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TITLE: Targeting Adenosine A2A Receptors in Parkinson's Disease

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CONTRACTING ORGANIZATION: Massachusetts General Hospital
Boston, MA 02114

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ABSTRACT:

In pursuit of an improved understanding and treatment of Parkinson's disease (PD) and related neuropsychiatric disease we have held a translational research conference entitled, "*Targeting Adenosine A_{2A} Receptors in Parkinson's Disease and other CNS Disorders*" in Boston, May 17-19, 2006. Recent insights into the CNS functions of the adenosine A_{2A} receptor, A_{2A} receptor neurotoxicology, and intriguing environmental clues have all converged to markedly enhance the potential of A_{2A} antagonists as a therapeutic strategy for PD. **The goal of this conference was to facilitate the translation of advances in A_{2A} receptor biology into improved therapy for PD by promoting the exchange of ideas on the basic science and clinical study of this molecular target.** The conference has accomplished or is successfully pursuing the following original aims (as indicated):

Aim 1: to summarize current knowledge of adenosine A_{2A} biology in the context of PD/CNS pathophysiology. The conference systematically reviewed and discussed 5 sequential themes (through 5 main scientific sessions):

A) **fundamental biology** of A_{2A} receptor function in the basal ganglia,
B) the basis for **symptomatic motor benefits** of A_{2A} antagonists,
C) A_{2A}Rs in **neuroprotection** against dopaminergic neuron toxicity,
D) the effects of A_{2A} receptors on **dyskinesias** and **non-locomotor CNS systems**,
E) **translating these insights** into effective, safe clinical trials for PD.

Opening and keynote addresses by Professors Bertil Fredholm (Karolinska Inst) and Paul Greengard (Rockefeller Univ) provided valuable perspective on the specific themes and topies of the meeting.

Aim 2: to integrate preclinical findings into the design of clinical trials targeting both disease-modifying and symptomatic benefits of A_{2A} antagonists for PD. This key translational concept was pursued not only though the meeting's the final theme/session (E), but also through a closing special lecture by Professor Stanley Fahn (Columbia Univ) on the development of sophisticated PD clinical trials for neuroprotection using agents that have known to have symptomatic efficacy. The translational therapeutics goal was also highlighted by the presentation of a patient's perspective with Professor Anne Young (Harvard University) at the opening of the conference.

Aim 3: to promote information exchange between academic, government and industry leaders that will improve prospects for an expeditious, unbiased and safe translation of A_{2A} adenosine biology into improved therapy for PD. This forum has encouraged long-term relationships as well as direct dialogue between basic scientists, clinical researchers and drug developers that will ultimately lead to more rigorous design and execution of clinical trials of A_{2A} antagonists for PD. The relationships were fostered by a high quality of discussion not only during the main scientific sessions, but also during two poster sessions (comprising ~50 presentations), 4 special lectures, receptions and group meals – all facilitating exchange of ideas over common interests in the conference goals. ~170 registrants (approx. double that of the our earlier 2002 conference on the this topic) from around the world spanned a range of academic (student as well as faculty), industry, media and government perspectives.

Aim 4: to widely disseminate lessons on A_{2A} antagonists not only as an attractive target for PD therapeutics, but more broadly as a model for translational research in neurodegenerative disease. This was achieved through inclusion of a diverse group of speakers and attendees at different career stages, and will be further pursued through the publication of the conference proceedings as a special issue of the scholarly review journal *Progress in Neurobiology* (Elseivier, publisher). In addition an invited meeting report is being developed for the online journal *Science STKE*.

Thus the conference has been of relevance to PD research and the USAMRMC mission at multiple levels:

- **Basic neuroscience significance** – seeking insights into adenosine receptor neurobiology
- **Therapeutic significance** - With A_{2A} antagonists emerging as promising therapeutic candidates based on their symptom-relieving action, the prospects for additional neuroprotective benefit may be of relevance not only for PD, but for stroke and other neurodegenerative diseases including ALS and Alzheimer's disease. The relevance of the conference to NETRP/USAMRMC extended beyond neurodegenerative disease to a broader range of health issues involving caffeine and A_{2A} receptors and including **environmental neurotoxicology, alerting and non-stimulant CNS effects of caffeine, addiction biology, and gender differences in how environmental factors impact disease risk.**

Table of Contents

Reportable Outcomes

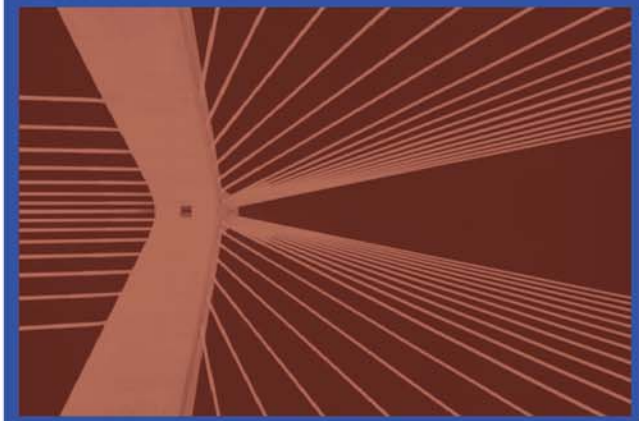
- Conference program [attached as appendix]
- Conference website [www.A2APD.org], to be maintained by MassGeneral Institute for Neurodegenerative Disease; featuring:
    a. conference overview
    b. **scientific program** with sessions/topics/~28 platform presenters
    c. posted/published **abstracts** ~75 including platform and poster presentations
    d. research travel **fellowship program**/awardees
    e. **sponsorship**, with USAMRAA/NETRP listed most prominently in proportion to support
- Conference report invited by Dr. Elizabeth Adler, editor ***Science STKE***; with reporting effort by science writer/conference faculty team of Drs. Lianna Orlando and Echo Chern.
  {status: in preparation}
- Conference proceedings/reviews in planned special issue of the high impact, scholarly review journal ***Progress in Neurobiology*** (Elsevier, publisher; Michael Zigmond, editor-in-chief; Michael Schwarzschild, guest editor), comprising 5 full-length reviews paralleling the 5 preclinical sessions of the conference. Supplemental funding application pending with USAMRAA/NETRP.
  {status: in preparation; manuscript submission due date July 17}

Conclusions

The conference and post-conference report and proceedings in development have achieved and are on track to achieve all the original aims outlined for this conference grant proposal. The conference is successfully facilitating the translation of our understanding of adenosine neurobiology into realistic therapeutic opportunities for Parkinson's disease and related neuropsychiatric disorders, of broad significance for military and public health.

Bridging adenosine neurobiology to better therapy for CNS disorders



A2A PD
Boston 2006




Images of Boston's Zakim Bunker Hill Bridge, 1999-2002, by Don Eyles

Targeting Adenosine A_{2A} Receptors in Parkinson's Disease and other CNS Disorders

an international research conference

May 17, 18 & 19, 2006
Boston, Massachusetts USA

Presented by

the MassGeneral Institute for Neurodegenerative Disease

Co-chairs

Thomas N. Chase, Jiang-Fan Chen, Michael A. Schwarzschild & Anne B. Young

Director
Michael A. Schwarzschild

Coordinator
Galina Slezinger

SECTION 1 – General Information

Photograph by Don Eyles February 2005

SPONSORSHIP

The *MassGeneral Institute for Neurodegenerative Disease* would like to recognize the following institutions and organizations for their generous financial support of the meeting:

The United States Army Military Research and Materiel Command's Neurotoxin Exposure Treatment Research Program

Schwarz Pharma

National Institute of Neurological Disorders and Stroke

Schering-Plough

Biogen Idec

Neurocrine Biosciences

Kyowa

CV Therapeutics

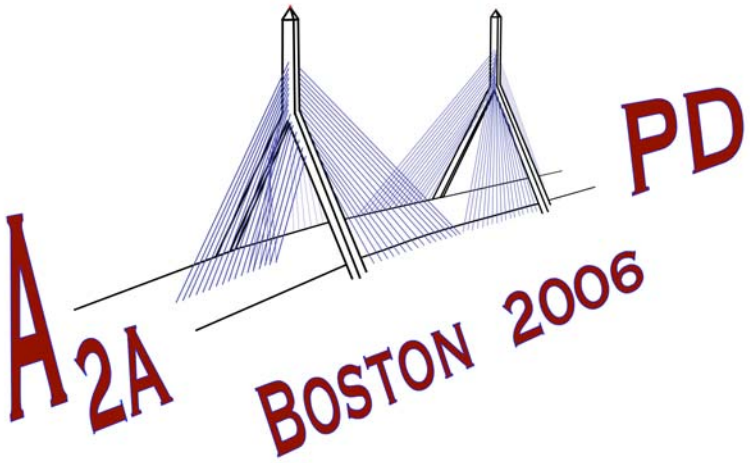
Roche

Lundbeck

ACKNOWLEDGEMENTS

To Don Eyles, thank you for donating your extraordinary photographic images of the Leonard P. Zakim Bunker Hill Bridge. Their artistry helps sharpen our focus on the translational goals of the conference. Don Eyles has been photographing the Big Dig since 1993. He can be contacted by email at "eyles@RCN.com".

Thank you to the conference presenters and attendees. Your commitment to pursuing basic and clinical neuroscience in the service of alleviating CNS disease is greatly appreciated.



Targeting Adenosine A_{2A} Receptors in Parkinson's Disease and other CNS Disorders

As symbolized by Boston's new Lenny Zakim Bunker Hill Bridge and its portrayal in the conference logo, the overarching goal of the meeting is to bridge our knowledge of A_{2A} receptors to the promise of improved treatments for Parkinson's and related neuropsychiatric disease. Put more practically, the conference seeks to facilitate the consideration, and if appropriate the development of A_{2A} receptor antagonists as a novel multi-faceted treatment for CNS disorders.

This translational research forum also endeavors to span the interests and resources of academia, government and industry in pursuit of this common goal. Thematically organized symposia (moving from adenosine biology across preclinical models to early clinical trials), a poster session, and other interactive venues have been designed to foster a fruitful exchange of ideas between all participants.

Welcome to the conference. I hope you will enjoy and build on the wonderful opportunities it offers.

Michael Schwarzschild
Boston, USA

GENERAL INFORMATION

Locations, Directions and Useful Phone/Contact Information

Ether Dome of Massachusetts General Hospital, 55 Fruit Street, Boston
(617) 726-2000 (general information at Massachusetts General Hospital)
NB – The Ether Dome amphitheatre is on the 4th floor of the Bulfinch Building. There are staircases on either side of Ether Dome at the center of the building and an elevator access in the north wing of the building; please see campus map below.

Shriners Center and Auditorium, 51 Blossom St., Boston, MA 02114
(617) 722-3000

Holiday Inn Select, 5 Blossom Street, Floor 15, Boston
(617) 742-7630

Museum of Science, Science Park, Boston, MA 02114
(617) 589-0100
information@mos.org
Walking directions from Holiday Inn to Museum of Science:
From the Charles/MGH T station (see "T" in lower left hand corner of conference map below) cross Storrow Drive towards the Charles River and turn right, walking with the river to your left. (You will see the Museum and Hayden Planetarium dome sitting over the river ahead of you.) Turn left at the Charles River Dam and State Police Station onto O'Brien. The Museum is on your left.
Walking time from the hotel to the museum is 20-30 minutes.
Parking for those driving: Parking at the Museum of Science is free for this evening event. Please take your parking ticket with you to the event to get it validated.

Harvard Faculty Club, 20 Quincy Street, Cambridge, MA 02138
(617) 495-5758
hfc@harvard.edu
To get to the Harvard Faculty Club (HFC; http://www.hfc.harvard.edu) for the post-conference dinner on Friday, one can take the "Red line" of the Transit ("T") system from "Charles/MGH" station (please see the lower left hand corner of the map below) outbound to the "Harvard Square" station. From there use the main exit, which is found at the far end of the platform (rear of outbound platform, front of inbound platform). Go upstairs and take a U-turn out of the exit. Walk up Massachusetts Avenue against the traffic. Quincy Street will be the first street on the left, several blocks up. The Faculty Club is located on the right side of the street, just after the gray house.

The "T" costs 1.25 USD in each direction, and rides can be purchased at the attendant booth at the "Charles/MGH" station. (You may wish to buy a second token for your return trip.) You can count on 30-35 min from the Holiday Inn to the HFC (including walking from the hotel to the "Charles/MGH" T station, and from the "Harvard Square" T station to the HFC. Along the way you will enjoy a nice view as the Red line takes you over the Charles River on the Longfellow Bridge, and you can even detour through Harvard Yard when walking to the HFC if you have a few extra minutes.

If you prefer to take a tax door-to-door, you can ask the driver to take you to "*the HFC at 20 Quincy Street in Cambridge near Harvard Yard*" and count on 15-20 min ride and $15-20 USD.

Galina Slezinger's cellular phone number:	(617) 968-5943
or office number:	(617) 726-1276
Michael Schwarzschild's office number:	(617) 724-9611

Massachusetts General Hospital: Main Campus

A2A & PD Conference

Walking directions from hotel to MGH's Ether Dome

Walking directions to Shriners Auditorium (main conf. venue)

Walking to and from coffee breaks

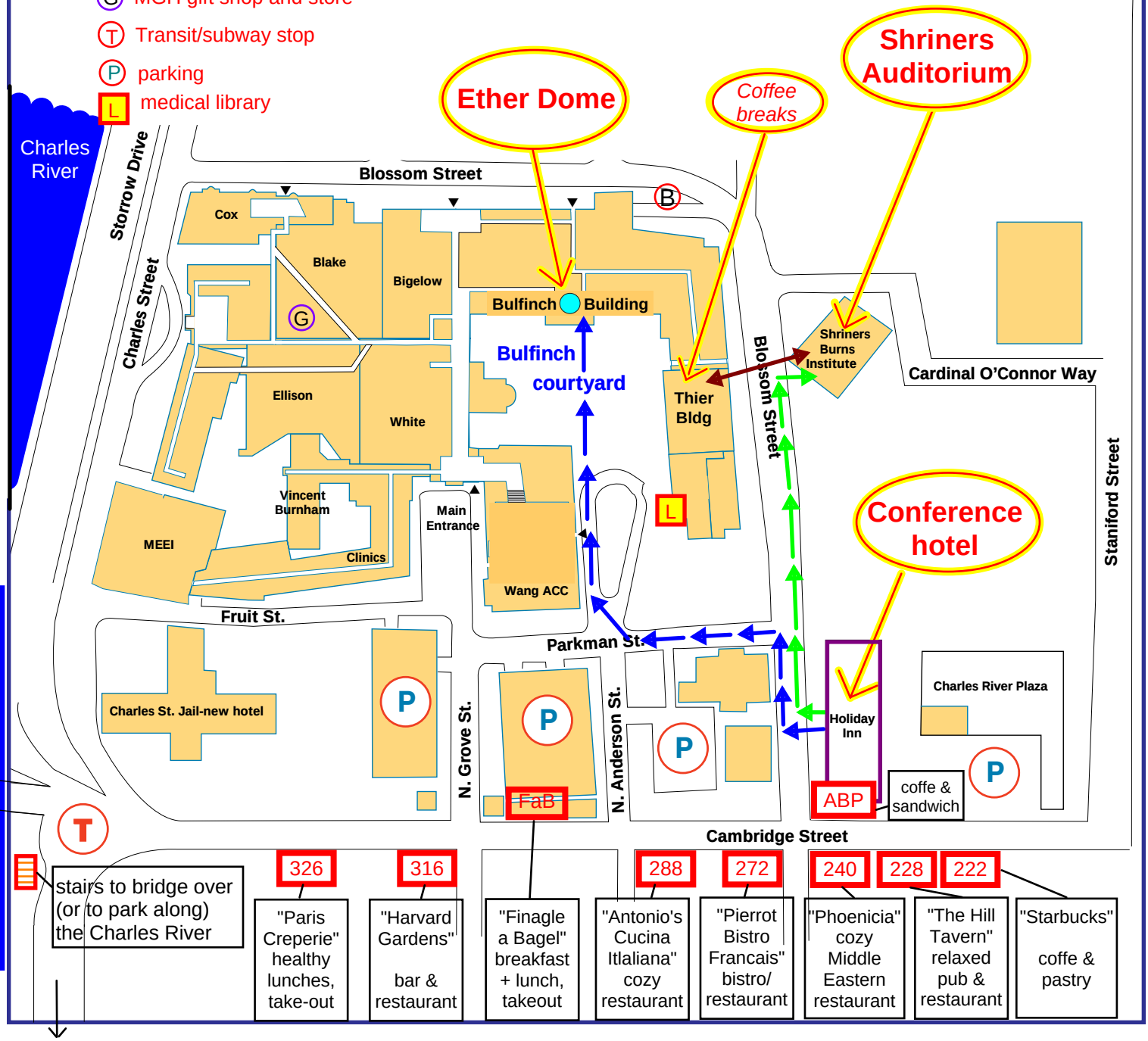
Ⓑ shuttle bus to the Navy Yard

Ⓖ MGH gift shop and store

Ⓣ Transit/subway stop

Ⓟ parking

Ⓛ medical library



SECTION 2 – ***Agenda***

Photograph by Don Eyles February 2005

Agenda
for

Targeting Adenosine A_{2A} Receptors in Parkinson's Disease and other CNS Disorders

May 17, 18 & 19, 2006

Locations:

Ether Dome of Massachusetts General Hospital, 55 Fruit Street, Boston

Shriners Auditorium, Mass General Hospital, Boston

Holiday Inn Select, 5 Blossom Street, Floor 15, Boston

Museum of Science, 1 Science Park, Boston

Wednesday, May 17, 2006

Opening session at historic *Ether Dome* of Massachusetts General Hospital

4:00PM	Welcome
4:10PM	Patient Perspective with Anne Young (Boston, USA)
4:25PM	*Opening Address:* "A_{2A} receptors in brain - some things we think we understand and some we definitely do not" Bertil Fredholm (Stockholm, Sweden)
4:50PM	Conference logistics

5:30PM	Welcome reception at MGH Bulfinch courtyard

Thursday, May 18, 2006

Shriners Auditorium, Blossom Street, Boston

7:30AM	*Breakfast at Thier Building, 1st floor Conference Room*
7:50AM	Registration (Shriners Center Lobby)
8:25AM	Welcome

A. General biology of A_{2A} receptor signaling
session chair: Bertil Fredholm

8:30AM	-"A_{2A} gene regulation and signal transduction" ***Yijuang Chern*** (Taipei, Taiwan)
8:55AM	-"Cellular Targets of A_{2A} Mediated Tissue Protection: the Spinal Cord Paradox**"** ***Joel Linden*** (Charlottesville, USA)
9:20AM	-"Systemic and CNS immune function of the A_{2A} receptor" ***Misha Sitkovsky*** (Boston, USA)
9:45AM	-"Heteromeric interactions - molecular aspects" ***Rafael Franco*** (Barcelona, Spain)
10:10AM	Moderator: ***Bertil Fredholm***

10:20AM	Coffee Break **at Thier Building Lobby**

B. A_{2A} & basal ganglia physiology
session chair: Serge Schiffmann

10:40AM	-"Functional heteromeric interactions between adenosine, dopamine & glutamate receptors" ***Sergi Ferré*** (Baltimore, USA)
11:05AM	-"A_{2A} signal integration in striatal neurons" Gilberto Fisone (Stockholm, Sweden)
11:30AM	-"A_{2A} in striatal neuron excitability & synaptic transmission/plasticity" ***Serge Schiffman*** (Brussels, Belgium)
11:55AM	-"Glial A_{2A} receptor function" ***Rodrigo Cunha*** (Coimbra, Portugal)
12:20PM	Moderator: **Serge Schiffmann**

12:30PM	**Lunch and POSTER SESSION at Holiday Inn, 15th Floor**

Thursday, May 18, 2006

C. A_{2A} & parkinsonian motor dysfunction/dyskinesia
session chair: Micaela Morelli

2:15PM	-"A_{2A} in rigidity & tremor" ***Jadwiga Wardas*** (Krakow, Poland)
2:40PM	-"Localizing antiparkinsonian mechanisms of A_{2A} antagonists" ***Micaela Morelli*** (Cagliari, Italy)
3:05PM	-"A_{2A} in L-dopa sensitization/dyskinesia models" ***Michael Schwarzschild*** (Boston, USA)
3:30PM	-"A_{2A} in primate models of PD and dyskinesia" ***Therese Di Paolo*** (Ste-Foy, Canada)
3:55PM	Moderator(s): ***Micaela Morelli***

4:05PM	Break

D. A_{2A} & neuroprotection in PD models
session chair: Michael Schwarzschild

4:15PM	-"Caffeine, estrogen & PD epidemiology" ***Alberto Ascherio*** (Boston, USA)
4:40PM	-"A_{2A} mechanisms and regional localization in PD neurodegeneration" ***Patricia Sonsalla*** (Piscataway, USA)
5:05PM	-"A_{2A} mechanisms and cellular localization in PD neurodegeneration" ***Jiang-Fan Chen*** (Boston, USA)
5:30PM	Moderator: ***Michael Schwarzschild***

5:40PM	FREE/TRAVEL TIME (Buses leave for Museum of Science from Holiday Inn at 6:30PM)
7:00PM	**Dinner and evening program at Museum of Science (Blue Wing)**
8:00PM	**"Modulation of adenosine A_{2A} receptor signaling"** **KEYNOTE ADDRESS: *Paul Greengard* (New York City, USA)**

Friday, May 19, 2006

Shriners Auditorium, Blossom Street, Boston

E. A_{2A} & non-dopaminergic neuron death
session chair: Jiang-Fan Chen

7:45AM	*Breakfast at* **Holiday Inn, 15th Floor**
8:00AM	Registration (Shriners Center Lobby)
8:30AM	-"A_{2A} in Huntington's disease and its models" ***Maria Rosaria Domenici*** (Rome, Italy)
8:55AM	-"A_{2A} in ischemic brain injury" ***Felicita Pedata*** (Florence, Italy)
9:20AM	-"A_{2A} in neuroinflammation" ***Jonathan Geiger*** (Grand Forks, USA)
9:45AM	-"A_{2A} in Alzheimer's disease and its models" ***Alexandre de Mendonça*** (Lisbon, Portugal)
10:10AM	Moderator: ***Jiang-Fan Chen***

10:20AM	Coffee Break **at Thier Building Lobby**

F. Non-motor A_{2A} targets in the CNS
session chair: Sergi Ferré

10:40AM	- "A_{2A} and drug addiction" ***Steven Goldberg*** (Baltimore, USA)
11:05AM	- "A_{2A} receptors regulate CNS responses to ethanol and addicting substances: Recent advances from cell biology to behavior" ***Ivan Diamond*** (Palo Alto, USA)
11:30AM	- "A_{2A} in pain" ***Ian Kitchen*** (Guildford, United Kingdom)
11:55AM	- "A_{2A} in sleep and arousal" ***Yoshi Urade*** (Osaka, Japan)
12:20PM	Moderator: ***Sergi Ferré***

12:30PM	**Lunch and POSTER SESSION at Holiday Inn, 15th Floor**

Friday, May 19, 2006

G. Clinical development of A_{2A} antagonists for PD
session chair: Tom Chase

2:15PM	-"Neuroimaging of A_{2A} receptor binding" ***Rosamaria Moresco*** (Milan, Italy)
2:40PM	-"Review of clinical experience with A_{2A} antagonists in Parkinson's disease." ***John Growdon*** (Boston, USA)
3:05PM	- "Progressing the A_{2A} antagonist BIIB014/V2006 to the clinic for the treatment of PD" ***Sean Lightowler*** (Vernalis, Wokingham, United Kingdom)
3:30PM	- "SCH 420814: Exploring PD and Related Movement Disorders" ***John Hunter*** (Schering-Plough, Kenilworth, USA)
3:55PM	Moderator: *Tom Chase*
4:05PM	**J. Stephen Fink Memorial Lecture** Introduction (***Anne Young***) "Designing PD clinical trials of symptomatic therapies for disease modification" ***Stanley Fahn*** (New York City, USA)

7:00 PM	Dinner at Harvard Faculty Club, Cambridge, MA

SECTION 3 – ***Platform Presentations***

Photograph by Don Eyles March 2003

Targeting Adenosine A_{2A} Receptors
in Parkinson's Disease
and Other CNS Disorders

May 17 - 19, 2006
Boston USA

presented by
MassGeneral Institute for Neurodegenerative Disease

A2A receptors in brain – some things we think we understand and some we definitely do not

Bertil B. Fredholm

Department of Physiology and Pharmacology, Karolinska Institutet, S-171 77 Stockholm, Sweden. (emailbertil.fredholm@ki.se)

As is obvious from the title of this presentation it is less a scientific paper than an Essay. As it is also very difficult to write an abstract of an Essay this one is very brief. I plan to very briefly recapitulate the basic facts of A_{2A} receptors in striatopallidal neurons and their functional significance and consider that as a "known". I will most likely very briefly touch on controversies relating to role(s) of A_{2A} receptors in regulating transmitter release. More time will be spent on highlighting some of the very important questions relating to the precise targets of the neuroprotective effects (of agonists and/or antagonists). The inescapable conclusion is that much exciting research remains to be discussed during the meeting and be performed when we return home from it.

