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Acute Effects of Anticholinesterase Agents on Pupillary Function

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Ezio Giacobini

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			antichloinesterases; pupillary function; vision; DFP

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

Three main directions of our research has been pursued. ~~First, we have accumulated~~ new pharmacological evidence for a mechanism of ACh release related to a muscarinic auto-receptor present in the rat iris. Secondly, ~~we have continued our~~ study of drug effect on release of ACh, adding new groups of drugs. Finally, ~~we have studied~~ the effect of aging on pupillary function and ACh metabolism. These three lines of work have each produced novel and intriguing results which are summarized in the enclosed section. The results described in this report have been communicated at several notional and internationsl meetings. The abstracts of the communication are attached to the final report.

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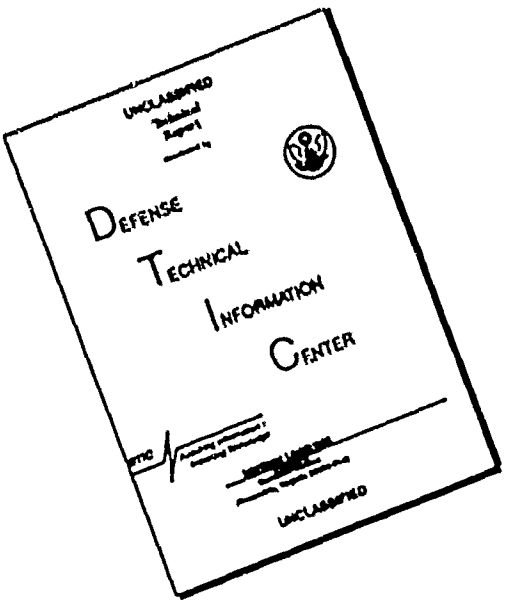

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CITATION OF PUBLISHED WORKS RECOGNIZING AFOSR

Mattio, T.G., J.S. Richardson and E. Giacobini, Effects of DFP on iridic metabolism and release of acetylcholine and on pupillary function in the rat. Neuropharmacology, 23(10):1207-1214, 1984.

Mattio, T.G., J.S. Richardson and E. Giacobini, Acute effects of cholinesterase inhibitors on uptake of choline in the rat iris. Neuropharmacology, 24(4):325-328, 1985.

Richardson, J.S., T.G. Mattio and E. Giacobini, Amitriptyline and imipramine inhibit the release of acetylcholine from parasympathetic nerve terminals in the rat iris. Canadian J. Physiol. Pharmacol., 62:857-859, 1984.

Giacobini, E., I. Mussini and T. Mattio, Aging of cholinergic synapses in the avian iris. In: Developmental Neuroscience: Physiological, Pharmacological and Clinical Aspects (F. Caciagli, E. Giacobini and R. Paoletti, eds.), Elsevier, New York, pp. 89-93, 1984.

Giacobini, E., T. Mattio and I. Mussini, Aging of cholinergic synapses in the avian iris. I. Biochemical studies. Neurobiology of Aging, 1986 (submitted).

ABSTRACTS - presented at 23 meetings (copies attached)

Dr. Ezio Giacobini
AFOSR Final Technical Report

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So. Ill. Univ. Sch. Med.
~~P.O. Box 3926~~
Springfield, IL 62708

PARTICIPANTS & INSTITUTIONS

Name T. Mattio
So. Illinois Univ. Sch. Med.
~~P.O. Box 3926~~
Springfield, IL 62708

Name I. Mussini
C.S. Biol. Fisiopat. Musc.
~~Ist. Patologia Generale~~
Univ. di Padova
35100 Padova ITALY

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AGING OF CHOLINERGIC SYNAPSES IN THE AVIAN IRIS.
Organizer: E. Giacobini
Participants: T. Mattio and I. Mussini

A hypothesis of aging of the cholinergic synapse has been proposed (Giacobini, Adv. Cell. Neurobiol., 3:173, 1983) which contemplates age-related changes in carrier-mediated mechanisms of uptake and release of the neurotransmitter and its precursor (choline) leading to "chemical denervation". Morphometric analysis of neuromuscular junctions in the chicken iris showed a significant reduction of the axonal junctional membrane at five years. A 50% decrease in the volume of vesicles per unit volume of the synapse was evident at three years. In addition, the 3-year tissue released significantly less ^{3}H-acetylcholine (^{3}H-ACh) than the 4-month tissue as determined by the area under the release curve. Also, the 3-year tissue showed a lower peak release of ^{3}H-ACh than the 4-month iris. The time needed for the 3-year tissue to reach its peak release was significantly longer than at 4-month and its rate of release was significantly slower. These neurochemical results correlate well with the morphological data which demonstrates that two important features for neurotransmitter release (vesicular volume and synaptic length) were decreased in the 3-year (or 5-year) old tissue. These results support the hypothesis that age-dependent decline in cholinergic transmission is related to modifications of presynaptic mechanisms of release and uptake of the neurotransmitter and its precursor.

