ) to have a benign preclinical toxicologic profile, was administered during controlled conditions to volunteers with moderately advanced PD.[33] Eleven patients with PD completed this 6-week, increasing-dose evaluation.

When given as monotherapy, KW6002 had no effect on the severity of parkinsonian signs at a daily dose of 40 or 80 mg. Similarly, neither dose of KW6002 altered parkinsonian or dyskinesia scores in patients who were receiving an optimal dose of L-dopa (always coadministered with carbidopa) by steady-state IV infusion. Conversely, KW6002 potentiated the antiparkinsonian action of low-dose L-dopa (which alone produced no antiparkinsonian response) at the 40- and 80-mg doses (both $p < 0.05$). At the higher KW6002 dose, the degree of antiparkinsonian response to low-dose L-dopa did not differ from that produced by optimal-dose L-dopa alone. All cardinal parkinsonian signs improved, most notably resting tremor ($p < 0.02$). Choreiform dyskinesias were 50% less severe during treatment with low-dose L-dopa plus high-dose KW6002 than with optimal-dose L-dopa alone ($p < 0.05$). KW6002 also prolonged the duration of antiparkinsonian action of optimal-dose L-dopa, with efficacy half-time values increasing by an average of approximately 75% at the best dose of KW6002 ($p < 0.05$). In our study, KW6002 alone or with L-dopa appeared safe and generally well tolerated. Specifically, notwithstanding the cardiovascular effects reported in preclinical studies of some A_{2A} antagonists,[34] no consistent change in heart rate or blood pressure was observed.

Therefore, KW6002 potentiated the antiparkinsonian action of low-dose L-dopa without exacerbating dyskinesias. Unlike standard therapies for this disorder, the addition of KW6002 to L-dopa produced substantially fewer dyskinesias than an equivalent antiparkinsonian dose of L-dopa given alone. Moreover, although KW6002 coadministration benefited all primary parkinsonian signs, again unlike typical dopaminomimetic agents, the amelioratory effect on resting tremor predominated. KW6002 also prolonged the antiparkinsonian action of L-dopa to a clinically significant degree. Therefore, the addition of KW6002 to L-dopa therapy would be expected to decrease fluctuations of the wearing-off type.[2] The tendency for dyskinesias to be less severe when KW6002 is coadministered with L-dopa may in part reflect the reduced amount of the DA precursor required to achieve the same antiparkinsonian response. Conversely, the associated prolongation in efficacy half-time suggests a direct effect of KW6002 on mechanisms subserving motor complications in patients with PD as appears to be the case in animal models of this disorder.

Concluding observations. KW6002 effects observed in parkinsonian animals and patients with PD likely reflect an inhibitory action at striatal A_{2A} receptors.[9,10,25] Previous studies support the view that the output of striatal spiny neurons undergoes characteristic alterations after exposure of their DA receptors to the nonphysiologic conditions attending denervation or intermittent stimulation.[2] Either situation triggers internal signaling cascades capable of modifying the phosphorylation state and thus the synaptic efficacy of nearby glutamatergic receptors.[6] Resultant changes, presumably including an increase in cortical excitation, affect spiny neuron firing rates and patterns in ways that degrade motor function.[35,36] Now it appears that signaling between striatal dopaminergic and glutamatergic receptors may be influenced by A_{2A} receptor-mediated mechanisms and thus that pharmacologic blockade of striatal A_{2A} receptors could serve as a novel approach to the amelioration of parkinsonian signs with a decreased risk for motor complications (see figure). More generally, the ability of one type of neurotransmitter receptor to influence the synaptic strength of others via signaling cascades that can be modulated by additional receptor types could serve as a molecular basis for synaptic integration in dendrites. During such circumstances, direct pharmacologic

targeting of these signaling enzymes could provide a new strategy for the management of brain disease.

The salutatory effects of KW6002 in our patients with PD were predicted by the results of prefatory animal model studies of A_{2A} antagonists.[20-25] Therefore, the present clinical observations appear to provide additional validation for results obtained in the 6-OHDA rat and the MPTP monkey models. They also illustrate the critical benefits to clinical trial design conferred by the mechanistic and behavioral insights provided by relevant animal model evaluations and by bridging proof-of-concept studies in a small number of carefully chosen and meticulously monitored target subjects. In view of the benign safety and tolerability assessments of KW6002, advancing to clinical trials at the phase IIB level appears warranted; similarly, our findings should encourage the development of other A_{2A} antagonists now in preclinical evaluation. At a more general level, the aforementioned events illustrate how focused preclinical studies linked with carefully designed proof-of-concept evaluations can mitigate certain of the rate-limiting impediments to expeditious neurologic drug development. The time from our initial laboratory studies to clinical trial completion was less than 3 years. Steps to further optimize this translational process are essential if the full benefits of current neuroscience research are to redound to the benefit of those in greatest medical need.

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