To the best of my knowledge and judgment I, the presenting author, report that:
The authors have no financial conflict of interest in the presentation of this work.

Targeting Adenosine A_{2A} Receptors
in Parkinson's Disease
and Other CNS Disorders

presented by
MassGeneral Institute for Neurodegenerative Disease

May 17 - 19, 2006
Boston USA

A_{2A} gene regulation and signal transduction

Yijuang Chern

Institute of Biomedical Sciences, Academia Sinica, Nankang, Taipei 115, Taiwan (email bmychern@ibms.sinica.edu.tw)

The A_{2A} adenosine receptor (A_{2A}-R) is a major target of caffeine, the most widely used psychoactive substance in the world. Stimulation of the A_{2A}-R results in activation of adenylyl cyclase and protein kinase C (PKC). Using PC12 as a neuron-like model system, we demonstrated that atypical PKCs (aPKCs) function downstream of protein kinase A (PKA) to mediate the protective effect of the A_{2A}-R. A_{2A}-R stimulation also rescued the blockage of NGF-induced neurite outgrowth when the NGF-evoked MAPK/CREB cascade was suppressed. In addition to the well-characterized conventional signaling pathways, several interacting proteins which bind to the C terminus of the A_{2A}-R have been reported and are proposed as contributing to various functions of the A_{2A}-R. Expression of the A_{2A}-R is transiently regulated in various areas of the developing rat brain, suggesting that adenosine acting on the A_{2A}-R may play an important role in brain development. Soon after neurogenesis, rat A_{2A}-R transcripts are heavily expressed by striatal neurons and colocalize with the D2 dopamine receptor in GABAergic striatopallidal neurons. Note that GABAergic striatopallidal neurons are the most vulnerable neurons during the progression of Huntington's disease (HD). Studies from several laboratories including ours have reported that the A_{2A}-R gene contains multiple independent promoters. The major difference in the transcripts from these alternative promoters is the length of their 5'-untranslated regions (5'UTRs). Interestingly, the 5'UTRs of at least two transcripts of the rat A_{2A}-R gene suppress A_{2A}-R expression at the translational level. This translational regulation of the rat A_{2A}-R gene appears to be a general mechanism in that the 5'UTR of the A_{2A}-R gene possesses strong interspecific homology. Using a transgenic approach, we also found that one of the promoters (P2) of the rat A_{2A}-R gene directs A_{2A}-R expression in the brain, but not in other peripheral tissues examined. Moreover, mutant Huntingtin (Htt; the causative gene of HD), with expanded polyQ, significantly suppressed the P2 promoter of the A_{2A}-R gene. An increase in the intracellular cAMP level or expression of a constitutively positive CREB mutant rescued the suppression by mutant Htt, suggesting involvement of the PKA/CREB pathway. Further characterization of selectively regulated A_{2A}-R promoters will provide an important foundation for the future clinical application of A_{2A}-R-related drugs.

Funded by grants from the National Science Council (NSC93-2320-B-001-009 and NSC94-2320-B-001-030) and Academia Sinica, Taipei, Taiwan.…

The author has not indicated whether a conflict of interest exists.

May 17 - 19, 2006
Boston USA

Targeting Adenosine A_{2A} Receptors in Parkinson's Disease and Other CNS Disorders

presented by
MassGeneral Institute for Neurodegenerative Disease

Can Parkinson disease studies benefit from the acute inflammation studies?

Michail V. Sitkovsky

New England Inflammation and Tissue Protection Institute, a Consortium at Northeastern University , Boston, MA, 02110, m.sitkovsky@neu.edu

In this presentation I will review the mechanisms that control activities of immune cells in inflamed and damaged tissue microenvironments during the acute phase of immune response. The genetic *in vivo* data show that A2A adenosine receptors play critical and non-redundant role in down-regulation of overactive immune cells. The A2A adenosine receptors on the surface of immune cells inhibit overactive immune cells and thereby provide tissue protection during acute inflammation. Much higher levels of inflammatory mediators and tissue damage have been observed using A2 receptors antagonists or genetic deletion of A2A adenosine receptors in mice.

The paradoxically opposite effects of A2AR in Parkinson disease vs acute inflammation suggest that Parkinson disease pathogenesis is opposite to acute inflammation and several testable scenarios will be offered for discussion.

The author has not indicated whether a conflict of interest exists.

May 17 - 19, 2006
Boston USA

Targeting Adenosine A_{2A} Receptors in Parkinson's Disease and Other CNS Disorders

presented by
MassGeneral Institute for Neurodegenerative Disease

Cellular Targets of A_{2A} Mediated Tissue Protection: the Spinal Cord Paradox

Joel Linden
Department of Medicine, Cardiovascular Research Cetner, University of Virginia, Charllotsville, VA, USA (email jlinden@virginia.edu)

Background: Activation of adenosne A_{2A} receptors (A_{2A}Rs) at the time of reperfuison following ischemia reduces necrosis in heart, liver, kidney and other tissues. Thorugh the use of A_{2A}R KO mice and bone marrow chimera it is clear that this protection is mediated predominantly by A_{2A}Rs on bone marrow-derived cells. Additional experiments using Cre/A_{2A}R-floxed mice, adoptive transfer of T cells in to Rag1 KO mice, and antibodies to deplete $CD4^+$ or $CD8^+$ T cells has implicated $CD4^+$ T cells as major targets of A_{2A} mediated tissue protection. We also have shown that A_{2A} agonists can reduce locomotor dysfunction when administered to rabbits or pigs at the time of reperfusion following spinal cord ischemia or starting 10 minutes after spinal cord trauma. In contrast to peripheral organs, in brain, deletion of the A_{2A}R reduces ischemic or traumatic injury. Here we describe initial studies demonstrating paradoxical effects mediated by A_{2A}Rs in the spinal cord. We domonstrate protective effects either by A_{2A} agonsits or by A_{2A}R deletion on locomotor function following (SCI) – the "spinal cord paradox".
Methods: We have devised the "mBBB score" as a new system for scoring hindlimb movement in mice that acurately asseses locomotor dysfunction. Mice are subjected to compression-induced SCI after laminectomy and are periodically evaluated over 6 weeks. A 1 x 2 mm region of spinal cord is compressed for 5 min with a 15 g weight applied to the dorsal surface of the cord at T12. The potent and selective A_{2A} agonist, ATL313, is administered IP for 5 minutes before injury or at various time after injury. ATL313 does not influence SCI in A_{2A}R knock out mice.
Results: Compared to vehicle controls, ATL313-treatment prior to or shortly after injury produces significant locomotor improvement that reaches a plateau within 7 days and is sustained until the end of the experiment at 42 days. Injury is reduced based on both locomotor and histologic critera. Paradoxically, locomotor function also is improved in A_{2A}R KO mice comparted to congenic wild type controls, but this imporvement develops slowly after injury, and is not fully manifest until 14 days after SCI. Ongoing expeirments with bone marrow chimera mice indicate that rapid imporvement in locomotor function is mediated by A_{2A} receptors on bone marrow-dervied cells, and slow improvement noted in A_{2A}R KO animals are mediated by a different populations of cells.
Conclusion: The brain is protected by A_{2A}R deletion from ischemic or traumatic injury. In contrast, peripheral tissues are protected by A_{2A} agonsits, and these effects are due to inhibition of inflammation during reperfusion injury. We show here for the first time that activation of A_{2A}Rs on inflammatory cells reduces locomotor dysfunction following SCI, but deletion of A_{2A}Rs on another population of cells also improves locomotor recovery. It will be necessary to better understand these paradoxical effects in order to optimally exploit adenosine receptor signaling in order to reduce spinal cord injury.
Funded by NIH R01HL37942.

To the best of my knowledge and judgment I, the presenting author, report that:
The authors may have a financial conflict(s) of interest as I have described here:

JL owns stock in Adenosne Therapeutics, LLC. The company is developing A_{2A} agonist compounds for the treatment of inflammatory diseases.

Targeting Adenosine A_{2A} Receptors in Parkinson's Disease and Other CNS Disorders

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Heteromeric Interactions. Molecular Aspects

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Whereas there is consensus on the role of dimerization of receptor tyrosine kinases for proper function, the physiological role of dimerization of G-protein-coupled or heptaspanning-transmembrane receptors (7TMR) is still a matter of debate. There is recent evidence pointing towards a prominent role of homo- and hetero-dimers for 7TMR function, including that of adenosine A_{2A} receptors. In addition, homo or heteromers targeted by bivalent ligands constitute a promising therapeutical approach for a number of human brain disorders.

The receptor-receptor molecular interaction is relevant to understand many aspects of 7TMR behavior. From a pharmacological point of view the binding of natural or synthetic ligands quite often exhibits cooperativity, i.e. non- linear Scatchard plots. Negative cooperativity cannot be explained assuming the existence of interconnected receptor forms and has been classically explained by assuming the existence of monomeric receptors coupled or not to G proteins; moreover, positive cooperativity cannot be explained by monomeric species. The recently devised "Two-State Dimer Model", takes into account the existence of receptor homodimers and can explain the binding behavior of agonists, inverse agonists ad antagonists to 7TMR exhibiting negative or positive cooperativity. Furthermore, the model provides the theoretical framework to understand 7TMR operation.

Dopamine and adenosine receptors have been a paradigm in the identification of receptor-receptor interactions. These receptors can form homodimers or heterodimers with a variety of other receptors. Interestingly, these dimers lead to both functional and pharmacological receptor diversity. For instance, the existence of heterodimers of dopamine D_1 and D_2 receptors or of adenosine A_1 and A_{2A} receptors, which are oppositely coupled to Gs (A_{2A} or D_1) or to Gi (A_1 or D_2), provides a new mechanism for the control of neurotransmission. But also another interesting aspect of receptor-receptor interactions is that the receptors modify the affinity for the hormone or ligand depending on the composition and constraints of the heteromer. A good example is given by caffeine, which targets A_{2A} receptors quite differently depending on whether they form homodimers or heterodimers. In fact heteromerization can explain some features of caffeine effects and tolerance.

Receptor-receptor interactions open also new perspectives from a therapeutical point of view. Individually, dopamine receptor agonists or adenosine receptors antagonists are used to combat Parkinson's disease (PD). To exploit the pharmacological potential of adenosine A_{2A} and dopamine D_2 receptor heteromers, we have developed bivalent ligands that can interact with the two receptors. These consist of D_2 receptor agonist and A_{2A} receptor antagonist pharmacophores linked by a spacer of variable size. Bivalent ligands with short spacers are able to interact simultaneously with the two receptors, and have higher affinity than the monovalent reagents. The compounds selected behave as D_2 receptor agonists and A_{2A} receptor antagonists. Furthermore, these bivalent ligands are able to detect $A_{2A}R/D_2R$ heteromers in striatum. Therefore, dopamine-adenosine bivalent ligands constitute novel tools to detect heteromers and to target them to improve the efficacy of PD therapies.

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Functional Heteromeric Interactions between Adenosine, Dopamine and Glutamate Receptors in the Striatum

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Heteromeric complexes of adenosine, dopamine and glutamate receptors play a key role in the modulation of striatal glutamatergic neurotransmission. Striatal glutamatergic synapses are mostly localized in the heads of the dendritic spines of striatal GABAergic efferent neurons. It can be assumed that there are two major distinct types of striatal glutamatergic synapses, localized on either GABAergic dynorphinergic or GABAergic enkephalinergic efferent neurons. Excitatory neurotransmission in dynorphinergic and enkephalinergic neurons is differentially modulated by heteromeric complexes of adenosine A_1, dopamine D_1 and NMDA receptors and adenosine A_{2A}, dopamine D_2 and metabotropic $mGlu_5$ receptors, respectively. Furthermore, we have recently demonstrated that adenosine A_1-A_{2A} receptor heteromers control glutamatergic neurotransmission in the striatal glutamatergic terminals. A_1-A_{2A} receptor heteromers allow adenosine to exert a fine-tuning modulation of striatal glutamatergic neurotransmission, by providing a switch mechanism, by which low and high concentrations of adenosine inhibit and stimulate, respectively, glutamate release.

Dopamine afferents make preferential synaptic contact with the neck of the dendritic spines, an arrangement that allows mesencephalic dopaminergic inputs to modulate cortico-limbic-thalamic glutamatergic excitatory inputs. D_1 receptors are Gs-olf protein-coupled receptors whose main signaling pathway is activation of the cAMP-PKA cascade. D_1 receptor-mediated PKA activation, with the consequent phosphorylation of NMDA receptors and the $GluR_1$ subunit of the AMPA receptor, provides a main mechanism by which dopamine modulates glutamate-dependent synaptic plasticity, the heterosynaptic modulation essential for stabilizing homosynaptic plasticity. However, this mechanism can only operate in the A_1-D_1-NMDA receptor-regulated glutamatergic synapses in dynorphinergic neurons, since D_2 receptor inhibits cAMP-PKA cascade in enkephalinergic neurons. But A_{2A} receptors are also Gs-olf protein-coupled receptors that signal through the cAMP-PKA cascade. We review recent evidence supporting the hypothesis that adenosine provides the heterosynaptic-like modulation necessary for stabilizing homosynaptic plasticity in the A_{2A}-D_2-$mGlu_5$ receptor-regulated glutamatergic synapses of the GABAergic enkephalinergic neurons.

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A_{2A} Signal Integration in Striatal Neurons

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A large proportion of striatal medium spiny neurons control the activity of the output stations of the basal ganglia via a polysynaptic circuit that includes globus pallidus and subthalamic nucleus. Adenosine A2A receptors are highly expressed in this indirect pathway, where they interact with dopamine D2 receptors. One important site of interaction is represented by the cAMP/protein kinase A (PKA) cascade. This signaling pathway is activated by A2A receptors and inhibited by D2 receptors. The dopamine- and cAMP-regulated phosphoprotein of 32 kDa (DARPP-32) is critically involved in mediating cAMP-dependent responses. Increased phosphorylation of DARPP-32 at Thr34 amplifies the effects of activation of the cAMP/PKA signalling cascade by inhibiting PP-1 and reducing dephosphorylation of downstream target proteins. Conversely, increased phosphorylation of DARPP-32 at Thr75 counteracts cAMP-mediated responses by inhibiting PKA. In the medium spiny neurons of the indirect pathway, the state of phosphorylation of DARPP-32 is controlled by the opposing actions of A2A receptors, which stimulate Thr34 phosphorylation, and D2 receptors, which reduce Thr34 phosphorylation. Furthermore, blockade of A2A receptors results in a large increase in the state of phosphorylation of DARPP-32 at Thr75, accompanied by reduced phosphorylation at Thr34. Changes in the state of phosphorylation of DARPP-32 produced by A2A receptor agonists and antagonists mediate the effects of these drugs on motor activity. The opposite regulation of the cAMP/PKA/DARPP-32 cascade exerted by A2A and D2 receptors is reflected by their contrasting regulation of glutamate AMPA receptors. Administration of haloperidol, a dopamine D_2 receptor antagonist, increases the phosphorylation of the GluR1 subunit of the AMPA receptor at the PKA site, Ser845. In contrast, administration of the dopamine D_2-like agonist, quinpirole, decreases GluR1 phosphorylation at Ser845. The increase in Ser845 phosphorylation produced by haloperidol is abolished in DARPP-32 knockout mice, or in mice in which the PKA phosphorylation site on DARPP-32 (i.e. Thr34) has been replaced by Ala. Blockade of A2A receptors with KW6002 prevents haloperidol-induced phosphorylation of DARPP-32 at Thr34 and GluR1 at Ser845. These results indicate that the opposite regulation of the cAMP/PKA/DARPP-32 cascade exerted by A2A and D2 receptors affects the state of excitability of medium spiny neurons by modulating glutamate AMPA receptor transmission and provide a molecular framework to explain the antagonistic interaction between dopamine and adenosine in the striatum.

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A_{2A} receptor in striatal neuron excitability and synaptic transmission/plasticity

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A_{2A} receptor is highly co-expressed with enkephalin and D_2 receptor in striato-pallidal neurons. A_{2A} antagonists acutely enhance motor behavior in animal models of PD and are therefore considered as potential PD therapeutics. To refine the knowledge on the role(s) of A_{2A} receptor in the control of striatal physiology, the modulation in striatal neuron excitability and corticostriatal synaptic transmission and synaptic plasticity by A_{2A} receptor and its interaction with D2 receptor were explored in rats, mice and A_{2A} receptor-deficient mice (A_{2A}-$R^{-/-}$). These studies were mostly focused on the accumbens nucleus which is a brain region involved in motivation, attention, reward and drug addiction. The output of the striatum is determined by the bursting activity in the medium-sized spiny GABAergic neurons (MSN). These bursts are driven by multiple corticostriatal inputs that depolarize MSN from their resting hyperpolarized membrane potential around -80 mV, which is recognized as the down-state, to a more depolarized level near -55 mV, called the up-state. These transitions between down- and up-state require channels regulated by several striatal transmitters. Perforated patch clamp recordings on acute rat brain slices were performed to characterize the role of D_2-A_{2A} receptors interactions in the modulation of transitions between down- and up –state. By its own, A_{2A} receptor activation induced no modification. In contrast, D_2 receptor activation abolishes the firing of MSN in the up-state and inhibits the down/up-state transition in a subpopulation of neurons and these effects are totally reversed by A_{2A} receptor stimulation. Moreover, it was also demonstrated that these effects involved a specific calcium channel of the CaV.1 subfamily and an anchoring protein of the Shank family.
Long term potentiation (LTP) could be evoked at the cortico-accumbal synapse. Although, the basal synaptic transmission was not dramatically modified, LTP was significantly reduced in slices from A_{2A}-$R^{-/-}$ mice. Confirming the involvement of this receptor, LTP is also reduced by treatment with A_{2A} antagonist during its induction in wild-type slices
Therefore, in the striatum, A_{2A} receptors, in interaction with D_2, play a key role in the control of transitions between down- and up-state and, hence, in the generation of action potential firing patterns, in the modulation of inhibitory synaptic transmission as well as in the induction of synaptic plasticity events. The modulation of these events, which are important for information processing within the nucleus accumbens and more largely in the striatum, should have a high impact on basal ganglia-related behaviors.
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Glial A_{2A} receptor functions

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Background: Adenosine is considered a prototypic neuromodulatory substance and the better established roles of the more abundant A_1 receptors are related with the control of synaptic transmission and excitability. Furthermore, the ability of A_1 receptors (A_1Rs) to control glutamate release and effects, to control calcium transients and membrane potential is widely assumed to represent the basis of the neuroprotection afforded by acute activation of A_1Rs. This has certainly contributed to bias our view on the role of A_{2A}Rs in the realm of the control of brain function, leading to the conclusion that the role of A_{2A}Rs in the control of brain function and mainly in the control of neuro-degeneration result from a direct action of neuronal A_{2A}Rs.
Aim: Here, we will review evidence indicating that A_{2A}Rs are also located in glial cells (astrocytes and microglia) and that these glial A_{2A}Rs may well play a role in the prominent control of neuro-degeneration in chronic noxious brain conditions.
Results: A_{2A}Rs are expressed both in astrocytes and microglia where they control the uptake and outflow of glutamate from astrocytes and the release of neurotrophic factors, such as NGF, or of inflammatory mediators, such as NO or COX-2 products (references in Fredholm et al., 2005, Int Rev Neurobiol 63:191). In chronic noxious conditions, A_{2A}Rs were identified in brain-resident microglia cells (Angulo et al., 2003, Brain Pathol 13:440), possibly as a result of their up-regulation in reactive microglia, which does not seem to occur in reactive astrocytes (unpublished). We found that 1 day after convulsive behaviour triggered by ip injection of kainate in mice, there is a neuronal damage accompained by astrogliosis and microgliosis and an up-regulation of A_{2A}Rs in non-neuronal cells. All these measures are abolished in A_{2A}R knockout mice (collaboration with JF Chen). Furthermore, SCH58261 (A_{2A}R antagonist) prevented the lipopolyssacharide-induced microgliosis and the release of IL-1β (a pro-inflammatory cytokine) that pre-dates neuronal dysfunction in the rat hippocampus (collaboration with MA Lynch).
Conclusion: These results prompt the hypothesis that the neuroprotection associated with A_{2A}R blockade may also involve glial A_{2A}Rs control of astrogliosis and microgliosis rather than solely or mainly a direct role of neuronal A_{2A}Rs. If these glial A_{2A}Rs are endowed with different properties, it might be possible to conceive novel strategies to selectively interfere with neuronal viability while minimizing effects on neuronal function, mainly in the basal granglia.

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The role of adenosine A_{2A} receptors in parkinsonian-like muscle rigidity and tremor: their interaction with mGluR5 receptors

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Background: Clinical features of Parkinson's disease (PD) include muscle rigidity and tremor. Recently a search for an alternative therapy of PD has focused on antagonists of adenosine A_{2A} and glutamatergic metabotropic mGluR5 receptors, which are active in different models of PD. It is known that A_{2A} and mGluR5 receptors form heteromeric complexes and interact with each other. Therefore the aim of the present study was to determine whether joint administration of the A_{2A} and the mGluR5 receptor antagonists SCH58261 and MTEP, respectively, synergistically influenced parkinsonian-like muscle rigidity and tremor in rats.
Methods: Muscle tone was estimated by a mechanomyographic method (MMG) for measuring the resistance of a rat's hind foot to passive movements. Muscle rigidity was induced by haloperidol (Hal, 0.5 mg/kg ip), and was recorded for 60 min starting at 30 min after its injection. MTEP and SCH58261 were given immediately and 10 min before Hal, respectively.
Parkinsonian-like tremor was induced by tacrine (2.5 mg/kg ip), and was measured for up to 60 min at 10-min intervals. The number of both tremulous jaw movements (TJM), defined as vertical deflections of the lower jaw resembling chewing but not directed at any particular stimulus, and bursts of jaw movements (at least 4 TJM) was assessed.
Results: SCH58261 at doses of 1 - 5 mg/kg caused a dose-dependent decrease in the Hal-induced muscle rigidity during both extension and flexion of the hind foot. At the same time, SCH58261 (5 mg/kg) decreased the number of tacrine-induced TJM and the number of bursts. Administration of MTEP (1 mg/kg) partially decreased the muscle rigidity evoked by Hal, but did not affect the tacrine-induced TJM. Combined treatment with SCH58261 (1 mg/kg) and MTEP (1 mg/kg) synergistically diminished the haloperidol-induced muscle rigidity.
Conclusions: The present study demonstrates that adenosine A_{2A} receptor antagonists reduce parkinsonian-like tremor and parkinsonian-like muscle rigidity in rats. Moreover, combined acute blockade of A_{2A} and mGluR5 receptors potentiates their beneficial effects in a model of muscle rigidity, which makes this pharmacological strategy a promising non-dopaminergic therapy in the treatment of motor deficits in Parkinson's disease.

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Localizing antiparkinsonian mechanisms of A_{2A} receptor antagonists in basal ganglia

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The present study evaluated the effects of the A_{2A} receptor antagonists SCH 58261 and ST 1535 in the unilateral 6-hydroxydopamine (6-OHDA) rat model of Parkinson's disease (PD) by combining behavioral and *in situ* hybridisation studies. In 6-OHDA lesioned rats, acute administration of SCH 58261 or ST 1535 counteracted the motor and sensory/motor impairments induced by the lesion and increased the turning behavior induced by L-DOPA. Administration of L-DOPA alone induced changes in selective striatal neurons different from those induced by the combined administration of SCH 58261 + L-DOPA indicating that a primary site of action of A_{2A} receptor antagonists is the striatum. Moreover, intrapallidal infusion of SCH BT2 a water soluble analogue of SCH 58261 altered neither motor behaviour nor produced postural asymmetry by itself, but it significantly potentiated the number of contraversive rotations induced by a threshold dose of L-DOPA suggesting that A_{2A} receptors located in the globus pallidus may also be involved in the antiparkinsonian effects of A_{2A} antagonists. In chronic studies, SCH 58261 or ST 1535 + L-DOPA induce lower dyskinetic movements than L-DOPA alone and did not induce long-term increase in GAD67 (the synthesizing enzyme of GABA) and dynorphin mRNAs in striatum. The results obtained provide evidence that A_{2A} receptor antagonists may be beneficial in motor impairment which characterize PD. Furthermore the neuronal modifications observed in rat basal ganglia after chronic treatment with SCH 58261 + L-DOPA as compared to L-DOPA alone, suggest that such treatment might not produce detrimental long-term responses in basal ganglia areas.