[Supported by AFOSR Grants 81-9229 and 83-0051, Nowatski Eye Res. Fdn., E.F. Pearson Fdn. and Natl. Res. Council of Italy).

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PHARMACOLOGY OF MUSCARINIC RECEPTORS CONTROLLING ACH RELEASE IN THE RAT IRIS. T. Mattio*, E. Giacobini and V. Hoban*. Department of Pharmacology, Southern Illinois University School of Medicine, Springfield, IL 62708 USA

The release of acetylcholine (ACh), both in central and peripheral nerve tissues, seems to be modulated by a presynaptic muscarinic receptor. In the albino rat iris, which contains a dense cholinergic plexus, we have demonstrated the presence of a muscarinic autoreceptor. The electrically stimulated release of ACh (50 Hz, 20 mA, 5 ms biphasic square wave) was increased in the presence of muscarinic antagonists. Pirenzepine increased ACh release in a dose dependent manner from 10^{-3} M (by 90%) to 10^{-7} M (by 27%). Scopolamine also showed a dose-dependent increase in ACh release. At 10^{-4} M and 10^{-5} M scopolamine increased ACh release by 88% and 41%, respectively. Atropine increased ACh release from 10^{-3} M (by 70%) to 10^{-7} M (by 55%). Oxotremorine decreased the stimulated release of ACh at 10^{-3} M and 10^{-5} M. Both pirenzepine and atropine antagonized this effect. 2-Aminopyridine, 3-aminopyridine and 3,4-diaminopyridine were without effect on ACh release, however, 4-aminopyridine increased ACh release by 55% at 10^{-3} M. Hemicholinium (10^{-3} M) increased the stimulated overflow of ACh in the iris. These results demonstrate the effects of muscarinic antagonists and agonist on the release of ACh. This release is controlled by a presynaptic muscarinic receptor which when stimulated decreases ACh release and when blocked increase ACh release. In the iris the aminopyridines are not as effective as has been found at neuromuscular junctions. Hemicholinium which prevents the reuptake of choline after ACh hydrolysis shows a marked increase in ACh overflow after electrical stimulation. This overflow is most probably due to the effect on uptake and not to an effect on the muscarinic autoreceptor. The rat iris offers several advantages in studies of actions of drugs on muscarinic receptors and ACh release.

(Supported by grant AFOSR-83-0051 to E.G.)

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Satellite meeting of Intl. Soc. Neurochem., Iraklion, Crete, Greece
5/26-29/85

THE ISOLATED IRIS AS A MODEL OF AGING. Giacobini, E., Dept. Pharmacology, Southern Illinois University School of Medicine, Springfield, IL 62708 USA

The iris contains a dense plexus of cholinergic nerve terminals in addition to noradrenergic and peptidergic endings. These cholinergic nerve terminals are located at a distance from their cell bodies in the ciliary ganglion. As cell bodies in the ciliary ganglion are all cholinergics, from the point of view of innervation the iris is a much more homogeneous and readily accessible tissue than the CNS and offers several advantages in pharmacological studies of drug action on selective populations of terminals. The avian iris muscle which is a striated muscle has provided an experimental model for the study of various aspects of development and differentiation (Giacobini, E., IN: Developmental Neurobiology of the Autonomic Nervous System, ed. by P.M. Gootman, Humana Press, Inc., 1985), denervation (Mussini, I. et al., Neuroscience, 12(1):53-66, 1984) and aging (Giacobini, E., Adv. Cell. Neurobiol., 3:173-214, 1982). Similarly, the isolated iris of the rat has been extensively utilized to characterize mechanisms of synthesis and release of acetylcholine (ACh) as well as to define the action of drugs on these systems (Mattio, T. et al., Neuropharmacology, 23(1):1207-1214, 1984). Mechanisms of choline (Ch) uptake, ACh release, as well as ACh synthesis and turnover can all be studied in the same isolated iris and at the same time morphometric measurements can be performed on the same preparation at various ages. High affinity Ch uptake and ACh release are both affected at early stages of aging in the avian iris. These functional defects can be correlated to a decrease in vesicular volumes and junctional appositional areas in the same synapse (Giacobini, E. et al., IN: Developmental Neuroscience; Physiological, Pharmacological and Clinical Aspects, ed. by F. Caciagli et al., 1984). (Supported by grants from Air Force Office of Scientific Res.; Nowatski Eye Research Fund, and S.I.U. Central Research Fund.)