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A_{2A} Receptors in Psychostimulant Sensitization and Models of L-Dopa Dyskinesia in PD

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Preclinical evidence that adenosine A_{2A} antagonists reliably reduce motor disability without inducing or exacerbating dyskinesias in parkinsonian primates has led to initial clinical data targeting advanced PD patients already experiencing dyskinesias and related motor complications. Little is known however about the role of adenosine A_{2A} receptors (A_{2A}Rs) in the maladaptive neuroplasticity underlying the development of L-dopa-induced dyskinesias.

Using complementary genetic (knockout, KO) and pharmacological approaches to A_{2A}R inactivation our laboratory initially addressed the role of the A_{2A}R in the maladaptive behavioral responses to chronic exposure to psychostimulants, which indirectly stimulate dopaminergic neurotransmission. We found that that locomotor sensitization induced by daily amphetamine treatment is absent in forebrain conditional A_{2A} KO as well as global A_{2A} KO mice, and that this behavioral sensitization could also be prevented in wild-type mice when the daily amphetamine is paired with a low dose of A_{2A} antagonist, KW-6002 or SCH 58261.

In parallel experiments conducted in hemiparkinsonian (unilaterally 6-hydroxydopamine lesioned) mice treated daily with L-dopa, we demonstrated that the development of contralateral rotational sensitization in wild-type mice (which shares some features of dyskinesias in PD) was attenuated in both global and the forebrain conditional A_{2A} KO mice. Again the KO phenotypes were matched by the ability of low doses of KW-6002 and SCH 58261 to reduce the extent of sensitization. Inactivation of forebrain A_{2A}Rs in the conditional (Cre/*loxP* system) KO mice also attenuated L-dopa-induced abnormal involuntary movements (AIMs), which may more closely model the dyskinesias of PD.

Similar studies of A_{2A} antagonist effects in animal models of L-dopa-induced dyskinesias in other laboratories have variably implicated a role of the A_{2A}R, perhaps reflecting dosing or species differences. Nevertheless, bolstered by a demonstration that KW-6002 can prevent the development of dyskinesias in parkinsonian primates, our findings raise the possibility that the early pairing of L-dopa with an A_{2A} antagonist in the treatment of PD may reduce the risk of developing dyskinesias. Moreover, the A_{2A}R may play a broader facilitative role in basal ganglia neuroplasticity in response to chronic dopaminergic stimulation.

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Adenosine A_{2A} receptors in human post-mortem brains and in primate models of Parkinson's disease and dyskinesias

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Brain adenosine A_{2A} receptors (A_{2A}R) have received increasing attention because of their interaction with the dopaminergic system and as potential targets for Parkinson's disease (PD) pharmacotherapy. We reported in postmortem brains that adenosine A_{2A} receptor mRNA and ^{3}H-SCH 58261 specific binding to adenosine A_{2A} receptor were increased in the putamen of dyskinetic PD patients compared to Controls. In the present study, A_{2A}R messenger RNA (mRNA) and receptor specific binding in the brain of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) monkeys were studied after chronic treatment with L-DOPA and drugs to prevent the development of dyskinesias. MPTP monkeys that received L-DOPA/benserazide developed dyskinesias, whereas among the MPTP monkeys who additionally received a selective NR1A/2B NMDA receptor antagonist, CI-1041 or a small dose of the dopamine receptor agonist, cabergoline only one developed mild dyskinesias in each group. Four normal monkeys and four MPTP-treated monkeys were also studied. A_{2A}R mRNA levels, measured by *in situ* hybridization, were increased in the caudate and putamen of saline-treated MPTP monkeys more in their rostral than their caudal parts when compared to controls and remained elevated in L-DOPA-treated MPTP monkeys. A_{2A}R mRNA levels of L-DOPA + CI-1041-treated monkeys were at control levels and decreased in the lateral rostral caudate and caudal putamen when compared to L-DOPA-treated and saline-treated MPTP monkeys respectively whereas in L-DOPA + cabergoline treated monkeys these levels remained elevated. A_{2A}Rs binding labeled by autoradiography with [^{3}H]SCH-58261 was lower in the L-DOPA + CI-1041 and L-DOPA + cabergoline treated MPTP monkeys compared to saline or L-DOPA treated MPTP and control monkeys in the rostral lateral and medial caudate and putamen. No effect of lesion or L-DOPA treatment was measured on [^{3}H]SCH-58261 specific binding. These findings suggest that increased adenosine A_{2A}R in striatopallidal pathway neurons is associated with the development of dyskinesias following long-term L-DOPA therapy in PD. This can be modeled in MPTP monkeys; drugs tested that prevented dyskinesias also modulated A_{2A} receptors.

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Caffeine, estrogen & PD epidemiology

Alberto Ascherio, MD, DrPH

Large epidemiological investigations have identified caffeine consumption as a strong inverse predictor of PD, independently from cigarette smoking. The risk of PD among men habitually consuming 2-3 cups of coffee per day is about half that of men who do not consume caffeine, whereas consumption of decaffeinated coffee is unrelated to PD risk. Results among women, however, are less clear. In women who do not use postmenopausal hormones, risk of PD declines with increasing caffeine intake, as observed in men. In contrast, in women using postmenopausal hormones we found no decrease in risk of PD with increasing caffeine intake, suggesting that female hormones may prevent the beneficial effect of caffeine. This unexpected result has recently been reproduced in the mouse MPTP model of PD. The existence of an interaction between caffeine and estrogen in modulating the risk of PD could provide new clues on their possible mechanisms of action -- estrogen has potent but still incompletely understood effects on the nigrostriatal dopaminergic system and is a competitive inhibitor of caffeine metabolism. Further, the fact that both caffeine and estrogen are being considered as candidates in clinical trials among individuals with PD gives to their potential interaction an immediate practical importance.

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A_{2A} Mechanisms and Regional Localization in an Animal Model of Parkinson's Disease

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Background: The blockade of A_{2A} receptors provides protection against neurodegeneration in several animal models of neurological disorders including Parkinson's disease. Caffeine, selective A_{2A} antagonists or genetic knock-out of A_{2A} receptors attenuates damage to dopaminergic (DA) neurons produced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine or 6-hydroxydopamine. We have found that A_{2A} receptor antagonists also protect against damage to DA neurons caused by a striatal infusion of the mitochondrial inhibitor malonate. We have conducted additional studies to define the mechanism of this protection.

Methods: Cannulas were placed unilaterally into the substantia nigra (SN) and/or striatum of rats using stereotaxic techniques. Studies were performed 3-5 days later. Neurotoxicity: Malonate and DMPX were co-infused into the striatum or alternatively, malonate was infused into the striatum and DMPX into the SN. Rats were euthanized 1 week or 1 month later. The striatum and SN were evaluated for loss of DA neurons. Microdialysis: A dialysis probe was placed into the SN the night before the study. After collection of 3-5 basal samples, malonate was infused into the striatum. Nigral microdialysate samples were collected for 5-8 hours.

Results: The infusion of DMPX into the SN, but not the striatum, provided protection against damage produced by a striatal infusion of malonate. The striatal application of malonate caused a significant elevation in nigral glutamate overflow. Destruction of the striatal GABA outflow pathways by quinolinic acid lesions (performed 3-5 days before malonate infusion) also provided protection to DA neurons against the malonate-induced damage.

Conclusion: These findings indicate that protection exerted by blockade of A_{2A} receptors occurs at the level of the SN and not the striatum. Moreover, the elevation in nigral glutamate following a striatal malonate infusion suggests that even a striatal metabolic stress makes the DA neurons more vulnerable to a possible excitotoxic action within the SN. Consistent with this possibility is that destruction of the striatal GABA outflow pathways provided protection against striatal malonate. We propose that the striatal metabolic stress facilitates activity in the indirect striato-subthalamo-nigral pathway leading to increased glutamate release in the SN that negatively impacts on viability of DA neurons.

To the best of my knowledge and judgment I, the presenting author, report that:
The authors have no financial conflict of interest in the presentation of this work.

Targeting Adenosine A_{2A} Receptors
in Parkinson's Disease
and Other CNS Disorders

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Adenosine A_{2A} receptors modulate psychomotor activity and brain injury by distinct cellular mechanisms

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Background: The adenosine A_{2A} receptor ($A_{2A}R$) has recently emerged as a leading non-dopaminergic therapeutic target for Parkinson's disease for its ability to regulate motor activity. Furthermore, $A_{2A}Rs$ influence brain injury outcome in variety of neurological disease models, presumably through modulation of glutamate release.
Methods: Using forebrain neuronal-specific $A_{2A}R$ knockout (KO) mice, we here provide the first direct evidence that $A_{2A}R$-mediated control of motor function and neuroprotection involve distinct cellular mechanisms.
Results: By crossing the floxed $A_{2A}R$ mice with the CaMKII-Cre transgenic line, we selectively depleted $A_{2A}R$ mRNA and protein in forebrain neurons to the background level of the global $A_{2A}R$ KO mice, as demonstrated by in situ hybridization, immunochemistry and receptor binding assays. This genetic deletion of $A_{2A}Rs$ in forebrain neurons abolished the psychomotor effect of the $A_{2A}R$ selective agonist CGS21680 and antagonist KW-6002 and of the non-selective antagonist caffeine, and largely attenuated the pyschostimulant effect of cocaine. This demonstrates the key role of forebrain neuronal $A_{2A}Rs$ in the modulation of psychomotor activity. In contrast, genetic deletion of the $A_{2A}R$ in forebrain neurons did not confer protection against ischemic brain injury by middle cerebral arterial occlusion or against MPTP-induced dopaminergic neurotoxicity, despite abolishing CGS21680-mediated presynaptic facilitation of glutamate release in forebrain $A_{2A}R$ KO mice. Furthermore, intracerebral ventricular administration of KW-6002 into forebrain $A_{2A}R$ KO mice reinstated neuroprotection against MPTP neurotoxicity.
Conclusion: These results provide the clearest data yet that $A_{2A}R$ activity in forebrain neurons is critical to control psychomotor activity, but not for neuroprotection against brain injury, indicating that $A_{2A}Rs$ modulate motor activity and brain damage by distinct cellular mechanisms. This opens up the new possibility of selectively manipulating $A_{2A}R$'s motor and neuroprotective effects by targeting different cellular elements.

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"Modulation of adenosine A2A receptor signaling"

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Adenosine, acting on A2A receptors, plays a major role in regulating glutamatergic and dopaminergic transmission in the neostriatum. Recent studies will be presented on the role of adenosine A1 receptors in the modulation of dopamine D1 and adenosine A2A receptor signaling in the neostriatum. In addition, we have recently identified a novel neuromodulator of A2A receptor signaling using a yeast-2 hybrid based approach. This modulator appears to interact directly with the A2A receptor and greatly amplify signaling through this receptor, as manifested by both electrophysiological and morphological responses.

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A_{2A} in Huntington's disease and its models

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Huntington's disease (HD), a neurological disorder characterized by chorea, dementia and psychiatric symptoms, is caused by a CAG trinucleotide expansion in exon 1 of the huntingtin gene (Htt) resulting in a selective loss of neurons in striatum and cortex. Adenosine A_{2A} receptors (A_{2A}-Rs) are highly expressed on GABAergic striatal neurons and have been suggested to play a pathogenetic role in HD. Recently, we demonstrated that the blockade of A_{2A}-Rs has a beneficial effect (most probably mediated by a presynaptic mechanism) towards quinolinic acid-induced neurodegeneration, a pathogenetic model of HD. However, in the model of HD induced by 3-nitropropionic acid (3-NPA), both A_{2A}-R agonists and antagonists have been shown to potentiate striatal neurodegeneration, and conflicting results have been found when 3-NPA was administered to A_{2A}-R knock-out mice. Moreover, in R6/2 mice (a genetic model of HD), an A_{2A}-R agonist attenuated some symptoms of the disease. These controversial results may depend on the fact that A_{2A}-Rs may mediated both beneficial and detrimental effects, and thus the final outcome obtained by activating or blocking A_{2A}-Rs might depend on the specific mechanisms that are "at work" under the different experimental conditions. Furthermore, the fact that changes in the transcription, in the expression and in the function of A_{2A}Rs have been reported in HD, adds further complexity to the matter.

In the attempt to clarify the role of A_{2A}-Rs in HD, we have recently evaluated whether the ability of A_{2A}-Rs to modulate excitotoxicity changed with the progression of the disease and whether chronic (1-3 weeks) treatment with the A_{2A}R antagonist SCH 58261 was beneficial in R6/2 mice.

We found that the A_{2A}-R agonist CGS21680 exerted opposite effects in HD and wild-type (WT) mice, reducing and potentiating, respectively, NMDA-induced toxicity in striatal slices. As for the treatment with SCH 58261, we found that the 1-week, but not the 3-weeks treatment schedule elicited beneficial effects on some behavioural and electrophysiological parameters.

These results confirm that the functions of A_{2A}-Rs may be profoundly altered by the HD mutation, and that such an alteration impacts on the ability of A_{2A}-Rs to modulate NMDA-dependent effects. They also suggest that the actual neuroprotective potential of A_{2A}-R antagonists, if any, may be limited to specific (and apparently narrow) time windows.

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Targeting Adenosine A_{2A} Receptors in Parkinson's Disease and Other CNS Disorders

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Adenosine A_{2A} Receptor Antagonism and Ischemic Brain Injury

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Background: Our previous results indicate that the adenosine A_{2A} antagonist SCH 58261, administered acutely soon after focal ischemia, reduces glutamate outflow and an acute motor disturbance in the first hours after ischemia and cortical necrotic damage 24 hours after ischemia.
Methods: Now the effect of a subchronic treatment of the A_{2A} receptor antagonist SCH58261 was studied. Focal ischemia was induced by middle cerebral artery occlusion (MCAo). SCH58261 (0.01 mg/Kg, i.p.) was administered 5 min, 6 hours and 15 hours after MCAo.
Results: Soon after ischemia, contralateral turning behavior was evaluated as the number of rotations per hour between 3 and 4 h after MCAo. In SCH58261-treated rats (n=14), the number of rotations per hour was significantly reduced with respect to vehicle-treated rats (n=13) (mean±S.E.: 116.9±34.6 vs 795.4±170.6, $p<0.0001$). SCH58261-treated rats (n=14) showed significant improvement of the neurological score (mean±S.E: 10.8±0.4 vs 8.8±0.5, $p<0.001$) and reduction in the extent of the ischemic damage by 26% in the cortex (41.1±2.8 mm^3 vs 55.6±3.9 mm^3, $p<0.02$) and by 45% in the striatum (12.8±1.9 mm^3 vs 23.2±2.7 mm^3, $p<0.01$) with respect to vehicle-treated rats. 24 hours after ischemia, phospho-p38 MAPK levels in the ischemic striatum of vehicle-treated rats (n=5) were increased by 500% compared to the contralateral non ischemic striatum. In SCH58261-treated rats (n=6), the phospho-p38 MAPK levels were significantly reduced by 70% in the ischemic striatum ($p<0.01$) with respect to vehicle-treated rats. In the striatum and cortex, the phospho-p38 immunopositive cells showed swollen and hyper-trophic cell bodies as well as processes and exhibited morphological features of activated microglia. In the same areas, 24 h after MCAo, astrocytes are moderately activated. Activated microglia, immunostained by OX-42 or isolectin B4, was present in the same cortical and striatal areas where phospho-p38 immunopositive cells were detected. SCH 58261 reduced phospho-p38 MAPK immunoreactivity in the striatum and cortex without changing the microglia cell morphology.
Conclusion: Results indicate that during ischemia, the protective effect of the adenosine antagonist SCH 58261 involves inhibition of phospho-p38 and indicate that treatment with A_{2A} antagonists may be a useful therapeutic approach up to several hours after ischemia.

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Targeting Adenosine A_{2A} Receptors in Parkinson's Disease and Other CNS Disorders

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Anti-inflammatory actions of adenosine: Implications for multiple sclerosis, intracerebral hemorrhage and HIV-1 dementia.

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Inflammatory events are at least associated with if not contributors to neurodegeneration observed in such acute conditions as hemorrhagic stroke as well as in such chronic conditions as multiple sclerosis and HIV-1 associated dementia. Involved are proinflammatory substances originating from brain-resident cells as well as cells that traffic into brain. For intracerebral hemorrhage, the A_{2A} receptor agonist CGS 21680 decreased numbers of TUNEL positive cells and inhibited TNF-α mRNA production by greater than 95% in the immediate area of the hematoma possibly through interactions with infiltrating neutrophils, mononuclear cells, resident microglia, astrocytes and neurons - all cells that express A_{2A} receptors. For multiple sclerosis, adenosine in general and adenosine A_1 receptors more specifically may play an important role because serum levels of TNF-α were significantly higher and those of adenosine were significantly lower in multiple sclerosis patients; stimulation of A_1 receptors in peripheral blood mononuclear cells from control subjects, but not relapsing-remitting multiple sclerosis patients, significantly inhibited TNF-α levels; and significantly reduced levels of A_1 receptors were found in brain, peripheral blood mononuclear cells and blood derived macrophages from multiple sclerosis patients. A_1 receptor null mice with experimental allergic encephalomyelitis developed a more pronounced progressive-relapsing form of the disorder, had increased expression of proinflammatory genes, and had a down-regulation of A_1 receptors compared to wild type mice. Furthermore, up-regulation of adenosine receptors following chronic caffeine ingestion lessened the severity of the experimental allergic encephalomyelitis. For HIV-1 associated dementia, only few reports have appeared implicating the adenosine system in the pathogenesis of HIV-associated dementia or suggesting the use of adenosine therapeutics for this condition. A_{2A} receptor activation with CGS21680 almost completely blocked HIV-1 Tat protein induced increases in TNF-α through an action mediated by protein phosphatases. Although inflammation plays an important role in neurodegenerative conditions and adenosine receptor manipulation has been shown to be anti-inflammatory and neuroprotective, it is less clear the extent to which the actions of adenosine receptor activation and blockade is neuroprotective because of its anti-inflammatory effects. [This work was supported by CIHR (HOP-8901), NIA (AG-17628), NIMH (MH-065431), NICHHD (HD-17449), and NCRR (P20 RR-017699).]

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Targeting Adenosine A_{2A} Receptors in Parkinson's Disease and Other CNS Disorders

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A_{2A} receptors in Alzheimer's disease and its models

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Evidence from animal models suggests that blockade of adenosine A_{2A} receptors may attenuate neuronal damage caused by beta-amyloid, the peptide associated with Alzheimer's disease (AD) pathology. Either caffeine, an adenosine A_1 and A_{2A} receptor antagonist, or ZM 241385, a selective adenosine A_{2A} receptor antagonist, prevent neurotoxicity induced by beta-amyloid in cerebellar granule cell cultures. Preliminary data indicate that blockade of adenosine A_{2A} receptors may also attenuate neurotoxicity induced by beta-amyloid using *in vivo* rat models.

In humans, there is evidence that caffeine may protect from AD. In a case-control study, patients with AD had an average daily caffeine intake of 73.9±97.9 mg during the 20 years that preceded diagnosis of AD, whereas the controls had an average daily caffeine intake of 198.7±135.7 mg during the corresponding 20 years of their lifetimes. Caffeine exposure during this period was significantly inversely associated with AD (odds ratio =0.40, 95% confidence interval =0.25–0.67). Caffeine may have a symptomatic effect or a genuine neuroprotective effect in AD. In healthy volunteers, caffeine is able to improve mood and to enhance psychomotor and cognitive performance. Using transcranial magnetic stimulation, a non-invasive technique that allows accessing of the excitatory and inhibitory properties of neuronal networks in humans, caffeine significantly reduced the cortical silent period, showing effects on neuronal excitability and on the efficiency of the communication among neuronal cells in the neocortex.

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Targeting Adenosine A_{2A} Receptors in Parkinson's Disease and Other CNS Disorders

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Adenosine A_{2A} Receptors and Drug Addiction

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The ventral striatum forms part of the brain circuitry involved in goal-directed behavior, the conversion of motivation into action. A common denominator in the neurochemical effects of most addictive substances, including opiates, ethanol, amphetamines, cocaine, nicotine in tobacco and Δ^9-tetrahydrocannabinol (THC) in marijuana, is striatal dopamine release, with preferential effects on the ventral striatum (nucleus accumbens shell). Furthermore, increased glutamatergic neurotransmission in the cortical-accumbens projection is emerging as an important mechanism involved in the secondary reinforcing effects of addictive drugs and drug-related environmental stimuli ("drug-seeking behavior"). Since the ventral striatum contains a high density of adenosine A_{2A} receptors, which strongly modulate excitatory synapses of GABAergic enkephalinergic neurons, adenosinergic modulation of drug-seeking and drug-taking behavior is likely.

We have recently demonstrated functional and physical interactions between A_{2A} and cannabinoid CB_1 receptors. These include demonstrations of both true A_{2A}-CB_1 heteromers using BRET techniques in mammalian co-transfected cells and A_{2A}-CB_1 heteromeric receptor complexes in rat striatum using co-immunoprecipitation techniques. In *in-vitro* studies with a human neuroblastoma cell line, CB_1 receptor signaling was completely dependent on A_{2A} receptor activation and, in *in-vivo* studies with rats, blockade of A_{2A} receptors counteracted motor depressant effects produced by intra-striatal administration of the cannabinoid receptor agonist WIN 55,212. In *in-vivo* studies with squirrel monkeys, the A_{2A} receptor antagonist MSX-3 markedly potentiated reinforcing effects of the CB_1-receptor agonists anandamide and THC, when drug-taking behavior was studied under a fixed-ratio schedule. This occurred at doses of MSX-3 with no effect on comparable responding for food. In contrast, MSX-3 did not reinstate extinguished anandamide or THC drug-seeking behavior, but immediately and almost completely suppressed drug-seeking behavior maintained by THC under a second-order schedule in which behavioral responses intermittently produced brief visual stimuli that were paired with THC injection only at the end of daily sessions. Finally, A_{2A} receptor antagonists can potentiate the discriminative effects of methamphetamine and cocaine in rats. We hypothesize that A_{2A} receptor antagonists can enhance the direct reinforcing effects of THC and other addictive drugs by potentiating dopaminergic neurotransmission, but can reduce drug-seeking behavior by presynaptic modulation of actions on glutamate release and endocannabinoid signaling.

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Targeting Adenosine A_{2A} Receptors in Parkinson's Disease and Other CNS Disorders

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A2A adenosine receptors regulate CNS responses to ethanol and addicting substances: Recent advances from cell biology to behavior.

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The highest levels of Gs(olf)-coupled A2A adenosine receptors (A2A) in the brain are expressed in the striatum, which includes the nucleus accumbens (NAcb), a mesolimbic brain reward region involved in alcoholism and addiction. In neural cell cultures, ethanol inhibits adenosine re-uptake and increases extracellular adenosine levels, thereby activating A2A receptors. Activation of A2A receptors increases cAMP production and PKA signaling. In the NAcb, A2A receptor expression occurs postsynaptically on the same neurons expressing Gi-coupled D2 dopamine (D2), mu opioid (MOR) and cannabinoid (CB1) receptors. These receptors are involved in addiction.

Activation of Gi-coupled receptors for 30 min. inhibits cAMP production. By contrast, activation for 10 min. first increases cAMP levels. We show in primary NAcb/striatum neurons that subthreshold levels of D2, MOR or CB1 agonists synergize to stimulate cAMP/PKA signaling in 10 min., inducing CRE-mediated gene expression 5 hours later. Synergy between receptors involved in addiction is mediated by free Gi-βγ subunits released from Gαi3. Free Gi-βγ subunits potentiate Gαs stimulated adenylyl cyclase (AC) activity. The Activator of G Protein Signaling, AGS3, appears to enhance free βγ stimulation of AC by binding to Gαi3 and preventing βγ from re-associating with Gαi3. Importantly, A2A blockade or degradation of adenosine by adenosine deaminase prevents all instances of drug-induced synergy in neuronal cultures.

Relapse is the most serious complication that prevents effective medical treatment of human addicts. Antisense knockdown or inhibition of individual components of the Gαi3/βγ/AGS3/cAMP/PKA signaling pathway prevents synergy for CRE-mediated gene expression in primary NAcb neurons. In an *in vivo* model of human relapse, knockdown of AGS3 in the NAcb core, but not in the NAcb shell, eliminates reinstatement of heroin-seeking behavior in addicted rats. Because A2A is required for ethanol and synergy-induced cAMP/PKA signaling in primary neurons, we asked whether A2A antagonists prevent addictive behaviors in rats. Indeed, A2a receptor antagonists (DMPX or MSX-3) administered directly into the NAcb or indirectly by i.p. injection reduce voluntary ethanol drinking and eliminate reinstatement of heroin-seeking behavior in addicted rats. These findings in rodent models of human alcoholism and heroin relapse suggest that A2A adenosine receptor antagonists could be useful therapeutic agents in the medical management of addicted patients.

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A patent under review related to the work being reported is held by the University of California, San Francisco. Dr. Diamond is Vice President, Neuroscience, CV Therapeutics. Lina Yao is a Senior Scientist in Neuroscience at CV Therapeutics.

Targeting Adenosine A_{2A} Receptors
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A_{2A} in pain

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The endogenous mediator adenosine has complex effects in pain, and can be either pronociceptive or antinociceptive depending on the site of administration and the receptor subtype activated. Most attention has been focussed on the effects on pain pathways of adenosine acting via the A_1 receptor and there is ample evidence that activation of spinal A_1 receptors results in antinociceptive effects in acute nociceptive tests and models of neuropathic and inflammatory pain. There is debate as to whether A_1 receptors on peripheral nerves are pro- or antinociceptive, but overall central A_1 receptors dominate and this is confirmed in A_1 receptor gene knockout mice which have an enhanced response to nociceptive stimuli and show reduced responses to morphine. The role of the A_{2A} receptor in pain is less clear and we, and others, have shown the receptor is absent in the spinal cord and very discretely expressed in striatal brain regions. The absence of the A_{2A} receptor in parts of the CNS associated with pain pathways suggests that any antinociceptive effects of drugs acting at the A_{2A} receptor are likely to be peripheral rather than central. Peripheral injections of A_{2A} antagonists reduce behavioural responses to nociceptive stimuli suggesting pronociceptive A_{2A} receptors on sensory nerves may enhance nociceptive signalling, and in support of this assertion the original phenotyping of A_{2A} receptor gene knockout mice showed reduced responses to thermal nociceptive stimuli. We have been studying in greater detail the hypothesis that the A_{2A} receptor on periperhal nerves plays a key role in modulating noiceptive traffic to the spinal cord and thus might be a novel target for analgesics. Mild nociceptive stimuli such as low temperature thermal tests and the tail pressure tests show no differences in responses in A_{2A} knockout mice. In contrast, high temperature thermal tests show reduced responses in the absence of the A_{2A} receptor, and there is also a reduced behavioural response in the first phase of the formalin test, a strong inflammatory pain stimulus. In addition to this, we have found greatly reduced binding of [^{3}H]MK801 to NMDA receptors in the spinal cord of A_{2A} knockout mice which is likely to reflect a change in pain signalling in these mice, as glutamate binding to NMDA receptors is the major transmitter released from primary afferent neurones synapsing in the spinal cord. In further support of a pronociceptive role of A_{2A} receptors we have also shown the selective A_{2A} antagonist SCH58261 to be antinociceptive in thermal tests at high temperatures. Lack of the A_{2A} receptor gene also manifests other alterations in pain pathways and we have shown changes in δ and κ opioid receptors in the spinal cord in parallel with altered antinociceptive responses to δ and κ opioid receptor agonists, suggesting that the lack of A_{2A} receptors during development causes changes in pain processing. In conclusion, our current understanding suggests that the A_{2A} receptor plays a role in pain processing, probably at peripheral sensory sites, and may be more important in regulating pain induced by high intensity stimuli. More studies are needed to determine the potential for novel analgesics acting at the A_{2A} receptor in high intensity pain conditions.

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Key roles of adenosine A_{2A} receptor in sleep-wake regulation

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Background: Adenosine is one of major humoral sleep-inducing substances and stimulates 4 distinct subtypes (A_1, A_{2A}, A_{2B}, and A_3) of receptors in the central nervous system. Although both A_1 receptors (A_1R) and A_{2A} receptors (A_{2A}R) are proposed to be involved in the sleep-inducing effect of adenosine, it is controversial which subtype of receptors plays a major role in the sleep-wake regulation.

Methods: We used stable analogs of adenosine, which selectively bind to A_1R or A_{2A}R, to examine the sleep induction after their intracerebroventricular (i.c.v.) infusion. We also determined the sleep-wake cycles of wild-type (WT), A_1R-knockout (KO), and A_{2A}R-KO mice under the basal conditions and after total sleep deprivation for 6 hr. Furthermore, we measured the arousal effect of caffeine in those three groups of mice.

Results: When infused i.c.v. into wild-type (WT) mice, cyclopentyl adenosine, an agonist of A_1R, did not change the sleep-wake cycle; whereas CGS21680, an agonist of A_{2A}R, induced potent non-rapid eye movement (non-REM) sleep. The i.c.v. infusion of A_{2A}R-agonist increased the expression of *c-fos* protein in the ventrolateral preoptic area (VLPO), one of the sleep centers, in a non-REM sleep-dependent manner. The activation of VLPO neurons was associated with a decrease in *c-fos* expression in the histaminergic tuberomammillary nucleus (TMN), one of the arousal centers. Activation of A_{2A}R evoked GABA release specifically in the TMN, but not in the cortex, to inhibit selectively the histaminergic system and induced non-REM sleep. Compared with WT mice, A_1R-KO mice did not show any changes in non-REM and REM sleep regulation under basal conditions and exhibited the same amount of rebound sleep after 6-hr sleep deprivation during the light period. On the other hand, A_{2A}R-KO mice showed a slight increase in non-REM and REM sleep during the dark period under the basal conditions and did not exhibit the non-REM sleep rebound after the 6-hr sleep deprivation. Moreover, caffeine inhibited non-REM and REM sleep in WT and A_1R-KO mice but not in A_{2A}R-KO mice at all.

Conclusion: These results indicate that A_{2A}R, but not A_1R, is important for sleep-wake regulation and that the neural network between VLPO and TMN regulates sleep and wakefulness by means of a 'flip-flop' mechanism.

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Targeting Adenosine A_{2A} Receptors
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In vivo imaging of adenosine A_{2A} receptor using molecular imaging technique

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PET based molecular imaging techniques allow the in vivo study of different biological targets in living subjects. We have recently developed and validate in selected clinical models a new ligand for the in vivo measurement of adenosine A_{2A} receptors named [C-11]SCH442416. Preclinical biodistribution studies indicated that this molecule demonstrated both in rodents and primate a kinetic and pharmacological profile adequate for a further application to human studies. This radioligand distributes in rats and monkey brain with a rank order that follows that of adenosine A_{2A} . it is characterized by a slow rate of metabolic degradation in brain and periphery and by an high specificity for the target site since its in vivo uptake is significantly reduced only by drugs acting on A_{2A} receptors. The administration of quinolinic acid, a neurotoxin that selectively destroy intrastriatal gabaergic neurons, induces a significant reduction of radioactivity accumulation indicating that the sensitivity of [^{11}C]SCH442416 is adequate to visualize modifications in A_{2A} receptors availability. This radioligand has been successfully applied in ex-vivo and in vivo studies for the characterization of long term effects of quinolinic acid administration on intrastriatal neurons degenerations.

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May 17 - 19, 2006
Boston USA

Targeting Adenosine A_{2A} Receptors in Parkinson's Disease and Other CNS Disorders

presented by
MassGeneral Institute for Neurodegenerative Disease

Progressing the A_{2A} antagonist BIIB014/V2006 to the clinic for the treatment of PD

Sean Lightowler

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BIIB014/V2006 is a novel, non-xanthine adenosine receptor antagonist that displays high affinity (binding Ki 1.3 nM) and selectivity (>50-fold) for the A_{2A} receptor subtype. BIIB014/V2006 has good oral activity in three species, in three distinct models used to assess its potential anti-Parkinson's efficacy: The mouse and rat haloperidol-induced hypolocomotion test (MED 0.1-1.0 mg/kg po); the rat unilateral 6-OHDA test (MED 3 mg/kg po); the marmoset MPTP model (MED < 5 mg/kg po).

Consistent with its efficacy in models of PD, *in vivo* pharmacokinetic studies in rats demonstrate that BIIB014/V2006 possesses good oral bioavailability, a long plasma half-life and good brain penetration. In the cynomolgus monkey BIIB014/V2006 also exhibits a long half-life and high exposure following oral administration.

In vitro DMPK studies indicate that BIIB014/V2006 is metabolised via multiple Cytochrome P450 (CYP) isoforms, indicating a low propensity for drug-drug interactions or variable pharmacokinetics due to CYP polymorphisms. Furthermore, the compound does not inhibit the major CYP isoforms *in vitro*.

Safety pharmacology studies have not revealed any effects that are unexpected from the intended CNS activity of BIIB014/V2006. BIIB014/V2006 is not genotoxic in standard tests. Repeated dose toxicity studies in rats and monkeys have demonstrated that high systemic exposures of BIIB014/V2006 are tolerated without obvious adverse effects.

BIIB014/V2006 was tolerated well in healthy male and female subjects in studies in which it was administered in single oral doses up to 100 mg and repeated dosing with up to 50 mg/day for 10 days. BIIB014/V2006 exhibited good systemic exposure and a mean terminal elimination half-life consistent with once a day dosing for the treatment of PD.

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Targeting Adenosine A_{2A} Receptors
in Parkinson's Disease
and Other CNS Disorders

May 17 - 19, 2006
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MassGeneral Institute for Neurodegenerative Disease

SCH 420814: A novel Adenosine A2a antagonist. Exploring Parkinson's Disease and beyond.

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Background: Antagonism of the adenosine A2a receptor represents a novel means of improving motor function in PD patients but without the motor complications (*e.g.* dyskinesia) as well other adverse events that are associated with current, predominantly dopaminergic, therapies.
Methods: SCH 420814 was investigated in several rodent (*e.g.* catalepsy, 6-OHDA) and primate (*e.g.* catalepsy, MPTP) models of PD. In the clinic, SCH420814 was investigated for safety in rising single and multiple dose studies in healthy human volunteers. A Phase 1b study to correlate plasma exposure with receptor occupancy was performed using the ligand [^{11}C]-SCH 442416 and measured by positron emission tomography (PET). Two Phase 2a studies then investigated the short-term exposure of SCH (bid dosing over 1-3d), dosed concomitantly with L-Dopa, in PD patients. Motor function was measured using the UPDRS Part III.
Results: SCH 420814 (0.1-3 mg/kg, PO) improved motor function in all animal models of PD. SCH (0.1-1 mg/kg) produced a dose-dependent reversal of haloperidol-induced catalepsy in rats and mice. In contrast to L-Dopa, SCH (1 and 3 mg/kg, PO) produced a dose-dependent improvement in motor function without dyskinesia in the MPTP cynomolgus monkey. A more pronounced effect was observed in animals with mild to moderate disease. In severe disease, potentiation of L-Dopa showed an improvement in function with a significant increase in time spent in the "on-state". In the Phase 1 RSD and RMD clinical safety studies, SCH was found to be well tolerated with all adverse events non-specific, mild and self-limiting. The profile was quite different to a dopaminergic agent with no nausea and vomiting, hypotention or any CNS events recorded up to the maximal dose. In the Phase 1b PET study, SCH produced displacement of [^{11}C]-SCH 442416 binding in the caudate-putamen that was time and dose-dependent. In the Phase 2a studies in PD patients, short-term exposure of SCH (bid dosing over 1-3d), dosed in combination with L-Dopa, produced an improvement in motor function as measured by the UPDRS Part III.
Conclusion: The potent and highly selective A2a antagonist SCH 420814 produces an improvement in motor function in both animal models of PD and in PD patients with apparent need for dose titration.

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Targeting Adenosine A_{2A} Receptors in Parkinson's Disease and Other CNS Disorders

presented by
MassGeneral Institute for Neurodegenerative Disease

Designing PD Clinical Trials of Symptomatic Therapies for Disease Modification: The J. Stephen Fink Memorial Lecture

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Background: Controlled clinical trials for slowing progression of PD began more than 20 years ago with the DATATOP study evaluating selegiline and alpha-tocopherol. This trial evaluated these medications in "de novo" subjects, and the trial design instituted a novel measurement as the primary outcome variable, namely the need for dopaminergic therapy. When the results revealed that selegiline has mild symptomatic effects, there was realization that interpretation of any disease-modifying effects was problematic. Thus, it became apparent that new methodologies would be needed to detect disease modification in the presence of drugs that also provided any symptomatic effect.
Methods: Following DATATOP, other primary outcome variables were utilized. In trials working with do novo subjects, measuring a change in UPDRS scores from the baseline evaluation has been used, including scores after washout of experimental medication and scores comparing early-start and delayed-start. Other trials evaluated subjects who were taking symptomatic drugs for PD. These trials utilized outcomes of change from baseline in UPDRS "practically-defined off" scores, change in neuroimaging of dopaminergic cell markers and change in other clinical features over time. "Futility" trials are variants of UPDRS change trials, using fewer subjects.
Results: With the proposal by Paul Leber (1997) that a delayed-start design has several advantages over a wash-out design to determine disease modification, there has been growing interest in using this approach to evaluate drugs that are known to have beneficial symptomatic effects on the disease being studied. Such trial designs in PD have the added burden that de novo subjects are aware that effective symptomatic therapies are available, so the severity of disease at baseline would be an important factor in limiting the amount of time for the parallel first phase that is placebo controlled. Too long a duration would likely lead to subjects terminating the study if symptoms have worsened to the point of requiring symptomatic relief. The second phase – both treatment arms now on active symptomatic therapy – needs to be of sufficient duration to allow the opportunity to discover if the symptomatic benefit remains apart in a parallel manner, if the two treatment arms converge over time, or if the delayed-start arm becomes superior to the early-start arm. These three outcomes would reflect disease modification, pure symptomatic effect, and disease worsening by the experimental drug, respectively. The degree of symptomatic benefit by the experimental drug would influence the duration of the two treatment phases, with a weak drug needing to keep the second phase short enough to avoid terminations by subjects wanting stronger symptomatic relief. A third phase – a long washout phase – is also desired in case the symptomatic effect of the experimental drug is so strong that it would overwhelm and thereby mask the manifestation of a disease modifying effect of the drug.
Conclusion: The delayed-start design appears to be the only acceptable design so far conceived that can allow a demonstration of a disease-modifying effect of a drug that also has symptomatic effects.
Funded by Columbia University

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SECTION 4 – ***Poster Presentations***

Photograph by Don Eyles July 2004

P-1

May 17 - 19, 2006
Boston USA

Targeting Adenosine A_{2A} Receptors in Parkinson's Disease and Other CNS Disorders

presented by
MassGeneral Institute for Neurodegenerative Disease

Novel Analogs of SCH 58261 as Potent and Selective Adenosine A_{2A} Receptor Antagonists

Craig D. Boyle[1]*, Unmesh Shah[1], Samuel Chackalamannil[1], Bernard Neustadt[1], Neil Lindo[1], Carolyn Foster[2], Leyla Arik[2], Ying Zhai[2], Jean E. Lachowicz[2], Kwokei Ng[3], Shiyong Wang[3], & Angela Monopoli[4]

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Background: Blocking A_{2A} receptors produces anti-Parkinsonian activity in animal models suggesting that A_{2A} receptor antagonists will be therapeutically beneficial in Parkinson's Disease. In addition to their potential as anti-Parkinson's drugs, recent evidence suggests that A_{2A} receptor antagonists may also possess anti-depressant activities. SCH 58261 was discovered as a potent A_{2A} receptor antagonist with modest selectivity over A_1.
Methods: This poster summarizes our efforts to use SCH 58261 as a structural template to identify more potent and selective A_{2A} antagonists with acceptable *in vitro* and *in vivo* profiles. The synthesis and design of SCH 58261 biaryl and heteroaryl analogs are described herein. *In vitro* evaluation of the compounds was accomplished via receptor binding assays against A_{2A} and A_1 receptors. Rat PK was measured, and *in vivo* activity was tested in a haloperidol challenge catalepsy assay.
Results: Biaryl and heteroaryl derivatives of SCH 58261 were discovered with superior binding profiles compared with the parent compound. In addition, the best analogs demonstrated acceptable plasma exposure as well as anticataleptic activity in rats dosed with haloperidol.

Funded by Schering-Plough Research Institute

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The authors are employed by Schering-Plough which funds this research. Most employees have company stock as well.

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Targeting Adenosine A_{2A} Receptors in Parkinson's Disease and Other CNS Disorders

May 17 - 19, 2006
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presented by
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Pharmacological Profile of SCH 412348 and SCH 420814, selective A_{2A} adenosine receptor antagonists

Jean Lachowicz[1]*, Ying Zhai[1], Lia Kwee[1], Leyla Arik[1], Jinsong Hao[2], Bernard Neustadt[2], William Greenlee[2], John Hunter[1] & Carolyn Foster[1]

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Background: A growing body of evidence supports the utility of A_{2A} receptor antagonists for treatment of Parkinson's Disease and other neuromotor disorders. The endogenous agonist adenosine is a ubiquitous nucleoside that mediates physiological functions via four adenosine receptor subtypes. Because of the wide tissue distribution of these receptors and the range of functions that they impact, subtype selectivity is a crucial requirement for a successful therapeutic agent. Here we report SCH 412348 and SCH 420814, two A_{2A} antagonists with high selectivity over A_1, A_{2B}, and A_3 receptor subtypes.
Methods: Membrane preparations from HEK293 or CHO cells expressing adenosine receptor subtypes were used for binding assays to determine affinity (K_i) values. For A_{2A} receptors, [^{3}H] SCH 58261 was used as the radioligand in competition assays. Binding kinetics were determined by measuring the effect of compounds on [^{3}H] SCH 58261 kinetics. K_B determinations were performed by incubating compounds and the A_{2A} agonist CGS 21680 with cells and measuring total cAMP production.
Results: SCH 420814 and SCH 412348 bind A_{2A} receptors with affinities of 1.3 and 0.6 nM. K_i values for SCH 420814 for A_1 and A_3 receptors are greater than 1000 nM. SCH 420814 binds competitively to A_{2A} receptors, as saturation binding of [^{3}H] SCH 58261 shows decreased affinity rather than B_{max} in the presence of the compound. SCH 420814 and SCH 412348 inhibit increases in cAMP mediated by CGS 21680 with K_B values of 1.3 and 0.26 nM, respectively. Both compounds display slower dissociation rates from the A_{2A} receptor than SCH 58261, with calculated off-rates of 30 and 90 minutes *in vitro*.
Conclusion: Using recombinant systems, we have demonstrated that SCH 412348 and SCH 420814 are high affinity A_{2A} receptor antagonists with high selectivity over other adenosine receptor subtypes. The increase in selectivity and decrease in dissociation rate gained by these compounds in comparison to SCH 58261 is expected to confer increased utility in models of Parkinson's Disease and other movement disorders.

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P-3

May 17 - 19, 2006
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Targeting Adenosine A_{2A} Receptors in Parkinson's Disease and Other CNS Disorders

presented by
MassGeneral Institute for Neurodegenerative Disease

Pharmacology of BIIB014/V2006, an A_{2A} antagonist for the treament of PD

S. Lightowler*, A.R. Knight, R. Upton, A. Misra, D. M. Sellwood, M. Reece, J. Lerpiniere, G. C. Stratton, R. J. Gillespie

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Background: During 1998 Vernalis initiated a drug discovery campaign to identify novel adenosine A_{2A} receptor antagonists for the treatment of PD. A screening cascade was constructed, which incorporated a range of pharmacological, DMPK, side effect and toxicity assays, and associated compound progression criteria. This paper describes the pharmacology assay data generated for BIIB014/V2006, a compound progressed successfully to clinical studies.
Methods: BIIB014/V2006 was tested in the following pharmacology assays:
a. Receptor affinity determination via displacement of radioligand to all four known human adenosine receptor subtypes (A_1, A_{2A}, A_{2B} and A_3).
b. Wide binding activity (1 µM) at 75 receptors and 7 enzymes.
c. Functional agonist/antagonist activity at human A_1, A_{2A}, A_{2B} and A_3 receptors by fluorimetric-imaging plate reader technology.
d. Reversal of hypolocomotion induced by the dopamine receptor antagonist, haloperidol, in rodents, as an indicator of its *in vivo* potency.
d. Potentiation of the effects of a sub-threshold dose of apomorphine on contralateral rotations in 6-OHDA unilaterally lesioned rats, as a predictor of its clinical efficacy as an adjunct treatment to L-DOPA or a dopamine receptor agonist.
e. Reversal of PD symptoms in dyskinetic MPTP lesioned, L-DOPA pretreated marmosets.
Results: BIIB014/V2006 displayed high affinity for, and potent antagonist activity at the adenosine A_{2A} receptor (binding Ki 1.3 nM), and a high degree of selectivity (>50-fold vs all other sites tested). The minimum effective dose for BIIB014/V2006 given orally before monitoring activity in haloperidol pretreated mice and rats was 0.1 mg/kg and 1 mg/kg, respectively. BIIB014/V2006, in combination with a sub-threshold dose of the dopamine agonist apomorphine, increased the number of contralateral and ipsilateral rotations in 6-OHDA unilaterally lesioned rats compared to apomorphine alone. Statistically significant effects were seen at 3 and 10 mg/kg orally, and there was a more pronounced effect on contralateral than ipsilateral rotations, indicating specific anti-parkinsonian activity. In MPTP lesioned, L-DOPA pretreated marmosets, BIIB014/V2006 produced the same magnitude of anti-parkinsonian effect as L-DOPA against disability, with an equivalent duration of action, but fewer dyskinesias.
Conclusion: BIIB014/V2006 is a novel, adenosine receptor antagonist that displays high affinity and selectivity for the A_{2A} receptor subtype. BIIB014/V2006 has good oral activity in three species, in three distinct models used to assess its potential for efficacy in Parkinson's disease.

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P-4

May 17 - 19, 2006
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Targeting Adenosine A_{2A} Receptors in Parkinson's Disease and Other CNS Disorders

presented by
MassGeneral Institute for Neurodegenerative Disease

Discovery of Potent and Selective Adenosine A2a Receptor Antagonists: Triazolo[1,5-*c*]pyrimidines

Hong Liu*, Bernard Neustadt, Carolyn Foster, Leyla Arik, Jean Lachowicz, Rosalia Bertorelli, Silva Fredduzzi, Kwokei Ng, Andrew Stamford & William Greenlee

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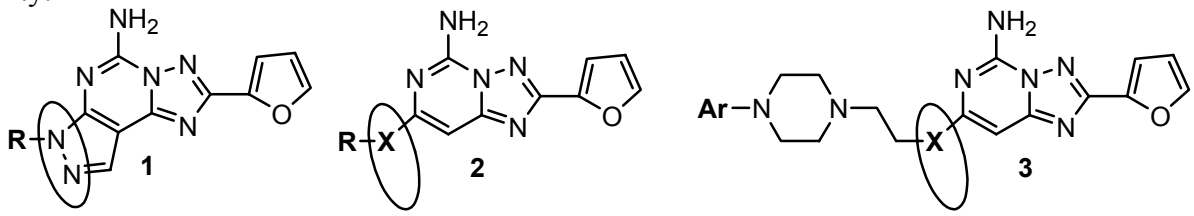
Background: Adenosine A2A receptor antagonists, by virtue of their modulation of the dopaminergic system, offer great potential in the treatment of Parkinson's disease and other neurological disorders. Pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidines of type **1** have been reported as adenosine A2A antagonist, and an agent of this type is currently undergoing clinical evaluation.

Methods: Among structural variations of this tricyclic system, bicyclic triazolo[1,5-c]pyrimidines of structure **2** were explored. Synthetic methods were developed which allowed access to a set of targets **2** incorporating varied hetero-atom linkers **X**. These compounds were assayed for A2a receptor affinity, receptor subtype selectivity, and oral activity in the rat haloperidol-induced catalepsy model.

Results: These compounds proved to be potent adenosine A2A receptor antagonists. However, only with a substituted N-atom as linker was high selectivity achieved versus the A1 receptor. SAR studies delineated additional structural requirements for high potency and selectivity.
With introduction of an aryl-piperazine side-chain as in structure **3**, potency and selectivity were retained. Most significantly, several targets of type **3** showed good plasma levels and potent oral anti-cataleptic activity.

Conclusion: The series of A2A antagonists of type **3** demonstrates oral activity and is intrincally more soluble than the series **1** which provided anti-Parkinson's clinical candidate Sch 420814. With the exception of significant hERG inhibitory activity, this series has the potential for clinical utility.