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TITLE RISE AND FALL OF THE CHOLINERGIC SYNAPSE

AUTHORS GIACOBINI, E., MUSSINI, I.* and MATTIO, T.

ADDRESS Dept. Pharmacol., Southern Illinois Univ. Sch. Med., Springfield, IL 62708 USA and * C.S. Biol. Fisiopat. Musc., Ist. Patologia Generale, Universita di Padova, 35100 Padova, Italy.

Based on the results of our studies on the ciliary ganglion iris preparation, a hypothesis of aging of the cholinergic synapse has been proposed (Giacobini, E., Adv. Cell. Neurobiol., 3:173, 1983). This hypothesis contemplates age-related changes in carrier-mediated mechanisms of uptake and release of the neurotransmitter and its precursor (choline) leading to "chemical denervation". Morphometric analysis of neuromuscular junctions in the iris showed a significant reduction of the axonal junctional membrane at five years. A 50% decrease in the volume of vesicles per unit volume of the synapse was evident at three years. Experiments were designed to determine the ability of the 3-year iris to undergo depletion-reloading-release of ^{3}H-acetylcholine (^{3}H-Ach). The 3-year tissue released significantly less ^{3}H-ACh than the 4-month tissue as determined by the area under the release curve (peak area). Also, the 3-year tissue showed a lower peak release of ^{3}H-ACh than the 4-month iris. The time needed for the 3-year tissue to reach its peak release was significantly longer than at 4-month and its rate of release was significantly slower. These neurochemical results correlate well with the morphological data which demonstrates that two important features for neurotransmitter release (vesicular volume and synaptic length) were decreased in the 3-year (or 5-year) old tissue. These results support the hypothesis that age-dependant decline in cholinergic transmission is related to modifications of presynaptic mechanisms of release and uptake of the neurotransmitter and its precursor. [Supported by AFOSR Grants 81-9229 and 83-0051, by grants from the Nowatski Eye Res. Fdn., E.F. Pearson Fdn. and Natl. Res. Council of Italy to the Unit for Muscle Biology (I. Mussini)].

SYMPOSIUM ON SYNAPTIC DEVELOPMENT
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THE NEUROMUSCULAR JUNCTION IN THE AVIAN IRIS: AN EXPERIMENTAL MODEL FOR STUDIES ON PERIPHERAL SYNAPSE PLASTICITY.

Isabella Mussini, Ezio Giacobini* and Thomas Mattio*
National Research Council Unit for Muscle Biology and Physiopathol . Istitute of General Pathology, University of Padova, Italy and * Department of Pharmacology Southern Illinois University, School of Medicine, Springfield, Ill.62708. USA.

The iris muscle fibers of the chick are innervated by nerve endings of " en grappe" type which are located in shallow depressions of the myofibers lacking secondary synaptic foldings. Early after hatching (a.h.) the nerve terminal arborization is formed by a few boutons grouped together or variably oblique across the muscle fiber. Starting two weeks the arrangement of the boutons becomes prevalently longitudinal. Their number increases continuously reaching a mean value of 15/neuromuscular junction (NMJ) at 4 months. A parallel increase occurs in the length of the synaptic area: in young adults (4 months) the diffuse "en grappe" type NMJ extends over a distance of more than 80 um on the muscle surface. Though a "mature" ultrastructural appearance is achieved since 2 weeks a.h., morphometric analyses reveal that evolutive changes are still occurring in the nerve endings. The axonal junctional membrane reaches a steady length 1 month a.h., while the synaptic vesicles volume increases up to 4 months. According to changes in neurochemical parameters (Giacobini, E., Adv. Cell. Neurobiol. 3:173, 1983), this period of continuous growth is followed by a period of synaptic regression. In old irises the NMJ shows a significant decrease in the boutons number as well as in the axonal junctional membrane and in the synaptic vesicles volume. This is already reduced by more than 50% at 3 years. The morphological results suggest a plasticity of the synapse in the avian iris. Its continuous remodelling from hatching to senescence is probably related to the increasing complexity of the myofiber architecture, at first, and then, to the progressive decline of the cholinergic mechanisms. (Supported by funds from Natl.Res.Council of Italy to the Unit for Muscle Biol.Physiopatol. and by AFOSR Grants 81-9229 and 83-0051, by grants from the Nowatski Eye Res.Fdn. and E.F. Pearson Fdn. to E.Giacobini).

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AGING OF CHOLINERGIC SYNAPSES IN THE AVIAN IRIS. E. Giacobini, T. Mattio* and E. Mussini*, Dept. Pharmacol., Southern Illinois Univ. Sch. Med., Springfield, IL 62708 USA and C.S. Biol. Fisiopat. Musc., Ist. Patologia Generale, Universita di Padova, 35100 Padova, Italy.