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May 17 - 19, 2006
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Targeting Adenosine A_{2A} Receptors in Parkinson's Disease and Other CNS Disorders

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Design and Comparison of Biological Assay Formats to Support the Pharmaceutical Development of Adenosine A_{2A} Antagonists

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Background: Antagonists of the human adenosine A_{2A} (hA_{2A}) receptor have been shown in the clinic to reduce the symptoms of Parkinson's disease (PD). Their ability to potentiate the efficacy of L-dopa and to possibly reduce the neurodegeneration that accelerates the disease makes this receptor a compelling target for therapeutic intervention. Identification of suitable, small molecule antagonists requires the development of robust screening and selectivity assays. The primary selectivity target for hA_{2A} is the adenosine A_1 (hA_1) receptor since disruption at this receptor may lead to undesirable cardiac effects. In this poster we describe multiple assay formats that can be used to screen compounds for inhibitory activity of either hA_{2A} or hA_1.
Methods: Three types of radioligand binding assays were designed using membranes from recombinant cell lines expressing hA_{2A} or hA_1. In addition to heterogeneous filter binding assays, homogenous SPA scintillation and SPA imaging assays were optimized and validated using wheat germ agglutinin (WGA) PVT SPA scintillation beads and WGA YOx SPA imaging beads, respectively. All optimized assays were subsequently used to quantify K_d's and K_i's for a series of agonists and antagonists, respectively.
Results: Three assay formats were designed to determine K_i values for a diverse panel of small organic molecules. The hA_{2A} filter binding and SPA imaging assays generated reproducible K_i values that showed excellent correlation between the two assay methods. A meaningful signal could not be generated for hA_{2A} using SPA scintillation beads and so a comparison to that assay format cannot be made. The K_is for hA_1 were within a factor of three across all three (filter binding, SPA scintillation and SPA imaging) radioligand binding formats. Taken together, these data suggest that compound evaluation for hA_{2A} or hA_1 can be conducted using any one of the aforementioned assay formats.
Conclusion: The data suggest that these assay formats can serve as platforms for both high-throughput screening (HTS) and for lead optimization of potential A_{2A} antagonists. With the exclusion of a WGA SPA scintillation assay, hA_{2A} antagonist activity was successfully measured in filter binding and SPA imaging bead assays. hA_{2A} antagonist selectivity was effectively determined in hA_1 assays based either on filter binding, SPA scintillation or SPA imaging bead methodologies. The consistent K_i values obtained in both the hA_{2A} and hA_1 assays show that multiple formats can be used to develop robust assays for the pursuit of selective A_{2A} antagonists.

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A2A PD Boston 2006

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Targeting Adenosine A_{2A} Receptors
in Parkinson's Disease
and Other CNS Disorders

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Pre-clinical drug metabolism and pharmacokinetic studies with BIIB014/V2006

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BIIB014/V2006 has high exposure after oral dosing and long half lives in several pre clinical species notably the rat and cynomologus monkey. In the rat, CNS exposure of BIIB014/V2006 has also been demonstrated in both whole brain and CSF.
The cross species metabolic profile of BIIB014/V2006 was qualitatively similar, with the cynomolgus monkey being most similar to human. As a consequence, the cynomolgus monkey was chosen as the non-rodent toxicology species for BIIB014/V2006.
In vitro DMPK studies indicated that BIIB014/V2006 is metabolised by multiple Cytochrome P450 isoforms (CYPs), suggesting a limited potential for drug-drug interactions or variable pharmacokinetics due to CYP polymorphisms. In addition, BIIB014/V2006 was demonstrated to show a low potential for inhibition or induction of the major CYP isoforms.
The plasma protein binding of BIIB014/V2006 was highest in human and cynomolgus monkey and lower in rat, rabbit and mouse, respectively. Pharmacokinetic data from efficacy studies in the rat were used to estimate the likely therapeutic levels needed to achieve efficacy in patients.
In absorption, distribution, metabolism and elimination (ADME) studies with [^{14}C]-BIIB014/V2006 in rats and cynomolgus monkeys the compound was found to be eliminated predominantly in the faeces, with BIIB014/V2006 being the major component in this matrix. No differences were observed between the sexes in the rate and route of elimination of BIIB014/V2006.
Quantitative whole body autoradiography (QWBA) studies in the rat indicated that [^{14}C]-BIIB014/V2006 was widely distributed throughout the body following both intravenous and oral dosing.

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Targeting Adenosine A_{2A} Receptors in Parkinson's Disease and Other CNS Disorders

presented by
MassGeneral Institute for Neurodegenerative Disease

IN VIVO PROFILE OF TWO NOVEL, SELECTIVE ADENOSINE A_{2A} RECEPTOR ANTAGONISTS, SCH 412348 AND SCH 420814.

Eric Parker*, Rosalia Bertorelli, Mary Cohen-Williams, Angelo Forlani, Silva Fredduzzi, William Greenlee, Jinsong Hao, Guy Higgins, Jean Lachowicz, Neil Lindo, Bernard Neustadt, Leonard Parra, Andrew Stamford, John Hunter, Geoffrey Varty.

Department of Neurobiology, Schering-Plough Research Institute, 2015 Galloping Hill Road, Kenilworth, NJ 07033, USA and Schering-Plough Research Institute, Milan, Italy . *Presenting author (e-mail Eric.Parker@SPCorp.com).

Background: Selective antagonists at the adenosine A_{2A} receptor may represent potential treatments for Parkinson's disease and other movement disorders. These studies describe the development and in vivo characterization of two novel, selective adenosine A_{2A} receptor antagonists, SCH 412348 and SCH 420814.
Methods: SCH 412348 and SCH 420814 were dosed orally in an acidified polyethylene glycol vehicle. Both compounds were first tested for their ability to attenuate A_{2A} agonist-induced hypolocomotion. Rats were treated with various doses of SCH 412348 and SCH 420814 and 3 hr later the rats were injected with the A_{2A} agonist, CGS-21680, which is know to induce a marked hypoactivity. Following identification of active doses, SCH 412348 and SCH 420814 were tested for their ability to attenuate haloperidol-induced catalepsy in the rat. Rats were dosed with haloperidol (1 mg/kg sc) and 30 min later injected with one of several doses of SCH 412348 or SCH 420814. The time spent cataleptic during a 2 min observation was then measured 60 and 240 min after treatment. Following these studies, brains were collected and ex-vivo binding studies were conducted using [3H]-SCH 58261 to establish the level of A_{2A} receptor occupancy within the striatum. Finally, both A_{2A} antagonists were tested for their ability to potentiate L-dopa-induced rotational behavior in 6-hydroxydopamine (6-OHDA)-lesioned rats.
Results: SCH 412348 and SCH 420814 dose-dependently attenuated CGS-21680-induced hypolocomotion with significant effects at doses of 0.1-1 mg/kg. Furthermore, both compounds were able to attenuate haloperidol-induced catalepsy at similar doses of 0.1-3 mg/kg. Ex-vivo binding studies demonstrated that approximately 80% receptor occupancy is required within the striatum to produce marked anticataleptic activity. Finally, SCH 412348 and SCH 420814 potentiated L-dopa-induced rotations in 6-OHDA-lesioned rats, again across dose ranges of 0.1-3 mg/kg. Duration of action studies in all assays suggest that effects are present for at least 8 hr.
Conclusion: These studies have demonstrated that SCH 412348 and SCH 420814 are potent, A_{2A} receptor antagonist in vivo, highlighted by their ability to attenuate the effects of the selective A_{2A} agonist, CGS-21680. Furthermore, in support of their potential to treat Parkinson's disease and other disorders associated with reduced dopaminergic activity, SCH 412348 and SCH 420814 are active in two models of hypodopaminergic function.

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P-8

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Targeting Adenosine A_{2A} Receptors in Parkinson's Disease and Other CNS Disorders

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Synthesis and Biological Evaluation of Adenosine A_{2a} Receptor Antagonists: Exploration of 2-(2-Furanyl)-7-Phenyl[1,2,4]Triazolo[1,5-*c*]Pyrimidin-5-Amine Analogs

Deen Tulshian*†, John Caldwell*†, Julius Matasi†, Lisa Silverman†, Leyla Arik‡, Mary Cohen-Williams‡, Ahmad Fawzi‡, Guy Higgins‡, Jean Lachowicz‡, Geoffrey Varty‡, and Hongtao Zhang‡

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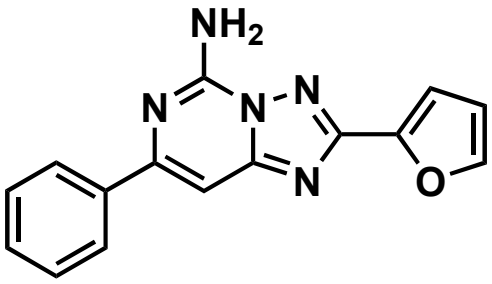
Parkinson's disease (PD) is a neurodegenerative motor disorder resulting from a deficiency of dopamine levels in the brain. Among existing therapies for Parkinson's disease are L-dopa and dopamine agonists which increase dopamine levels in the brain; yet, this therapy is limited by side effects and lack of efficacy over an extended time. Adenosine A_{2a} antagonists offer a potential effective treatment since they display an improvement in symptoms associated with Parkinson's disease in numerous animal models.

A summary of the synthesis and SAR of 2-(2-furanyl)-7-phenyl[1,2,4]triazolo[1,5-c]pyrimidin-5-amine analogs, such as **1**, is detailed within. Several examples possess single-digit nanomolar potency at the A_{2a} receptor and high (greater than 100 fold) selectivity over the other adenosine receptor subtypes.



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The authors may have a financial conflict(s) of interest as I have described on the next page

Authors are employee of the Schering-Plough Institute which funded the research

P-9

Targeting Adenosine A_{2A} Receptors in Parkinson's Disease and Other CNS Disorders

May 17 - 19, 2006
Boston USA

presented by
MassGeneral Institute for Neurodegenerative Disease

BIIB014/V2006: Pharmacokinetics in young and elderly healthy subjects

Andrew Wade*[1], Arash Bakhtyari[2], Richard Thomas[2], Steven Warrington[3], Robert Shaw[1], Kirsteen Donaldson[1], Kirsty Anthony[1], John Hutchison[1]

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Background: BIIB014/V2006 is a potent, selective, non-xanthine A_{2A} receptor antagonist, active at low oral doses in animal models and being developed for treatment of Parkinson's disease. Five studies have investigated pharmacokinetics of BIIB014/V2006 in human subjects.
Methods: Studies, in healthy young and elderly, used a double blind, randomized, placebo-controlled design. In a crossover study, 10 young males received single oral doses of 5, 15 and 50 mg, and 10 given 10, 25 and 100 mg BIIB014/V2006. A further 8 young and 6 elderly males/5 elderly females received a single 10 mg dose BIIB014/V2006 and placebo. In a parallel-group study, young males received a single dose then 10 days repeated dosing with 5, 10, 20 or 50 mg BIIB014/V2006/day (6/cohort) or placebo (2/cohort). Elderly subjects received a single dose on Day 1, then 10 days repeated dosing with 50 mg BIIB014/V2006/day (6 male, 6 female) with Sinemet-110® on Day 0 (monotherapy), and Sinemet-110® and placebo randomized on the 8th and 9th day of repeated dosing (combination therapy). Food effect was studied in 12 males (>49 years; open-label crossover). Safety and tolerability were assessed in all studies.
Results: BIIB014/V2006 plasma concentrations were measurable at all doses. Absorption was rapid (T_{max} 2 to 4 h), and dose proportionality seen up to 15 mg (fasted). Exposure between 15 and 100 mg increased but less-than-dose proportionally. Food improved absorption, increasing C_{max} (not AUC) after a 10 mg dose, and proportionality extended to 50 mg when given with food. BIIB014/V2006 had a moderate apparent clearance and a low to moderate apparent volume of distribution giving a half-life of 10 to 25 h, allowing once daily dosing. There was slight accumulation on repeated dosing but no evidence of self-induction or inhibition. Time to steady state was 5 days, consistent with half-life. Although advanced age (≥ 65 years old) influenced disposition of BIIB014/V2006 at high doses, steady-state was still achieved by day 5 and dose reductions are not required in this population. A levodopa/carbidopa combination (Sinemet 10/100®) delivered the same exposure of levodopa when co-administered with BIIB014/V2006 as when given alone. No clinically significant abnormalities were seen in vital sign, ECG, safety laboratory or cognitive function tests.
Conclusion: The pharmacokinetics of BIIB014/V2006 in healthy subjects are appropriate for further development of BIIB014 as a single daily treatment of Parkinson's disease. BIIB014/V2006 is well tolerated after single doses up to 100 mg and repeated dosing up to 50 mg/day for 10 days.
Funded by Vernalis (R&D) Ltd and Biogen Idec Inc.

To the best of my knowledge and judgment I, the presenting author, report that:

The authors may have a financial conflict(s) of interest‡ as I have described here:
Andrew Wade, Robert Shaw, Kirsty Anthony and John Hutchison are employees of Vernalis (R&D) Ltd.; Kirsteen Donaldson is clinical consultant to Vernalis (R&D) Ltd.

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Targeting Adenosine A_{2A} Receptors in Parkinson's Disease and Other CNS Disorders

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Pharmacological Characterization of the Potency and Selectivity of Six A_{2A}-Antagonists for Human and Rat A_{2A} Adenosine Receptors.

Ming Yang*, Sandriyana Soelaiman, Rao Kalla, Jeff Zablocki, Lina Yao, Ivan Diamond, Luiz Belardinelli, & John C Shryock

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Background: A_{2A}-antagonists may play an important role in the treatment of striatal degenerative disease (Parkinson's disease). A_{2A}-antagonists have been developed from both xanthine and non-xanthine precursors. To date, the potency and selectivity of these compounds at A_{2A} receptors have been determined mostly by competition with the agonist radioligand [^{3}H]CGS21680, but [^{3}H]CGS21680 may bind to sites other than A_{2A} receptors. Moreover, data comparing the species dependence of A_{2A}-antagonist potency and selectivity for human vs. rat A_{2A} receptors are unavailable.
Methods: Competition binding assays were performed by using either the A_{2A} selective antagonist radioligand [^{3}H]MSX-2 or the agonist radioligand [^{3}H]CGS21680. Radioligands for A_1, A_{2B} and A_3 receptor binding assays were [^{3}H]DPCPX, [^{3}H]ZM241385 and [^{3}H]MRE3008F20, respectively. Membranes were prepared from human A_{2A} or A_{2B} receptor stably-transfected HEK293 cells, human A_1 or A_3 receptor stably-transfected CHO cells, PC12 cells or rat striatum. cAMP assays were performed using a DiscoveRx assay kit.
Results: The rank order of antagonist potency to displace [^{3}H]MSX-2 binding to the human cloned A_{2A} receptor was: SCH58261 > Biogen-34 > Ver-6623 > MSX-2 > KW-6002 >> DMPX. The rank order of potency at rat A_{2A} receptor differed from that for human cloned A_{2A} receptor, with Biogen-34 > SCH58261 > MSX-2 > KW-6002 > Ver-6623 >> DMPX. The affinites of KW-6002 and MSX-2 at the rat A_{2A} receptors were higher when the competing radioligand was [^{3}H]CGS21680 than when it was [^{3}H]MSX-2. Similar results were obtained in cAMP assays. Biogen-34 and KW-6002 exhibited the highest selectivity for human or rat A_{2A} vs. human or rat A_1 receptor, respectively. Both MSX-2 and Biogen-34 were highly selective for human A_{2A} vs. A_{2B} receptors. Most of the compounds had low affinities for the human A_3 receptor.
Conclusion: SCH58261 was the most potent tested antagonist of the human A_{2A} receptor, whereas Biogen-34 was the most potent antagonist of the rat A_{2A} receptor. KW-6002 had low affinity for the human A_{2A} receptor but high affinity for the rat A_{2A} receptor. DMPX had relatively low affinity and selectivity for both human and rat A_{2A} receptors. The orders of potency and selectivity of antagonists at human and rat A_{2A} receptor are not identical, therefore caution should be used when selecting reference A_{2A}-antagonists and radioligands for the characterization of A_{2A} receptors of different species.
Funded by: CV Therapeutics, Inc.

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P-11

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Targeting Adenosine A_{2A} Receptors in Parkinson's Disease and Other CNS Disorders

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Tricyclic theophylline derivatives as A_{2A} Adenosine Receptor Ligands.

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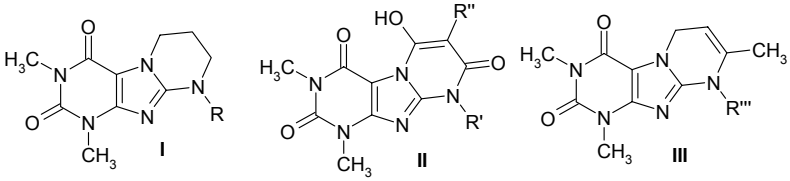
Background: A_{2A} adenosine receptor (AR) antagonists may be promising new drugs in neurodegenerative diseases such as Parkinson's and Alzheimer's disease. Methylxanthines such as theophylline and caffeine have been known to enhance locomotor activity; however, these compounds are nonselective AR antagonists and have weak affinity for A_{2A}AR. 8-Styrylxanthine derivative **KW-6002** (istradefylline) was discovered and now is in the phase II clinical trials.
Methods: Compounds envisaged as constrained bioisosteric analogs of 8-styrylxanthines: pyrimido[1,2-*a*]purinediones (**I**), 6-hydroxypyrimido[1,2-*a*]purinetriones (**II**) 6,9-dihydropyrimido [1,2-*a*]purinediones (**III**) were designed, synthesized and investigated in radioligand binding assays. Physicochemical properties of the ligands have been evaluated *in silico* in order to investigate structure-activity relationships using programs CAChe 6.1., HyperChem 7.5 and Alchemy2000.
Results: Compounds (**I**) with the N-phenylalkyl substituents have shown submicromolar affinity and selectivity toward A_{2A}AR. Introduction of the heteroatoms oxygen and nitrogen into the spacer connecting the aryl substituent with the tricyclic structure, has led to a decrease in affinity. In the series of derivatives (**II**) the only compound that showed moderate affinity toward A_{2A}AR, possessed a long alkyl substituent R' and a benzyl group R''. The latter functional group turned out to be crucial, since after removing the benzyl moiety, a decrease in affinity was observed. Derivatives of the group (**III**) compounds possessing alkyl substituent R''' showed better affinity than their benzyl analogues, and halogen substituents in the phenyl ring appeared not to be favourable for affinity. At the same time, the most active compound completely lost selectivity. From SAR studies the most favourable conformation for A_{2A}AR binding could be derived.



Conclusion: The third, annelated ring generally led to decrease in A_{2A}AR affinity of xanthine derivatives and the presence of oxygen heteroatoms in the third ring did not improve for the affinity. Introduction of an alkyl substituent and partial dehydratation of the pyrimidine ring decreased A_{2A}AR affinity but also led to an increase in A_1AR affinity. Long N-alkyl and N-phenylalkyl substituents were crucial for A_{2A}AR binding properties although sometimes led to the loss of selectivity.

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P-12

Targeting Adenosine A_{2A} Receptors in Parkinson's Disease and Other CNS Disorders

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AAV-Cre/*loxP* conditional KO of adenosine A_{2A} receptors in striatal neurons

Augusta Pisanu*, Miguel Sena-Esteves & Michael A. Schwarzschild

Department of Neurology, Massachusetts General Hospital, Charlestown, MA USA; *Presenting author (apisanu@partners.org.)

Gene knockout (KO) approaches to receptor function complement traditional pharmacological methods by complete specificity and inactivation. However, standard KO strategies globally eliminate the targeted receptor, and thus their use for investigating the role of receptors in the adult brain can be confounded by developmental or systemic phenotypes. The transgenic Cre/*loxP* conditional KO system can achieve partial control over the timing and distribution of receptor inactivation using a specific promoter to direct *cre* expression, e.g., to study the effects of the adenosine A_{2A} receptor ($A_{2A}R$) in postnatal forebrain. To achieve an even higher degree of precision in eliminating the $A_{2A}R$ from discrete regions on one side of the brain, and in pursuit of a better understanding of $A_{2A}R$ involvement in neurodegeneration, we adopted an AAV-Cre/*loxP* system. Adeno-associated virus (AAV)-Cre vectors were stereotactically infused into homozygous *floxed $A_{2A}R$* adult mice, resulting in a conditional KO of the $A_{2A}R$ at the site and time of infection.

Working with several serotypes of AAV-Cre and AAV-GFP, injected into the striatum of *floxed $A_{2A}R$* mice, we demonstrated an infection and GFP expression largely restricted to the targeted striatum for AAV1 serotype (Fig. A) but not AAV1/8 (which produced a widespread infection extending into the overlying cortex). Focusing on AAV1 serotype we characterized the titer-dependence and time-course of neuronal Cre expression and $A_{2A}R$ loss, visualized by IHC (as in Fig. B). Cre expression was detectable 8 days post-infusion of AAV vectors but the loss of $A_{2A}R$ was not evident until the 16th day, reaching a maximum extent at the 32nd day post-injection.

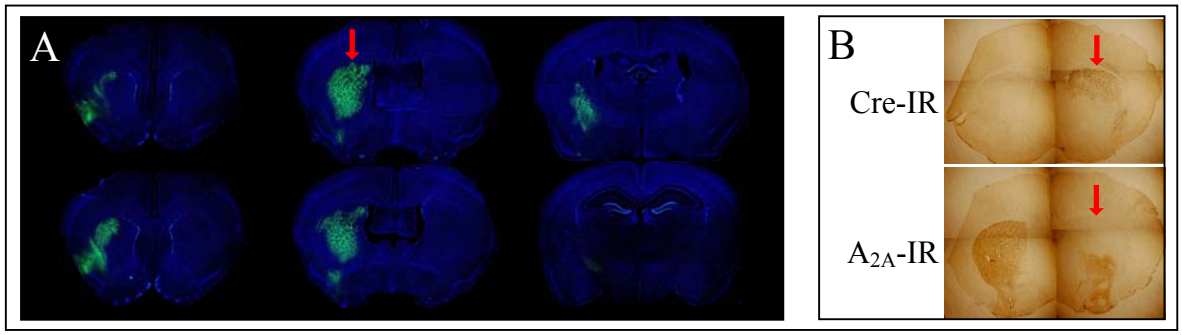

Figure: (**A**) Fluorescence of rostral→caudal coronal sections stained with a Hoechst 33258 one month after AAV1-GFP infusion (arrow). (**B**) Striatal Cre expression and the coincident elimination of striatal $A_{2A}R$ in a *floxed $A_{2A}R$* mouse one month after unilateral intrastriatal injection of AAV1-Cre (arrow).

The AAV-Cre/*loxP* conditional KO system provides a precise tool with which to explore the neurobiology of adenosine receptors and their pathophysiology in models of CNS disease.

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To the best of my knowledge and judgment I, the presenting author, report that:

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P-13

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Targeting Adenosine A_{2A} Receptors in Parkinson's Disease and Other CNS Disorders

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Characterization of the developmentally regulated, forebrain-specific A_{2A} receptor knockout mice

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[1]Department of Neurology, Boston University School of Medicine, Boston, MA; [2]Department of Internal Medicine, University of Virginia, Charlottesville, VA; [3]Neurology Dept, Mass. General Hosp., Boston, MA *Presenting author (email ekr19@bu.edu)

The A_{2A} receptor has recently emerged as a novel therapeutic target for neurodegenerative disorders such as Parkinson's disease (PD). We have demonstrated previously that global genetic knockout (KO) of the A_{2A} receptor attenuates damage in the MPTP model of PD and the 3-NP model of Huntington's Disease (HD). However, the global A_{2A}R KO is limited by the lack of tissue-specificity and confounding developmental effects. To circumvent these limitations, we have developed a forebrain neuron-specific A_{2A} KO by crossing the CaMKIIa-Cre transgenic line to a transgenic line with a floxed A_{2A}R gene. As the first step toward characterizing this KO line, we have evaluated the tissue specificity and developmental regulation of Cre-mediated deletion of the A_{2A}R. (1), we analyzed the deletion of the A_{2A}R gene by determining Cre-mediated recombination in 6 different brain regions and 6 peripheral organs by PCR analysis. The Cre-mediated recombination was detected specifically in forebrain region (including the cerebral cortex, striatum, hippocampus, olfactory bulb, and hypothalamus) but not in the cerebellum. (2), immunohistochemical analysis showed that in WT mice A_{2A}Rs were expressed at a high level in the striatum, nuclear accumbens and olfactory tract and in lymphocytes in the spleen. In contrast, in forebrain A_{2A}R KO mice, A_{2A}R expression in the striatum, nuclear accumbens and olfactory tract completely disappeared but the A_{2A}R expression in lymphocytes of spleen cells was preserved. (3), Cre-mediated recombination in the striatum was detectable by PCR analysis at postnatal day 15, peaked at day 50, and persisted thereafter. (4), the A_{2A}R specific antagonist KW6002-induced motor activity increased postnantally with a peak at day 50 in WT littermates. In contrast, KW6002-induced motor activity in forebrain KOs disappeared gradually during postnatal development, in parallel with the expression of Cre-mediated recombination in the striatum. Together, these results demonstrate that we have developed an A_{2A}R KO line with developmentally regulated, selective inactivation of the A_{2A}R in the forebrain region.

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P-14

A2A PD BOSTON 2006

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Targeting Adenosine A_{2A} Receptors in Parkinson's Disease and Other CNS Disorders

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Electrophysiological evidence for an antagonistic modulatory role of adenosine A_{2A} receptor on dopamine D_2 receptor regulation of down- and up-state transitions in the nucleus accumbens

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The nucleus accumbens is a brain region involved in motivation, attention, reward and drug addiction. The output of this area is determined by the bursting activity in the medium-sized spiny GABAergic neurons (MSN), which is central to basal ganglia function and dysfunction. These bursts are driven by multiple corticostriatal inputs that depolarize MSN from their resting hyperpolarized membrane potential around -80 mV, which is recognized as the down-state, to a more depolarized level near -55 mV, called the up-state. These transitions between down- and up-state require channels regulated by several striatal transmitters acting through G protein coupled receptors. These transmitters are therefore in position to tightly control the excitability and firing patterns of the MSN. Among these neurons, enkephalin and dopamine D_2 receptor-expressing neurons are highly enriched in adenosine A_{2A} receptor. This co-expression of the D_2 and A_{2A} receptors supports strong interactions between them but their physiological significance at the cellular level remains partially unclear.

In the present study, perforated patch clamp recordings on acute rat brain slices were performed to characterize the role of D_2-A_{2A} receptors interactions in the neuromodulation of transitions between down- and up -state by using the D_2 receptor agonist norapomorphine (NPA) and the A_{2A} receptor agonist CGS 21680.

In our *in vitro* model of state transitions, application of 5 µM NMDA allows the MSN to shift from its hyperpolarized resting potential to a depolarized plateau inducing a continuous action potentials firing. CGS 21680 (1 µM) induced modification neither in state transition nor in the firing frequency. In contrast, NPA (10 µM) abolishes the firing of MSN in the up-state and inhibits the down/up-state transition in a subpopulation of neurons (70%). The specificity of these effects was confirmed by the use of the selective D_2 antagonist, sulpiride (10 µM). Interestingly, these effects of 10 µM NPA are totally reversed by 1 µM of CGS 21680 and, this action was blocked by the selective A_{2A} receptor antagonist, SCH 58261 (1µM).

These results suggest that, in MSN, D_2 and A_{2A} receptors play a key role in the control of transitions between down- and up-state and, hence, in the generation of action potential firing patterns. The modulation of these transitions, which are important for information processing within the nucleus accumbens, should have a high impact on basal ganglia-related behaviors.

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P-15

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Targeting Adenosine A_{2A} Receptors in Parkinson's Disease and Other CNS Disorders

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Adenosine A_{2A} / Dopamine D_2 receptor interaction is enhanced at the level of DARPP-32 phosphorylation in the dopamine-depleted "weaver" mouse.

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DARPP-32 (Dopamine and cyclic AMP Regulated Phosphoprotein mw. 32,000) is a phosphoprotein of the dopaminergic system, regulating the excitability of striatal neurons. Adenosine A_{2A} receptor agonists induce phosphorylation of DARPP-32 at Thr^{34} residue. In this study, we examined if the antagonistic interaction between adenosine A_{2A} and dopamine D_2 receptors, which are colocalized on the striatopallidal neurons, is disturbed in the "weaver" mutation, a genetic model of dopamine deficiency. In striatal slices of "weaver" and control animals, we examined the effect of CGS21680 (specific A_{2A} receptor agonist) and Quinpirole (D_2 receptor agonist) on the phosphorylation state of DARPP-32. The detection was performed by quantitative Western blot analysis using the monoclonal antibody mAb-23, which is specific for the Thr^{34} phosphorylated form of DARPP-32. Our results revealed that: a) CGS21680 (1μM) caused a significant increase in DARPP-32 phosphorylation state in control and "weaver" mice, which was at a similar level. b) The A_{2A} mediated phosphorylation was significantly reduced by Quinpirole in control mice at the concentration of 100 nM, while in mutant "weaver' mice at the concentration of 50 nM. These results suggest that the A_{2A}/D_2 receptor interaction at the level of phosphorylation state of Thr^{34}-DARPP-32 is potentiated in mutant "weaver" mice.

Based on the colocalization of A_{2A} adenosine receptors and metabotropic glutamate receptors of group I (mGlu, group I) on the spiny neurons of striatum, we examined the interaction between the two receptor types in regulating the phosphorylation state of Thr^{34}-DARPP-32. Preliminary experiments in normal mice showed that DHPG, a specific agonist of group I mGlu receptors, causes a concentration-dependent reduction of the CGS21680 (1μM) induced phosphorylation of DARPP-32. This fact supports the evidence of A_{2A}/group I mGlu receptor interaction in striatal spiny neurons, which will be further investigated in the "weaver" mice.

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P-16

Targeting Adenosine A_{2A} Receptors in Parkinson's Disease and Other CNS Disorders

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Dissociation of adenosine A_{2A} receptors homodimers after antagonist binding.

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Background: There is increasing evidence that G protein-coupled receptors can exist as oligomers, possibly dimers. Association of these molecules can involve heterodimerisation, in which different members of superfamily are shown to be present, as well as homooligomerisation, in which multiple copies of the same receptor are shown to coexist. Recently, adenosine A_{2A} receptors have been proved to form homodimers constitutively and it seems that homodimers are the functional form of the receptors present on the cell membrane. There are no currently accepted rules to predict the impact of ligand binding on these receptors association.
Methods: HEK293 cells were co-transfected by plasmids pEYFP-N1 and pECFP-N1 bearing human A_{2A} receptors in 1:1 ratio. Forty-eight hours post-transfection, cells were washed twice in PBS, detached, centrifuged and resuspended in isotonic buffer. Measurements were performed using a Fluorolog 3 spectrofluorimetr (Jobin Yvon, France). After control experiment without ligand, antagonist in the final concentration of 10^{-8} or 10^{-7} M was added, samples were incubated in 37 ^{o}C and fluorescent emission was measured. All the measurements were performed using excitation at 433 nm in order to excite cyan fluorescent protein (CFP) and the emission was detected at 450-550 nm.
Results: We have examined influence of seven pyrimido[2,1-*f*]purinedione derivatives with defined adenosine receptors affinities, on A_{2A} receptors complexes in transfected HEK293 cells using fluorescence resonance energy transfer (FRET) method. Six of the tested compounds (KD32, KD64, KD67, KD114, KD164 and KD217) possess antagonistic activity (in submicromolar concentrations) against adenosine A_{2A} receptors. Two of them (KD217 and KD253) possess also antagonistic activity against adenosine A_1 receptors in similar concentrations. Two of the tested compounds (KD32 and KD114) were found to induce reduction in FRET signal.
Conclusion: Our results show, that adenosine A_{2A} receptors complexes dissociate after binding of these two compounds. Furthermore, we noticed, that the rate of dissociation depends on the ligand concentraton and changes in time, suggesting changes in receptors sensitivity and localisation in the plasma membrane. Full understanding of these mechanisms may contribute to development of new therapeutics for major neurological (eg. Parkinson's Disease) and psychiatric disorders.

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P-17

Targeting Adenosine A_{2A} Receptors in Parkinson's Disease and Other CNS Disorders

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Cross-modulation of radioligand binding kinetics to A_{2A} adenosine and D_2 dopamine receptors

Ain Uustare[1]*, Anton Terasmaa[2], Martin Lepiku[1] & Ago Rinken[1]

[1] *Institute of Organic and Bioorganic Chemistyr, Tartu University, Tartu, Estonia;* [2] *Laboratory of Clinical and Translational Studies, National Institute of Alcohol Abuse and Alcoholism, NIH, Bethesda, MD USA.* *Presenting author (email deffi@ut.ee)

Alteration of ligand binding kinetics to dopamine D_2 and adenosine A_{2A} receptors by the activation of the corresponding coexpressed receptors has been studied in means of kinetic analysis. The binding of dopaminergic agonist [^{3}H]quinpirole and A_{2A}-specific antagonist [^{3}H]ZM241385 to rat striatal membranes was homogeneous and characterized with the constants K_d = 1.5±0.1 nM, B_{max} = 115±2 fmol/mg protein and K_d = 0.14±0.01 nM, B_{max} = 1620±40 fmol/mg protein, respectively. The kinetic analyses of the binding of these ligands revealed that they had at least two consecutive and kinetically distinguishable steps, where fast equilibrium of complex formation between receptor and agonist is followed by slow isomerization equilibrium. Activation of adenosine A_{2A} receptors by CGS 21680 caused an increase of the [^{3}H]quinpirole binding rate, altering mainly the formation of the receptor-ligand complexes as well as the isomerization rate of this complexes (k_i), but the deisomerization rate (k_{-i}) remained unchanged. Activation of D_2 receptors by quinpirole had no influence on the ligand binding properties to A_{2A} receptors. The potencies of ZM241385 and quinpirole were studied also by their abilities to modulate adenylate cyclase activity. ZM241385 inhibited CGS 21680 induced cAMP accumulation with K_A = 6.6 nM and quinpirole inhibited forskolin stimulated cAMP accumulation with IC_{50} = 5.0 nM, which both are in better agreement with the fast affinity constants measured from the kinetic analysis in comparison with the binding constants obtained with standard equilibrium binding experiments. This means also that the isomerization step of the radioligand-receptor interactions may shed the modulation between the receptors in heterodimeric complexes, regulating their activities in radioligand binding experiments.

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P-18

Targeting Adenosine A_{2A} Receptors in Parkinson's Disease and Other CNS Disorders

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Modulation of Naphtha [1,2-d] thiazol-2-amine induced free cytosolic Ca^{2+} concentration by adenosine A_{2a} receptor in human embryonic kidney (HEK 293) cell line

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Background: Activation of adenosine A_{2a} receptor leads to the elevation of free cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) mediated by phosphoinositide turnover-dependent Ca^{2+} release from intracellular organelles. Since thiazoles and thiadiazoles exhibit considerable adenosine receptor antagonist activities, naphtha [1,2-d] thiazol-2-amine was designed and synthesized to study its effect on phosphoinositide turnover-dependent Ca^{2+} release from intracellular organelles in HEK 293 cells expressing A_{2a} receptor.

Methods: HEK 293 cells were stably transfected with pcDNA3.1 (+) containing human A_{2a} receptor cDNA using $CaPO_4$ method. Cells were loaded with 10µM Fura-2AM for 45 min at 37 ^{0}C. Excitation ratios (340/380nm) were recorded in a 4.1mM Ca^{2+} medium with caffeine and naphtha [1,2-d] thiazol-2-amine. Naphtha [1,2-d] thiazol-2-amine was prepared from the cyclization of N-naphthyl thiourea.

Results: Western blot analysis (using anti-HA-mouse-FITC) indicated the presence of moderate levels of A_{2a} receptor proteins in stably transfected HEK 293 cells. Naphtha [1,2-d] thiazol-2-amine significantly modulates the intracellular Ca^{2+} i.e. $[Ca^{2+}]_i$ level (Grynkiewicz et al. 1985) at the concentration of 5nM, 50nM and 500nM respectively in a 4.1mM Ca^{2+} medium. $[Ca^{2+}]_i$ level was very high initially (2022.5nM) and typically reduced after treatment with naphtha [1,2-d] thiazol-2-amine (1390.8nM at 5nM, 397.8nM at 50nM and 384nM at 500nM). Re-exposure of naphtha [1,2-d] thiazol-2-amine with same effective concentration (50nM) did not show any difference in $[Ca^{2+}]_i$ response from that to the first exposure. The results were comparable to the caffeine, which is known to possess adenosine A_{2a} receptor antagonist activity. In 4.1mM Ca^{2+} medium, a constant reduction in $[Ca^{2+}]_i$ level was observed in the presence of caffeine at 5nM (952nM), 50nM, (268.8 nM) and 500nm (214 nM) respectively.

Conclusion: The present study on the human A_{2a} receptor stably transfected HEK 293 cell line provided evidence for significantly modulating the intracellular Ca^{2+} i.e. $[Ca^{2+}]_i$ level in the presence of naphtha [1,2-d] thiazol-2-amine. The studies illustrated that the modulation of intracellular Ca^{2+} i.e. $[Ca^{2+}]_i$ level by A_{2a} receptor antagonists may lead to explore the signaling pathways triggered by adenosine receptors. Furthermore, roles played by induction of immediate early genes in signaling cascades may also be investigated.

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Targeting Adenosine A_{2A} Receptors in Parkinson's Disease and Other CNS Disorders

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In vivo stimulation of adenosine A_{2A} but also of adenosine A_1 receptors evokes zif/268 mRNA expression in striatum and cortex of the "weaver" mouse: a genetic model of dopamine deficiency.

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Adenosine A_1 and A_{2A} receptors are co-localized and interact antagonistically with dopamine D_1 and D_2 receptors respectively, on the medium sized spiny neurons of striatum regulating the activity of the "direct" and "indirect" pathway of the basal ganglia. In vivo activation of adenosine A_{2A} receptors elicits a strong expression of the immediate early gene zif/268 mRNA in striatum and motor cortex of the "weaver" mouse, a genetic model of dopamine deficiency [Ekonomou et al., 2004 *Neuroscience*, 123: 1025]. In order to clarify the signal transduction pathway mediating zif/268 induction, we examined the phosphorylation state of [Thr-34]-DARPP-32 and of [Thr202/Tyr204]-p44/42-MAPKs (Mitogen Activated Protein Kinases) in striatal membranes of the "weaver" mouse, after in vivo stimulation of A_{2A} receptors by western blot analysis. Preliminary results demonstrate a significant upregulation of the phosphorylation levels of DARPP-32 in striatum of the "weaver" but not of the control mice, indicating a supersensitivity of A_{2A} adenosine receptors, which under dopamine deficiency potentiate signaling, probably by activation of the A_{2A}/cAMP/PKA/DARPP-32/PP-1 signal transduction pathway.
Stimulation of adenosine A_1 receptors (negatively coupled to G-proteins) by CPA, an A_1 receptor agonist, attenuates the D_1-mediated zif/268 mRNA expression as detected by the in situ hybridization technique, in striatum of the "weaver" mouse. This confirms the A_1/D_1 interaction taking place on striatal neurons of the "direct" pathway. Unexpectantly CPA, when administrated alone, induces zif/268 mRNA expression in striatum and all layers of barrel field cortex of the "weaver" but not of control mice. The zif/268 mRNA induction is not counteracted by ZM241385, an adenosine A_{2A} receptor antagonist, confirming that it is A_1 receptor mediated. However, the zif/268 expression is significantly reduced a) by Quinpirole, a dopamine D_2 receptor agonist, and b) by U73122, an inhibitor of phospholipase C (PLC). In conclusion, our results suggest a) an A_1/D_2 receptor interaction in striatum, indicating a probable crosstalk between "direct" and "indirect" pathway b) a supersensitivity of adenosine A_1 receptors, which could probably rely on a switch in their function under dopamine deficiency, using probably the PLC signal transduction pathway.
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Targeting Adenosine A_{2A} Receptors in Parkinson's Disease and Other CNS Disorders

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Electrophysiological Responses to Adenosine and Caffeine in Hippocampal Slices from the Adenosine A_1-A_{2A} Double Knockout Mouse

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Background: Endogenous levels of adenosine are sufficient to activate tonically the adenosine A_1 and A_{2A} adenosine receptor subtypes. These endogenous levels of adenosine have been implicated in phenomena ranging from pain to the motor symptoms of Parkinson's disease. Both receptor subtypes are present in the hippocampus and exert opposing effects on synaptic transmission. Caffeine is a commonly ingested non-selective antagonist at adenosine A_1 and A_{2A} receptors. Here we present an initial characterization of electrophysiological responses in acute adult hippocampal slices obtained from mice lacking both the adenosine A_1 and A_{2A} receptor.
Methods: We characterized electrophysiological responses in acute adult hippocampal slices obtained from adenosine A_1 / A_{2A} receptor double knockout mice. The influence of both adenosine and caffeine on synaptic transmission were explored using field recording in CA1 and analysis of paired pulse responses in this region.
Results: During field recordings, bath application of adenosine (50 μM) to wild-type slices produced a reliable and significant decrease in the field responses of the CA1 region of the hippocampus, a result observed in many previous experiments and due to the dominant influence of the inhibitory adenosine A_1 receptor under normal conditions in hippocampal slices. In contrast, application of adenosine (50 μM) had no inhibitory effect, and, if anything, showed a trend toward a slight increase in synaptic transmission in the double knockout slices. As seen in previous studies, caffeine (250 μM) produced a reliable increase in synaptic transmission in the wild type slices (due to removal of the endogenous A_1 inhibitory influence), yet had no effect on the double knockout slices.
Conclusion: Hippocampal slices from the adenosine A_1-A_{2A} receptor double knockout mouse show no significant electrophysiological responses to standard concentrations of either a non-selective agonist (adenosine) or a non-selective antagonist (caffeine). The findings strengthen exististing evidence for a predominant role of the A_1 receptor in adenosinergic modulation of hippocampal synaptic transmission *in vitro*. They also highlight the potential of this congenic A_1-A_{2A} double knockout mouse (when compared to single knockout as well as wild-type littermates) for dissecting the relative contributions of the individual receptors to the role of adenosine in CNS physiology and disease models.
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Targeting Adenosine A_{2A} Receptors in Parkinson's Disease and Other CNS Disorders

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Systemic and Intra-Accumbens Injections of The Selective Adenosine A_{2a} Antagonist MSX-3 Reverse The Locomotor Suppression Induced By Haloperidol In Rats: Possible Relevance to Parkinsonism.

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Background: There is considerable evidence of antagonistic interactions between adenosine A_{2A} receptors and dopamine D_2 receptors in neostriatal areas, and antagonists of the A_{2A} receptor have been shown to exert antiparkinsonian effects in animal models. The D_2 antagonist haloperidol produces motor effects in rats that share many characteristics with human parkinsonian symptoms, including suppression of locomotion, which may be related to akinesia and bradykinesia. **Methods:** The present experiments were conduced to study the ability of the novel adenosine A_{2A} antagonist MSX-3 to reverse the locomotor effects of acute or subchronic administration of haloperidol in rats. MSX-3 is a water-soluble phosphate pro-drug of MSX-2, which is the active antagonist of A_{2A} receptors. **Results:** Systemic (i.p.) injections of MSX-3 (2.5-10.0 mg/kg) were capable of reversing the suppression of locomotion induced by either acute or repeated (i.e., 14 day) administration of 0.5 mg/kg haloperidol. Bilateral infusions of MSX-3 directly into the nucleus accumbens core (2.5 µg or 5.0 µg in 0.5 µl per side) produced a dose-related increase in locomotor activity in rats treated with 0.5 mg/kg haloperidol either acutely or repeatedly. There were no overall significant effects of MSX-3 infused directly into the dorsomedial nucleus accumbens shell or the ventrolateral neostriatum. **Conclusions:** Taken together, these results indicate that antagonism of adenosine A_{2A} receptors can reverse the locomotor suppression produced by D_2 antagonism, and that this effect may be mediated by A_{2A} receptors in the nucleus accumbens core. In conjunction with other published studies, the present results emphasize that different striatal subregions are involved in distinct aspects of motor function. Although antiparkinsonian drugs are typically given systemically, and the therapeutic target would generally be a broad improvement across diverse motor symptoms, it is nevertheless reasonable to argue that different therapeutic effects (i.e., increases in locomotion, decreases in rigidity or tremor) are related to actions on distinct striatal subcircuits. The present results would suggest that the nucleus accumbens is a critical striatal site for the restoration of locomotion seen after administration of adenosine A_{2A} antagonists.

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Targeting Adenosine A_{2A} Receptors in Parkinson's Disease and Other CNS Disorders

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The co-administration of caffeine and trihexyphenidyl improve the motor activity in rat with dopaminergic lesion.

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Background: The A_{2A} receptors antagonists have been proposal as therapy alternative from the Parkinson disease (PD). This drugs improvement the activity motor in animal models of the PD. The cholinergic antagonist drugs ameliorate PD symptoms. However this drug can cause acute intellectual impairment. The co-administration of an A_{2A} receptors antagonists and an anticholinergic drug will be improvining the motor activity.
In this work we study the effect of the co-administration of caffeine and trihexyphenidyl on motor activity in rat with dopaminergic lesion
Methods: We used male rats Wistar (250-300g). All animals were lesioned with 2 µl of the 6-hidroxydopamine in SNc. Stereotaxic injections were placed 4.5 mm anterior to interaural line, 2.1 lateral to midline and 6.5 mm ventral to surface of the skull. We used the staircase model and the turn behavior test. The doses were 5 mg/Kg of caffeine and 0.1 mg/Kg of trihexyphenidyl (THF) for 16 days. In the staircase model, we count the number of pellets grasped and eaten for each animal. The drugs were co-administrated for 10 days in staircase model and 16 days in turn behavior test.
Results: The turn behavior was decreased by 54% and 12% when we administration alone caffeine and trihexyphenidyl, respectively. However the co-administration produced a decrease of 58%. In the staircase model we find an increase in the skill motor of a 30% in the group with co-administration respect to groups of caffeine and THF alone**.**
Conclusion: The co-administration of A_{2A} antagonist like caffeine and an antagonist cholinergic like THF in rat with dopaminergic lesion are able of improve the ability motor. Finally these data suggest that A_{2A} receptor antagonist and an anticholinergic drug may be therapeutically beneficial in preventing parkinsonian symptoms.

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Targeting Adenosine A_{2A} Receptors in Parkinson's Disease and Other CNS Disorders

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Reduction of haloperidol induced motor impairments in Naphtha [1,2-d] thiazol-2-amine treated mice evocative of its potential as A_{2a} receptor antagonists.

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Background: Parkinson's Disease (PD) exhibits symptoms of motor dysfunction such as tremor, akinesia and rigidity. In the present study, the role of naphtha [1,2-d] thiazol-2-amine in modulating the haloperidol induced motor impairments in male mice was studied. Previously, naphthathiazole-2-amine showed significant modulation of intracellular Ca^{2+} i.e. $[Ca^{2+}]_i$ level on the human A_{2a} receptor stably transfected in HEK 293 cell line as compared to caffeine.

Methods: The role of naphtha [1,2-d] thiazol-2-amine in reduction of haloperidol induced catalepsy, akinesia and swim test was studied. Adult Balb/c male mice (6 weeks, 25-30g) were injected with caffeine (group I) and naphtha [1,2-d] thiazol-2-amine (group II) at dose of 10 mg/kg intraperitoneally 30 minutes before haloperidol injection. Group III and IV were injected with control (saline+1%acacia) and haloperidol (5mg/kg) respectively.

Results: Six mice were studied in each group. The rigidity or inability to correct an externally induced posture of the hind limbs was measured in 0-5 score. Haloperidol treated mice (group IV) developed catalepsy after 45 minutes while in caffeine treated mice **(**group I) and naphtha [1,2-d] thiazol-2-amine treated mice **(**group II), the catalepsy was observed after 90 minutes and 75 minutes respectively. Haloperidol administration caused impaired ability to initiate movements and showed maximum latency period in seconds to move all the four limbs. Caffeine treated mice showed minimum latency period in seconds, however naphtha [1,2-d] thiazol-2-amine treated mice exhibited mild latency period, and showed significant increase in latency period as compared to group IV. Significant increase in swim score was observed in Group II mice as compared to group I mice. Animals exhibited immobility after 45 minutes in group IV whereas mice in group I and group II became immobile after 90 minutes and 60 minutes respectively.

Conclusion: There is a high degree of correlation between dopamine depletion and motor impairment induced by haloperidol. Reduction in the motor impairment in the presence of caffeine or naphtha [1,2-d] thiazol-2-amine may be related to the binding of these compounds with adenosine A2a receptors. The study concludes that naphtha [1,2-d] thiazol-2-amine may be explored as potential A_{2a} receptor antagonists.

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Targeting Adenosine A_{2A} Receptors in Parkinson's Disease and Other CNS Disorders

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Antiparkinsonian potential of the Adenosine A_{2A} antagonist (SCH-58261) in the 6-OHDA rat model of Parkinson's disease: interaction with the dopaminergic agonist quinpirole

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Background: In striato-pallidal neurones, dopamine D_2 receptors are co-localized with adenosine A_{2A} receptors. Antagonistic A_{2A}/D_2 interactions have been reported in several *in vivo* preclinical models. In the present study, we investigated the ability of the adenosine A_{2A} antagonist SCH-58261 to modulate the activity of the dopaminergic D_2 agonist quinpirole in the unilateral 6-OHDA-lesioned rat model.
Methods: In a randomized experiment (n=256), vehicle or three different doses of SCH-58261 (1, 3 and 10 mg/kg) were administered before the injection of vehicle or quinpirole (three doses: 0.01, 0.03 and 0.1 mg/kg). Compounds were administered intraperitoneally 15 minutes before the quinpirole injection (ip) and contralateral/ipsilateral rotations were recorded for 180 min (dependent variable). Data were analyzed separately for each dose of quinpirole by a mixed model analysis of variance, incorporating the three hours of the test as the within-subject factor (time, 3 levels) and the dose of SCH-58261 as the between-groups factor (pretreatment, 4 levels).
Results: The results show that SCH-58261 alone significantly increases, dose-dependently both contralateral and ipsilateral circling, in comparison with a vehicle-treated group. Co-administration of SCH-58261 to an inactive dose of quinpirole (0.01 mg/kg), increases contralateral circling in a dose-dependent manner but induces less ipsilateral rotations than SCH-58261 alone. The A_{2a} antagonist, when co-administered with a subactive dose of quinpirole (0.03 mg/kg), significantly and dose-dependently increases and prolongs quinpirole-induced contralateral rotations.
Conclusion: These data demonstrate the potential antiparkinsonian activity of A_{2a} antagonists *per se* and highlight their ability to prolong dopaminergic activity in the 6-OHDA-lesioned rat model.

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Targeting Adenosine A_{2A} Receptors in Parkinson's Disease and Other CNS Disorders

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Local injection of caffeine produces motor improvements in the forepaw stepping test in a 6-hydroxydopamine rat model of Parkinson's disease.

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Background: Research has suggested that adenosine receptor antagonists are therapeutic in Parkinson's Disease (PD) either because of their interaction with L-DOPA in the dorsal striatum (direct pathway) or their inhibition of the overactive striatal-pallidal (indirect) pathway at the striatum and the external segment of the globus pallidus (GP_E). To test for the primary therapeutic action of adenosine antagonists, we measured the behavioral effects of local infusions of caffeine, an A_1, A_{2A}, and A_{2B} antagonist, into both the dorsal striatum and the external segment of the globus pallidus (GP_E) in the forepaw stepping rat model of PD.
Methods: Unilateral injections of X ul 6-hydroxydopamine (6-OHDA) into the medial forebrain bundle (MFB) produced a rat hemiparkinsonian model in 20 Long-Evans rats. Caffeine (1 µl of 1.0, 2.0, and 4.0 µg/µl or 1.0 µµl of 0.9% saline solution) was injected into the external segment of the globus pallidus (GP_E) and will be injected into the striatum through guide cannulas. The forepaw stepping test was the quantitative measure of motor performance and improvements.
Results: When injected into the GP_E, 2.0 and 4.0 µµg, but not 1.0 µµg caffeine improved stepping with the contralateral paw compared to saline ($p < 0.0009$). Data will be collected this semester on caffeine's behavioral effects when injected in the striatum, and we hope to examine the interaction of these central injections of caffenie with systemic L-DOPA.
Conclusion: As the effects of these central injections of caffeine are larger than those of systemic injections, these data highlight the importance of the GP_E in mediating these therapeutic effects of caffeine. A comparison of these effects to those produced by injections into the dorsal striatum will be important in indicating if the role of the GP_E is unique. The need to understand where and how adenosine antagonists work therapeutically in PD is especially exigent as these drugs enter human testing and become a new line of monotherapy or are combined with L-DOPA
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Targeting Adenosine A_{2A} Receptors in Parkinson's Disease and Other CNS Disorders

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Blockade of A_{2A} receptors in globus pallidus potentiates the effects of L-DOPA in unilaterally 6-hydroxydopamine-lesioned rats

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Background: Adenosine A_{2A} receptor antagonists ameliorate motor impairment and potentiate the effects of dopamine agonists in animal models of Parkinson's disease (PD). Dopamine-containing brain areas are highly enriched in A_{2A} receptors, the large majority of them being expressed on striatopallidal medium-sized spiny neurons and so far experimental evidence have suggested that striatum is the main area involved in the antiparkinsonian activity of A_{2A} antagonists. However, the globus pallidus (GP), the major target nucleus of the striatopallidal pathway, expresses A_{2A} receptors which regulate the extracellular concentrations of GABA in this area, an effect which might be involved in the counteraction of PD motor impairment by A_{2A} antagonists.
Methods: In order to evaluate whether antagonism of pallidal A_{2A} receptors results in antiparkinsonian effects, rotational behaviour in unilaterally 6-hydroxydopamine-(6-OHDA)-lesioned rats was assessed following the infusion of the A_{2A} antagonist SCH BT2 (5µg/1µl), a water-soluble analogue of SCH 58261, into GP, alone or in combination with a systemic subtreshold dose of L-DOPA (3 mg/kg i.p.).
Results: SCH BT2 alone neither altered motor behaviour nor produced motor asymmetry, whereas it significantly potentiated contraversive turning induced by L-DOPA.
Conclusion: These results indicate that A_{2A} blockade in GP sinergically amplify the effects of L-DOPA in 6-OHDA-lesioned rats suggesting that pallidal A_{2A} receptors participate in the antiparkinsonian effects of A_{2A} antagonists and providing new insights to the study of the neuronal substrates involved in the motor improving effects of these drugs.

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Targeting Adenosine A_{2A} Receptors
in Parkinson's Disease
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The effects of the adenosine A_{2a} antagonists MSX-3 and KW6002 on pimozide-induced motor effects in rats: assessment of potential treatments for parkinsonism.

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Background: In addition to the neuropathological and neurodegenerative forms of parkinsonism, there is considerable evidence that parkinsonian symptoms can be produced by various drug treatments. Typical antipsychotics, such as haloperidol and pimozide, have been shown to produce motor side effects such as parkinsonian tremor. Considerable research has revealed an antagonistic interaction between adenosine A_{2a} and dopamine D_2 receptors in the basal ganglia, which is related to the production of parkinsonian symptoms. **Methods:** Two experiments assessed the effects of the selective adenosine A_{2a} antagonists MSX-3 and KW 6002 on subchronic pimozide-induced motor syndromes in rats ultizing a battery of behavioral tests in which tremulous jaw movements (TJMs), catalepsy using the bar test, and locomotion in small stabilimeter cages, were recorded. Additionally, Fos-related protein expression was measured to study cellular interactions between D_2 receptors and A_{2a} receptors in the neostriatum. In the first experiment rats received a single daily i.p. injection of 1.0 mg/kg pimozide for 8 consecutive days. On day 8 all rats were treated with 1.0 mg/kg pimozide and received an injection of MSX-3 (1.25 – 10.0 mg/kg) or saline 3 hrs and 40 min following their daily pimozide injection. TJMs, catalepsy and locomotion were assessed and rats were immediately perfused. In the second experiment rats received a single daily i.p. injection of 1.0 mg/kg pimozide for 8 consecutive days. On day 8, rats received an injection of KW6002 (1.25 – 10.0 mg/kg) or vehicle and TJMs, catalepsy and locomotion were assessed in the same manner as described above for experiment 1. **Results:** Systemic administration of both MSX-3 and KW 6002 significantly reversed pimozide-induced tremulous jaw movements, catalepsy, and locomotor suppression. **Conclusion:** The present studies demonstrated that behavioral tests related to pimozide-induced changes in motor function can be useful for characterizing the potential antiparkinsonian effects of adenosine A_{2a} antagonists. Investigations of the neurochemical and behavioral interactions between adenosine A_{2a} and dopamine D_2 receptors could aid in the development of novel non-dopaminergic strategies for the treatment of idiopathic Parkinson's disease and drug-induced parkinsonism.
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Targeting Adenosine A_{2A} Receptors in Parkinson's Disease and Other CNS Disorders

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A_{2A} receptor antagonists improve akinesia and sensory-motor deficits in the unilateral 6-OHDA model of Parkinson's disease

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Background: Preclinical studies in different animal models of Parkinson's disease (PD) have demonstrated the ability of adenosine A_{2A} receptor antagonists to potentate L-DOPA-induced contralateral turning; however, very little is known about effects of those compounds when administered alone. To this aim we evaluated the effects of the adenosine A_{2A} receptor antagonists SCH 58261 and ST 1535 on akinesia and sensory-motor deficits induced by a 6-hydroxydopamine (6-OHDA) lesion.
Methods: 14 days after unilateral lesion with 6-OHDA in the medial forebrain bundle rats have been monitored for akinesia, gait impairment and sensory-motor deficits induced by the 6-OHDA lesion through the following tests: 1) initiation time of stepping; 2) adjusting test (stepping with forepaw was measured as the paw was dragged backwards and laterally); 3) the vibrissae-elicited forelimb (of the same side) placing test (an index of sensory-motor integration deficits). The effects of acute administration of a low dose (chosen to be subthreshold for induction of rotation) of L-DOPA (6 mg/kg i.p.) and the A_{2A} receptor antagonists SCH 58261 (5 mg/kg i.p.) and ST1535 (20 mg/kg i.p.), were evaluated at 3 weeks post-lesion.
Results: 1) The 6-OHDA lesion induced marked and long-lasting impairment in the initiation of stepping movement with the paw contralateral to the lesioned side. Administration of L-DOPA as well as SCH 58261 were effective in reversing this deficit. ST 1535 was also effective, although the improvement was less pronounced. 2) The unilateral 6-OHDA-lesioned rats stepped less often with the forepaw contralateral to the lesion than with their unimpaired ipsilateral forepaw. L-DOPA and SCH 58261 enhanced stepping with the contralateral paw in a similar way, whereas, ST 1535 induced a less marked improvement. 3) 6-OHDA-lesioned rats never placed on the table surface the 6-OHDA contralateral forelimb after brushing of vibrissae. L-DOPA restored the placement of contralateral forelimb; SCH 58261 and ST1535 were also effective but to a less extent than L-DOPA.
Conclusion: these results support the therapeutic potential of A_{2A} receptor antagonists and evidenced that blockade of A_{2A} receptors are effective in improving akinesia, gait deficits and sensory–motor impairments even without L-DOPA combined administration.

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P-29

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Targeting Adenosine A_{2A} Receptors in Parkinson's Disease and Other CNS Disorders

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The Selective Adenosine A_{2a} Antagonist MSX-3 Suppresses the Tremulous Jaw Movements Induced by Dopamine Antagonism and Dopamine Depletion: Studies With a Rodent Model of Parkinsonian Tremor.

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Background: Tremulous jaw movements are defined as rapid and repetitive vertical deflections of the lower jaw that are not directed at any particular stimulus. These movements occur within the 3-7 Hz frequency that is characteristic of parkinsonian resting tremor, and can be induced by a number of conditions that parallel the neurochemistry of the pathology of parkinsonism, including dopamine (DA) depletions in the ventrolateral neostriatum, acute or sub-chronic injections of DA antagonists, and administration of the DA-depleting agent reserpine. It has been suggested that tremulous jaw movements meet a reasonable set of validation criteria for use as an animal model of parkinsonian tremor. Previous studies have shown that tremulous jaw movements can be used to assess the effects of several classes of antiparkinsonian drugs, including L-DOPA, DA agonists, muscarinic antagonists, the atypical antipsychotic clozapine, and adenosine A_{2A} antagonists. **Methods:** In the present experiments, tremulous jaw movements were induced by repeated administration of 0.5 mg/kg of the DA antagonist haloperidol, and by 1.25 mg/kg of the DA depleting agent reserpine. The novel adenosine A_{2A} antagonist MSX-3 was coadministered with these drug treatments in order to determine if adenosine A_{2A} antagonism could reverse the tremorogenic effects of interference with DA transmission. MSX-3 is a water-soluble phosphate pro-drug of MSX-2, which is the active antagonist of A_{2A} receptors. **Results:** Systemic (IP) injections of MSX-3 (2.5-10.0 mg/kg IP) were capable of reversing the tremulous jaw movements induced by repeated (i.e., 14 day) administration of 0.5 mg/kg haloperidol. In addition, MSX-3 (10.0-20.0 mg/kg IP) suppressed the tremulous jaw movements induced by acute administration of 1.25 mg/kg reserpine. **Conclusions:** These results indicate that antagonism of adenosine A_{2A} receptors with MSX-3 can suppress the tremulous jaw movements induced by interference with DA transmission. This profile of activity is consistent with the hypothesis that antagonism of adenosine A2A receptors can result in a tremorolytic effect in a rodent model of parkinsonian tremor.

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Targeting Adenosine A_{2A} Receptors in Parkinson's Disease and Other CNS Disorders

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The neuroprotection by caffeine in the MPTP model of Parkinson's disease is lost in adenosine A_{2A} knockout mice.

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Background: Prospective epidemiological studies have raised the possibility of caffeine conferring protection against Parkinson's disease. This hypothesis is strengthened by our previous findings that caffeine attenuates MPTP-induced dopaminergic neurotoxicity in mice. Moreover, antagonists of the A_{2A} subtype of adenosine receptor ($A_{2A}R$), but not of the A_1R, provided similar protection. To further investigate the dependence upon and location of the $A_{2A}R$ in caffeine's neuroprotection, we examined the effect of caffeine on MPTP neurotoxicity in standard (global) $A_{2A}R$ knockout (A_{2A} KO) mice as well as tissue-specific (conditional) A_{2A} KO mice.
Methods: Postnatal forebrain neuron-specific and astrocyte-directed conditional A_{2A} KO mice were generated by using the Cre-*loxP* system based on the specificity of *CamKII*α and *GFAP* gene promoters, respectively. Tissue-specific disruption of the $A_{2A}R$ was confirmed by PCR and western blot. Locomotion, scored as the number of adjacent photobeam breaks (*Ambulation*), was determined 3 hr before and 3 hr after caffeine/saline injection in wide-type and knockout mice. In neuroprotection experiment, caffeine or saline were administered 10 minutes before MPTP treatment (40 mg/kg ip single injection). One week later, striatal dopamine content was determined by HPLC.
Results: Caffeine-stimulated locomotion is significantly decreased in forebrain neuron-specific $A_{2A}R$ KO mice, similar to what we found previously in global $A_{2A}R$ KO mice. MPTP treatment (40 mg/kg single injection) produced similar dopamine depletion in knockout mice and their respective wide-type littermates. On the other hand, caffeine pretreatment (25 mg/kg ip) significantly attenuated MPTP-induced striatal dopamine loss in wild-type mice. This neuroprotection by caffeine, however, is lost in global $A_{2A}R$ KO mice. Similarly, caffeine attenuated MPTP-induced dopamine depletion in control but not forebrain neuron-specific $A_{2A}R$ KO mice. On the other hand, caffeine's attenuation of MPTP neurotoxicity is present in both control and astrocyte-directed $A_{2A}R$ KO mice.
Conclusions: Taken together, these data suggest that caffeine's neuroprotection against MPTP neurotoxicity is dependent on the $A_{2A}R$, particularly those located in forebrain neurons.

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Targeting Adenosine A_{2A} Receptors in Parkinson's Disease and Other CNS Disorders

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Cell-type Selective Inactivation of the Adenosine A_{2A} Receptor Reveals a Critical Contribution of A_{2A} Receptors in Bone Marrow-derived Cells to 3-Nitropropionate-induced Striatal Damage in Mice

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Endogenous adenosine acting at the adenosine A_{2A} receptor (A_{2A}R) can modify brain injury in variety of neurological disorder models. However, neuroprotection against striatal damage by both A_{2A}R activation and inactivation raise an intriguing possibility that A_{2A}Rs in distinct cellular elements may have different (or opposing) effects. In this study, we developed two novel transgenic models to dissect out cell-type specific action of A_{2A}Rs against striatal damage by the mitorchondrial toxin 3-nitropropionic acid (3-NP). While global inactivation of A_{2A}Rs exacerbated neurological deficit behaviors as well striatal damage, selective inactivation of A_{2A}Rs in forebrain neurons (using the loxP-Cre strategy) did not affect neurological deficit behavior and failed to confer neuroprotection against 3-NP-induced striatal damage. Striatal damages by either intrapretoneal injection of 3-NP or intrastriatal injection of malonate were indistinguishable between forebrain A_{2A}R KO and WT littermates, suggesting that A_{2A}R activity in forebrain is not a critical contributor to modulation of striatal damage. Furthermore, we created chimeric mice with selective inactivation of A_{2A}Rs in bone marrow-derived cells (BMDCs) by transplantating bone marrow cells derived from either global A_{2A}R KO mice (KO→WT) or WT littermates (WT→WT) into C57BL/6 mice. Interestingly, striatal damage induced by 3-NP were also exacerbated by selective inactivation of A_{2A}R activity in BMDCs (comparing KO→WT with WT→WT mice). Together, these results suggest that A_{2A}Rs in BMDCs but not A_{2A}Rs in forebrain neurons are the important contributor to striatal damage induced by mitochondrial dysfunction.

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Targeting Adenosine A_{2A} Receptors in Parkinson's Disease and Other CNS Disorders

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Chronic treatment with caffeine attenuates traumatic brain injury in controlled cortical impact model of mice

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Background: The adenosine A_{2A} receptor ($A_{2A}R$) is expressed at high levels in the brain and plays an important role in the modulation of brain injury in response to variety of neuronal insults. However, the role of the $A_{2A}R$ in the development of traumatic brain injury (TBI) is not clear. In this study, we examined the effects of acute and chronic treatment with caffeine on neurological behaviors and brain damage after acute TBI, which was induced by controlled cortical impact (CCI) in Kunming (KM) mice.

Methods: KM mice were divided into three treatment groups: (1) water (no-caffeine) treatment; (2) oral caffeine (0.25 g/L) for 3 weeks; or (3) acute intraperitoneal injection of caffeine (15mg/kg) 30 minutes before TBI. The mice were then subjected to controlled cortical impact or no-CCI. CCI was performed by dropping a 20.0g-weight from 50 cm height above the ground onto the brain. We also determined the level of glutamate in cerebral spinal fluid (CSF) and the mRNA level of TNF-α□IL-1β in injured brain at 12 hours post-TBI, and neurological deficit score and brain water contents at 24 hours post-TBI.

Results: Twenty-hour hours after TBI, the brain water contents of the injured cerebral cortex of the mice in the CCI group were significantly higher than that of the non-CCI group ($P<0.01$). Importantly, brain water contents of the mice with chronic caffeine treatment (79.4%±0.91□,n=6) were significantly lower than that of the mice without caffeine treatment (82.65%±1.54□,n=10) after TBI ($P<0.01$). Furthermore, the neurological deficit scores of the mice with chronic caffeine treatment (1.14±0.38,n=7) were significantly lower than that of the mice without caffeine (1.8±0.41,n=15) after the TBI ($P<0.01$). However, the neurological deficit scores and the brain water contents were indistinguishable between the mice with acute treatment of caffeine (1.81±0.40,n=16) and the mice without caffeine treatment. In agreement with the water content results and the neurological deficit scores, at 12 hours after the TBI, the glutamate level in the CSF of mice with chronic caffeine treatment (7.68±2.47μM, n=6) was markedly lower than that of the mice without caffeine treatment (47.84±29.85μM, n=6) and mice with acute caffeine treatment (39.41±28.44μM, n=6) ($P<0.01$). However, at 12 hours after TBI, mRNA levels of TNF-α and IL-1βin injured brain tissue of the mice with chronic caffeine treatment were higher than those of the mice without caffeine treatment ($P<0.05$).

Conclusion: Chronic treatment with caffeine reduces neurological damage after TBI. This protective effect may mainly result from the inhibition of glutamate release by inactivating the A_{2A} receptor.

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Targeting Adenosine A_{2A} Receptors in Parkinson's Disease and Other CNS Disorders

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Neuroprotective effect of the selective A_{2A} receptor antagonist SCH 58261 after cerebral ischemia: effects on MAPKs

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Background: Our recent results demonstrated that the selective A_{2A} receptor antagonist, SCH 58261, reduces glutamate outflow and acute motor disturbance in the first hours after ischemia. Twenty-four hours after ischemia, SCH 58261 protectes against neurological deficit, brain damage and p38-MAPK activation in microglial cells localized in the ischemic striatum and cortex. Activation of MAPKs is reported in ischemia. While p38-MAPK activation is involved in cell suffering and death, ERK-MAPK activation is associated with cell proliferation and differentiation. The role of JNK-MAPK is still controversial.
Methods: Permanent focal cerebral ischemia was induced by right middle cerebral artery occlusion (MCAo) in the rat. SCH 58261 (0.01 mg/kg, i.p.) was administered 5 min, 6 h and 15 h after MCAo. Neurological deficits were evaluated 3 and 4 h after MCAo as number of rotations per hour and 24 h after ischemia as sensory-motor tests. Then, rats were sacrificed by transcardiac perfusion with 4% paraformaldehyde solution. Brains were cut in 30-µm-thick coronal slices to evaluate ischemic brain damage by acetate cresyl violet staining, to evaluate activation of ERK1/2- and JNK-MAPK and to stain microglia and oligodendrocytes by specific antibodies.
Results: After ischemia, vehicle-treated rats showed an acute motor disturbance, i.e. a contralateral turning behavior and a clear neurological deficit. SCH 58261-treated rats showed a significant improvement both in turning behaviour (rotation/h: mean±S.E.M: 116.9±34.6 vs 795.4±170.6, drug-treated vs vehicle-treated rats, $p<0.001$) and in neurological deficit (score: 10.8±0.4 vs 8.8±0.5, $p<0.001$). Moreover, SCH 58261 significantly reduced both cortical (41.1±2.8 mm^3 vs 55.6±3.9 mm^3, $p<0.02$) and striatal (12.8±1.9 mm^3 vs 23.2±2.7 mm^3, $p<0.01$) damage. In vehicle-treated rats 24 h after MCAo, phospho-ERK1/2 immunopositive cells, showing morphological features of reactive microglia, were localized in the cortex and in the striatum of the ischemic hemisphere. Phospho-JNK immunopositive cells were present in the corpus callosum and in the white matter of the striatal nuclei of the ischemic hemisphere. Phospho-JNK positive cells colocalized with oligodendrocytes. Evaluation of the effect of SCH 58261 on ERK1/2- and JNK-MAPK activation is now in progress.
Conclusion: The protective effect of the selective A_{2A} receptor antagonist SCH 58261 may involve modulation of MAPKs.
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Targeting Adenosine A_{2A} Receptors in Parkinson's Disease and Other CNS Disorders

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Systemic Injections of the Selective Adenosine A_{2a} Agonist CGS 21680 Impair Motor Functions that are Important for Feeding Behavior in Rats.

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Background: Several lines of evidence indicate that adenosine A_{2A} receptors are involved in the regulation of motor functions. Antagonists of the adenosine A_{2A} receptor have been shown to exert antiparkinsonian effects in animal models, and adenosine A_{2A} receptor agonists such as CGS 21680 suppress locomotion and impair various aspects of motor function. The present experiments were conduced to study the effects of the adenosine A_{2A} agonist CGS 21680 on specific parameters of food intake. **Methods:** Rats were observed in 30 min sessions that allowed for the measurement of food intake, time spent feeding, and feeding rate. Food intake and feeding rate have been shown in previous work to be sensitive to the effects of systemically administered dopamine (DA) antagonists and striatal DA depletions, and this effect has been interpreted as being consistent with the hypothesis that striatal DA depletions produce motor impairments that interfere with feeding. In addition, rats also were observed for drug-induced sedation using a sedation rating scale. **Results:** Systemic (i.p.) injections of CGS 21680 (0.025, 0.05, 0.1 mg/kg) produced a dose related suppression of the amount of food consumed during the 30 min test. This effect was largely dependent upon a slowing of the rate of feeding (in grams per min), and there was only a modest suppression of time spent feeding. The doses of CGS 21680 that suppressed feeding also were associated with the presence of sedation as measured by a sedation rating scale. **Conclusions:** The adenosine A_{2A} receptor agonists CGS 21680 suppressed food intake and feeding rate, and produced a pattern of effects similar to that previously shown for DA antagonists and striatal DA depletions. In contrast, appetite suppressant drugs have been shown to have more profound effects on time spent feeding, as opposed to rate of feeding. In conjunction with other published studies, the present results suggest that adenosine A_{2A} receptor stimulation suppresses feeding because of motor impairments, sedative effects, or a combination of both actions. Previous studies have shown that ventrolateral striatal DA depletions are associated with impairments in skilled motor functions that are necessary for food handling, and it is possible that local injections of CGS 21680 into this striatal subregion would suppress feeding rate and impair reaching, grasping and food handling.
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Targeting Adenosine A_{2A} Receptors in Parkinson's Disease and Other CNS Disorders

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Systemic Injections of the Adenosine A_{2a} Agonist CGS 21680 reduce lever pressing behavior: interaction with the work requirements of the task.

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Background: Considerable evidence indicates that nucleus accumbens dopamine (DA) is a critical component of the brain circuitry regulating work output in food-seeking behaviors. In addition, an emerging body of evidence indicates that striatal DA systems interact with adenosine A_{2A} receptors, and these interactions may also regulate the behavioral functions of the nucleus accumbens. The following experiments were designed to characterize the effects of the adenosine A_{2A} agonist CGS 21680 on effort-related functions known to be modulated by the nucleus accumbens. **Methods:** The present experiments study the effects of the adenosine A_{2A} agonist CGS 21680 on the performance of two variable interval (VI) schedules of reinforcement with fixed ratio (FR) requirements attached. On one schedule, the rats were reinforced for the 1st lever press after an average interval of 60 sec (VI60 FR1). On the other schedule, the rats were reinforced for the 10^{th} lever press after an average interval of 60 sec (VI60 FR10). **Results:** Systemic (i.p.) injections of CGS 21680 (0.05-0.2 mg/kg) dose-dependently reduced lever pressing in both schedules. However, statistical analysis revealed a significant dose-by-schedule interaction, showing that the task with a higher work requirement (VI60 FR10) was more sensitive to the suppressant effects of CGS 21680, than the task with a lower work requirement (VI60 FR1). In addition, the ED_{50} for the VI60 FR10 schedule was 0.05564 mg/kg, while the ED_{50} for the VI60 FR1 schedule was 0.09467 mg/kg. **Conclusions:** These results indicate that stimulation of adenosine A_{2A} receptors produces deficits on operant behaviors that are related to the work requirements of the task. The pattern of effects produced by CGS 21680 in the present studies was similar to the effects previously shown with accumbens DA depletions. Taken together, these results suggest that DA and adenosine systems are part of the neural circuitry that modulates effort-related functions.

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Role of A_{2A} Adenosine Receptors in the Development of Dopamine-Mediated Behavioral Sensitization in 6-Hydroxydopamine Lesioned Rats

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Background: Rats with unilateral 6-hydroxydopamine (6-OHDA) lesions of the nigrostriatal dopamine pathway exhibit behavioral sensitization following repeated injections with dopamine agonists, a phenomenon called 'priming.' When 6-OHDA lesioned rats are treated with three priming injections with D1 agonist SKF38393 (10 mg/kg), D2 agonist quinpirole (1 mg/kg) or D1/D2 agonist apomorphine (0.5 mg/kg), they exhibit robust contralateral rotational behavior following a challenge 7-12 days later with a low dose of D2 agonist quinpirole (0.25 mg/kg). Previous studies have suggested that the A_{2A} adenosine receptor may play an important role in the development of behavioral sensitization in the dopamine-depleted system. Adenosine and dopamine exert opposite effects on motor activity with dopamine agonists and adenosine antagonists stimulating motor behavior. In the striatum, A_{2A} receptors are co-expressed with D2 receptors, but are segregated from D1 receptor-expressing neurons. In this study, we used the A_{2A} adenosine antagonist 8-(3-chlorostyryl) caffeine (CSC) to test whether A_{2A} receptors are involved in the development of behavioral sensitization induced by D1, D2 or D1/D2 dopamine agonists.
Methods: Male Sprague-Dawley rats received a unilateral stereotaxic injection of the neurotoxin 6-OHDA into the left medial forebrain bundle. Three weeks later, rats were treated with three priming injections, 3-6 days apart, with A_{2A} antagonist CSC (0.3, 1 or 5 mg/kg) administered 30 minutes prior to SKF38393 (10 mg/kg), quinpirole (0.25 or 1 mg/kg) or apomorphine (0.1 or 0.5 mg/kg). Seven to twelve days after the third priming injection, 6-OHDA rats were challenged with low dose quinpirole (0.25 mg/kg) and contralateral rotational behavior was recorded.
Results: Administration of high dose CSC (5 mg/kg), but not lower doses of CSC (0.3 and 1 mg/kg), enhanced the ability of SKF38393 to prime quinpirole-mediated rotational behavior. In contrast, administration of CSC (5 mg/kg) together with apomorphine (0.1 or 0.5 mg/kg) or quinpirole (0.25 or 1 mg/kg) had no effect on the ability of these D1/D2 or D2 agonists to prime quinpirole-mediated rotational behavior.
Conclusion: Blocking A_{2A} adenosine receptors enhanced D1-mediated priming, but did not affect D1/D2- or D2-mediated priming, suggesting that A_{2A} adenosine receptors play a role in the development of D1-mediated behavioral sensitization in 6-OHDA lesioned rats.

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Genetic Inactivation of A_{2A} Receptors but not A_1 Receptors Attenuates Psychostimulant Effects of Cocaine

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Background: Adenosine A_1 and A_{2A} receptors (A_1R, $A_{2A}R$) are expressed at high levels in the brain regions with dopaminergic innervation and have profound interaction with the dopaminergic system. While recent pharmacological and genetic studies have implicated the A_1Rs and $A_{2A}Rs$ in modulation of psychostimulant actions, the exact roles of these receptors in regulation of acute and chronic psychostimulant effects are not clear.
Methods: In this study, we took advantage of newly developed three gene knockout (KO) models (mice deficient in A_1Rs or $A_{2A}Rs$ or both) to determine the contribution of these receptors to acute psychostimulant effect of cocaine and to behavioral sensitization after repeated treatment with cocaine.
Results: Behavioral analysis showed that spontaneous locomotor activities of $A_{2A}R$ KO, A_1R KO and A_1R-$A_{2A}R$ double KO mice were slightly lower than that of their wild-type (WT) littermates (n=8 per group, all in congenic C57BL/6 genetic background). The mice were treated daily with cocaine (25 mg/kg, i.p.) for 8 days and monitored for their locomotor activities on the 1st and 8th days for 30 min immediately after the injection. Consistent with our previous studies, on the 1st day, cocaine-induced locomotor activities were significantly lower in $A_{2A}R$ KO mice compared to their WT littermates. In contrast, cocaine-induced locomotor activities were indistinguishable between A_1R KO mice and their WT littermates. Furthermore, inactivation of both the A_1R and $A_{2A}R$ in the double KO produced similar attenuation of cocaine-induced motor activity as the $A_{2A}R$ KO mice. On the 8th day, locomotor sensitization induced by repeated treatment with cocaine was similarly attenuated in $A_{2A}R$ KO and A_1R-$A_{2A}R$ double KO mice. Again, repeated treatment with cocaine induced identical behavioral sensitization in A1R KO mice and their WT littermates.
Conclusion: These results suggest that $A_{2A}Rs$ play an important role in the acute and sensitized motor responses to cocaine whereas A_1Rs are not essential for these effects.

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Adenosine A_{2A} antagonists in a rat model of L-DOPA-induced dyskinesia

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Background: A_{2A} antagonists effect on L-DOPA-induced dyskinesia has been studied in pre-clinical animal models as well as in clinical trials. Recent clinical trials on the A_{2A} antagonist KW6002 as adjunct to L-DOPA-therapy showed an increase in dyskinesia which was associated with an increase in "on-time". A rat study by Lundblad et al. showed no effect of an A_{2A} antagonist on the development of L-DOPA-induced dyskinesias and a primate study by Bibbiani et al. reported that co-administration of an A_{2A} antagonist offered complete protection from the development of apomorphine-induced dyskinesias. This study further adresses the dyskinesiogenic potential of A_{2A} antagonist therapy in a rat model of L-DOPA-induced dyskinesia.

Methods: Drug naïve 6-OHDA-lesioned rats were dosed daily with 2-6mg/kg L-DOPA, 2-6mg/kg L-DOPA + 2mg/kg KW6002 or vehicle for 21 days. The abnormal involuntary movements (AIM) were scored every 20 minutes for 3hrs on 4 parameters: locomotor, oro-lingual, axial and limb.

Results: Rats treated with L-DOPA and KW6002 had significantly more dyskinesias compared with the L-DOPA-treated group. This effect was seen for all parameters (limb, axial, orolingual and locomotor dyskinesias). No vehicle-treated animals showed any dyskinesia. Daily dosing of KW6002 produced dyskinesias even in combination with a threshold dose of 2mg/kg L-DOPA. No dyskinesia was seen for KW6002 as mono-therapy.

Conclusion: A_{2A} antagonist treatment had a low dyskinesiogenic potential as stand-alone. However, the concept that A_{2A} antagonists protect from the development of L-DOPA induced dyskinesias was not supported by the rat dyskinesia data in the present report.

Bibbiani F, Oh JD, Petzer JP, Castagnoli N, Jr., Chen JF, Schwarzschild MA, Chase TN. A2A antagonist prevents dopamine agonist-induced motor complications in animal models of Parkinson's disease. Experimental neurology, 2003;184(1):285-94.

Lundblad M, Vaudano E, Cenci MA. Cellular and behavioural effects of the adenosine A2a receptor antagonist KW-6002 in a rat model of l-DOPA-induced dyskinesia. Journal of neurochemistry, 2003;84(6):1398-410.

The authors have not indicated whether a conflict of interest exists.

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Targeting Adenosine A_{2A} Receptors
in Parkinson's Disease
and Other CNS Disorders

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Adenosine A2a blockade prevents synergy between MOR and CB1 in NAc neurons and eliminates heroin seeking behavior in addicted rats

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Relapse is the most serious limitation of effective medical treatment of opiate addiction. Opiate-related behaviors appear to be modulated by cannabinoid (CB1) receptors through poorly understood cross-talk mechanisms. Opiate and CB1 receptors are co-expressed in the nucleus accumbens (NAc) and dorsal striatum. These regions also have the highest density of adenosine A2a receptors (A2a) in the brain. We have been investigating the postsynaptic signaling mechanisms of μ-opiate (MOR) and CB1 receptors in primary NAc/striatal neurons. Here we present evidence that MOR and CB1 act synergistically on cAMP/PKA signaling in NAc/striatal neurons. In addition, we find that synergy requires adenosine and A2a. Importantly, an A2a antagonist administered either directly into the NAc or indirectly by i.p. injection eliminates heroin-induced reinstatement in rats trained to self-administer heroin, a model of human craving and relapse. These findings suggest that A2a antagonists might be effective therapeutic agents in the management of abstinent heroin addicts.

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To the best of my knowledge and judgment I, the presenting author, report that:
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P-40

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Targeting Adenosine A_{2A} Receptors in Parkinson's Disease and Other CNS Disorders

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Adenosine A_{2A} Antagonism Reverses the Effects of Dopamine Antagonism on Instrumental Output and Effort-Related Choice: Implications for Studies of Psychomotor Slowing

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Background: Organisms frequently make effort-related decisions based upon differential assessments of motivational value and response costs. Energy-related dysfunctions, such as psychomotor slowing and apathy, are critically involved in various clinical syndromes. Considerable evidence indicates that dopamine, particularly in nucleus accumbens, regulates effort-related processes. Dopamine antagonism and accumbens dopamine depletions cause rats performing on choice tasks to reallocate their instrumental behavior away from food-reinforced tasks that have high response requirements. Previous studies have shown that there is a functional interaction between DA and adenosine A_{2A} receptors in striatal areas, including the nucleus accumbens. The present experiments were conducted to determine if adenosine A_{2A} antagonism could reverse the effects of dopamine antagonism on instrumental behavior and effort-related choice. **Methods:** The adenosine A_{2A} antagonist MSX-3 was investigated for its ability to reverse the effects of the dopamine antagonist haloperidol (0.1 mg/kg) on fixed ratio 5 instrumental lever pressing, and on response allocation using a concurrent lever pressing/chow feeding choice task. **Results:** Haloperidol significantly suppressed fixed ratio 5 responding, and with rats responding on the concurrent choice task it altered choice behavior, significantly reducing lever pressing for food and increasing chow intake. Injections of MSX-3 (0.5-2.0 mg/kg) produced a dose-related reversal of the effects of 0.1 mg/kg haloperidol on both tasks. The high dose of MSX-3, when administered in the absence of haloperidol, did not significantly affect responding on either task. **Conclusions:** Haloperidol significantly suppressed fixed ratio 5 responding, and with rats responding on the concurrent choice task it altered choice behavior, significantly reducing lever pressing for food and increasing chow intake. Injections of MSX-3 (0.5-2.0 mg/kg) produced a dose-related reversal of the effects of 0.1 mg/kg haloperidol on both tasks. The high dose of MSX-3, when administered in the absence of haloperidol, did not significantly affect responding on either task.

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Targeting Adenosine A_{2A} Receptors in Parkinson's Disease and Other CNS Disorders

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Neonatal caffeine enhances the breathing response to hypoxia and increases adenosine and dopamine receptors mRNA levels in chemosensitive structures of adult rats

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Background: Neonatal caffeine treatment (NCT) is commonly used to treat apnea of prematurity in clinics. We recently demonstrated that NCT persistently augments the ventilatory response to hypercapnia in juvenile rats (Montandon et al., 2006, *Ped Res*). However, the long term influence of NCT on the breathing response to hypoxia is unknown. The objectives of this study were to expand upon these initial findings and to address the consequences of NCT on the ventilatory response to hypoxia of adult rats. The impact of NCT on carotid body (a structure sensitive to oxygen) development was assessed by quantifying expression of adenosine A_1 and A_{2A} receptors, dopamine D_2 receptor, and tyrosine hydroxylase mRNA.
Methods: To this aim, ventilatory measurements were performed using plethysmography on freely-behaving awake adult rats previously subjected, during the neonatal period (postnatal days 3 to 12), once a day to either water (control) or caffeine 15 mg/kg (NCT) administration. After resting ventilatory recordings, animals were subjected to hypoxia (O_2=12%) for 20 min. Tyrosine hydroxylase, adenosine A_1, A_{2A} and dopamine D_2 receptors mRNA quantifications in the carotid body (the nervous structure that contains most of the oxygen sensing cells) and in the striatum were estimated by RT-PCR.
Results: NCT enhanced the immediate breathing frequency response to hypoxia by 20% approximately during the first 10 minutes. Results of RT-PCR analyses showed that NCT augments tyrosine hydroxylase (by 35%, $P<0.0001$), adenosine A_{2A} (by 22%, $P=0.0005$) and dopamine D_2 (by 43%, $P<0.0001$) receptor mRNA levels in the carotid bodies. However, increases of adenosine A_{2A} receptors and tyrosine hydroxylase mRNAs were not observed in the striatum.
Conclusion: These results demonstrate that a caffeine treatment occurring during the first week of life has long term consequences on the respiratory control system that persist until adulthood. Thus, The increase of the breathing response to hypoxia induced by NCT might be due to an increase of adenosine A_{2A} receptor expression observed only in the carotid bodies of NCT animals. This persistent enhanced sensitivity to oxygen might predispose children or adult to respiratory instability, especially during sleep.

Founded by the Jeanne-and-Jean-Louis-Levesque' Chair in Perinatalogy and the Canadian Research Chair in Respiratory Neurobiology.

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Targeting Adenosine A_{2A} Receptors in Parkinson's Disease and Other CNS Disorders

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ADENOSINE A_{2A} RECEPTORS MEDIATE THE EFFECT OF CAFFEINE TO IMPROVE REACTION TIME IN A MODEL OF ATTENTION IN RATS

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Background: Caffeine produces effects on cognitive function particularly relating to aspects of attention such as reaction time. Considering the plasma exposure levels following regular caffeine intake, and the affinity of caffeine for known pharmacological targets, these effects of caffeine are likely mediated by either the adenosine A_1 or A_{2A} receptor.
Methods: In the present studies, two rat strains [Long Evans (LE) and CD] were trained to asymptote performance in a test of selective attention, the 5-Choice Serial Reaction Time Task (5-CSRTT). Next, the effects of caffeine were compared to the selective A_{2A} antagonists, SCH 412348 and KW-6002 (Istradefylline), and the A_1 antagonist, DPCPX. Further studies compared the psychostimulant effects of each drug. Additionally, we tested the effects of the A_{2A} agonist, CGS-21680, on 5-CSRTT performance. Given the antipsychotic potential of A_{2A} agonist, we also studied the interaction between CGS-21680 and amphetamine in this task.
Results: Caffeine (3-10 mg/kg) improved reaction time in both LE and CD rats, with no effect on accuracy, an effect replicated by SCH 412348 (0.1-1 mg/kg) and KW-6002 (1-3 mg/kg), but not DPCPX (3-30 mg/kg). At least with SCH 412348, these effects were observed at doses that were not overtly psychostimulant. In contrast, CGS-21680 (0.03-0.3 mg/kg) increased reaction time and increased omissions. Interestingly, at a comparatively low dose of 0.03 mg/kg, CGS-21680 attenuated the increased premature responding produced by amphetamine (1 mg/kg).
Conclusion: The present results suggest that the attention-enhancing effects of caffeine are mediated through A_{2A} receptor blockade, and selective A_{2A} receptor antagonists may have potential as therapies for attention-related disorders. Furthermore, the improvement in response control in amphetamine-treated rats following CGS-21680 pretreatment supports the view that A_{2A} agonists may have potential as novel antipsychotics.

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Targeting Adenosine A_{2A} Receptors in Parkinson's Disease and Other CNS Disorders

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Distribution of adenosine A_{2A} receptors in de novo Parkinson's disease using TMSX PET — a preliminary study—

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Background: We developed a radioligand, [7-methyl-^{11}C]-(E)-8-(3,4,5-trimethoxystyryl)-1,3,7-trimethylxanthine (TMSX), for mapping the adenosine A_{2A} receptors for mapping the adenosine A2A receptors by positron emission tomography (PET) (Ishiwata K et al., J Nucl Med, 2000). This study is the first report for distribution of adenosine A_{2A} receptors in the patients with Parkinson's disease (PD) using the TMSX PET.
Methods: We studied six patients with de novo PD (three men and three women, mean age ± SD, 63.0 ± 6.2). Diagnoses were based on their medical histories including physical and neurological examination, laboratory tests, and MRI. To ensure the early diagnosis of PD, we also confirmed the low density of dopamine transporters and the normal or high density of dopamine D_2 receptors in the putamen of all patients, using ^{11}C-2β-carbomethoxy-3β-(4-fluorophenyl)tropane and ^{11}C-raclopride PET. A dynamic series of decay-corrected PET scans was performed for 60 minutes. Parametric images of binding potential (BP) for TMSX were generated without arterial blood sampling using a Logan plot and EPICA (Naganawa M et al., IEEE Trans Bio Med, 52, 2005). Circular ROIs of 10-mm diameter were placed in the parametric images over anterior and posterior putamen for each subject. Values for BP in these regions were calculated as mean of pixel value in the circular ROIs.
Results: In PD patients, the BP was significantly lower on the more affected side of the posterior putamen than the less affected side (paired t test, $p<0.05$).
Conclusion: Release of dopamine is reduced asymmetrically in the putamen of early PD. In the posterior putamen with PD, dopamine D_2 receptors are up-regulated as compensation for the reduced dopamine release. The adenosine A_{2A} receptors showed the reaction that was opposite to the dopamine D_2 receptors.

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Targeting Adenosine A_{2A} Receptors in Parkinson's Disease and Other CNS Disorders

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Modulation of Glutamatergic-Dopaminergic Function in 6-OHDA Rat Model of PD

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Background: Degeneration of dopaminergic neurons in the substantia nigra pars compacta is considered a process eventually leading to development of Parkinson's disease (PD).
Recent studies have shown the potential role of glutamatergic function in the induction-progression of excitotoxic damage in the basal ganglia and is considered a predominant mechanism of dopamine neurons death in PD.
The purpose of this investigation was to evaluate modulation of dopaminergic and metabotropic glutamatergic receptor function in a 6-OHDA rat model of Parkinson's disease by using *in-vivo* microPET imaging.
Methods: Seventeen male Sprague Dawley rats (8-10 weeks old) were used for the experiment: 13 rats were administered with 6-0HDA (8 µg/2 µl, injected in the right middle brain) and 4 rats injected with saline solution were used as controls. Four weeks after lesioning, rotational behavior was determined in six PD rats and four controls with a single injection of D-amphetamine (AMPH, 5 mg/kg i.p).
MicroPET imaging were conducted to establish the entity of the lesion. All rats were imaged with [^{11}C]-2β-carbomethoxy-3β-(4-fluorophenyl)tropane [^{11}C]CFT a sensitive and selective ligand of dopamine transporters (DAT). Eight rats were imaged with [^{11}C]raclopride [^{11}C]RAC to indirectly determine the DA release. Glutamate function was investigated in all rats by injection of [^{11}C]-2-methyl-6-(2-phenylethynyl)-pyridine [^{11}C]MPEP a selective and sensitive antagonist of mGluR5.
PET data were acquired dynamically in 3D mode (P4 Concord Microsystems, Inc) in the whole brain for 60 min after transmission imaging to correct for attenuation (10 min using a rotating ^{68}Ge/^{68}Ge source). For imaging studies rats were anesthetized with isoflurane® (1.5-2% with 1L/min O_2) and positioned in the scanner in a prone position with the head placed into a stereotaxic dedicated head-holder. Radioligands were injected as bolus trough catheterized tail vein.
Time dependent activity distribution curves (TAC) were determinated of selected brain areas. Borderline of region of interest (ROI) were drawn in all coronal slices where the specific brain areas where seen TAC's were processed as percent of the injected activity in each ROI.
Binding potential (BP) was calculated by using the cerebellum as a reference tissue. Two months post lesion rats were deeply anesthetized (80 mg/kg of ketamine i.p.) and trans-cardially perfused with saline solution followed by 4% paraformaldehyde. Brains were sliced into 35 µm coronal sections, and investigated with anti-tyrosine hydroxylase. TH-stained striatal sections were examined in left and right SN to evaluate the homogeneity and extension of the 6-OHDA lesion. Counts of TH-positive cells in sham and 6-OHDA rats were compared by unpaired two-tailed *t*-test. Differences were considered statistically significative at $p<0.05$. Optical density was also evaluated using Image/J software.
Results: The [^{11}C]CFT-PET studies showed decreased dopamine transporter function. [^{11}C]CFT-BP was decreased compared to the intact left striatum (25.4±13.4% with a range of 15% to 49%). In the hippocampus area [^{11}C]CFT-BP was decreased (4.0±3.2%), and in the cortex BP was enhanced (2.6±5.9%) indicating an enhancement of ipsilateral cortical dopamine function.
The [^{11}C]RAC-BP showed D2 receptor up-regulation in the right striatum (2.4±3.1%), in the hippocampus (3.1±4.3%), and in the cortex (6.9±6.3%), compared to the intact left side.
Correspondingly, [^{11}C]MPEP-BP was enhanced in the right striatum (4.4±6.5%), in the hippocampus (6.3±4.3%), and in the cortex (10.2±11.4%).

Decrease of [^{11}C]CFT-BP in the right striatum showed also an exponential correlation to the AMPH-induced ipsilateral turns. The Net ipsilateral rotations is 35.32E(0.0679xΔ[^{11}C]CFT-BP)–35.32 (at 95% confidence level). A linear inverse correlation can be resolved between changes of mGluR5 and DAT function: Δf (mGluR5) = (1/k) Δf (DAT).
TH-immunostaining in the right striatum and substantia nigra pars compacta showed a significant degeneration of dopaminergic neurons compared to the intact side (*p=0.00248)* in all examined rats. Optical density of TH fibers in the striatum showed a significant difference between left and right lesioned side (*p=0.087*).
Conclusion: [^{11}C]CFT-PET at 4 weeks after 6-OHDA lesion, showed a decrease of BP in the right lesioned side compared to the intact left striatum.
Enhanced accumulation of [^{11}C]RAC is an indication of functional up-regulation of dopamine D2 receptors.
In the lesioned striatum [^{11}C]MPEP accumulation was increased indicating enhanced mGluR5 function.
These data demonstrate modulation of mGluR5 and dopaminergic function.
Modulation of glutamatergic and dopaminergic function might be an implication of Parkinson disease like degeneration.

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SECTION 5 – ***Registrants*** *(as of 5/12/06)*

Photograph by Don Eyles August 2003

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