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[Supported by AFOSR Grants 81-9229 and 83-0051, by grants from the Nowatski Eye Res. Fdn., E.F. Pearson Fdn. and Natl. Res Council of Italy to the Unit for Muscle Biology (I. Mussini)].

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AGING OF CHOLINERGIC SYNAPSES IN THE AVIAN IRIS

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We have made use of the ciliary ganglion-iris preparation of the aging (1.5-9 yrs) chicken as a model of senescent peripheral cholinergic synapses. Neuromuscular junctions in the iris of aging chickens show early (1.5 yrs) morphological signs of damage such as, reduction and polymorphism of synaptic vesicles and increase of neurofilaments and mitochondria. Accumulations of cytoplasmic organelles and lysosomes are seen in the axoplasm of the nerve fiber. At later stages (5-9 yrs), the nerve ending is enveloped by Schwann cells infiltrating and filling the synaptic cleft. Quantitative morphometric changes in the ratio describing the relationship between volumes of terminals and volumes of synaptic vesicles show a progressive decrease in the volume occupied by synaptic vesicles. The ability of the cholinergic synapses to take up ^{3}H-choline and release the formed ^{3}H-acetylcholine (ACh) in response to high K^{+}-depolarization is impaired at 5 yrs resulting in a significant depletion of the ^{3}H-ACh releasable pool. These experiments seem to point out for the first time a selective functional defect in the cholinergic synapse during aging. (Supported by AFOSR Grant NL-144 and by Howatski Eye Research Fund to E.G.)

ADDRESS OF THE AUTHOR WHO WILL
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o Giacobini
ent of Pharmacology
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GIACOBINI, E., MUSSINI, I. and MATTIO, T.
"Aging of Cholinergic Synapses in the Avian Iris"
Department of Pharmacology, Southern Illinois University School of Medicine, Springfield, Illinois 62708

We have made use of the ciliary ganglion-iris preparation of the aging (1.5-9 yrs) chicken as a model of senescent peripheral cholinergic synapses. Neuromuscular junctions in the iris of aging chickens show early (1.5 yrs) morphological signs of damage such as, reduction and polymorphism of synaptic vesicles and increase of neurofilaments and mitochondria. Accumulations of cytoplasmic organelles and lysosomes are seen in the axoplasm of the nerve fiber. At later stages (5-9 yrs), the nerve ending is enveloped by Schwann cells infiltrating and filling the synaptic cleft. Quantitative morphometric changes in the ratio describing the relationship between volumes of terminals and volumes of synaptic vesicles show a progressive decrease in the volume occupied by synaptic vesicles. The ability of the cholinergic synapses to take up ^{3}H-choline and release the formed ^{3}H-acetylcholine (ACh) in response to high K^{+}-depolarization is impaired at 5 yrs resulting in a significant depletion of the ^{3}H-ACh releasable pool. These experiments seem to point out for the first time a selective functional defect in the cholinergic synapse during aging. (Supported by AFOSR Grant NL-144 and by Nowatski Eye Research Fund to E.G.)

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J.S. RICHARDSON, T.G. HATTIO* and E. GIACOBINI*
Pharmacology and Psychiatry, University of Saskatchewan, Saskatoon; and Pharmacology, Southern Illinois University, Springfield.

The release of acetylcholine from rat iris is inhibited by a presynaptic muscarinic autoreceptor.

The release of acetylcholine (ACh), both in central and peripheral nerve tissues, seems to be modulated by a presynaptic muscarinic autoreceptor that is responsive to exogeneous muscarinic agents. In the albino rat iris, we have demonstrated the presence of a muscarinic autoreceptor and elucidated its role after acetylcholinesterase (AChE) inhibition by diisopropylfluorophosphate (DFP). The electrically stimulated release of ACh (50 Hz, 20 mA, 5 ms, biphasic square wave) from the rat iris is temperature, Na^+ and Ca^{++} dependent. The addition of 10^{-4} M and 10^{-5} M scopolamine in the superfusion buffer, increased ACh release by 190% and 150%, respectively. In the presence of 10^{-4}, 10^{-5} and 10^{-6} M DFP in the buffer, ACh release was significantly decreased and AChE activity was inhibited by more than 90%. The inhibition of ACh release was totally reversed by scopolamine (10^{-6} M) indicating the involvement of a muscarinic autoreceptor. The accumulation of ACh in the synaptic cleft after DFP, seems to activate the muscarinic autoreceptor and to produce a feedback inhibition of additional ACh release. Since the isolated iris of the rat does not contain nerve cell bodies, this muscarinic autoreceptor would appear to be on presynaptic nerve terminals.
(Supported by USAFOSR).

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A SPECIFIC FUNCTIONAL DEFECT OF PERIPHERAL CHOLINERGIC SYNAPSES DURING AGING

Mussini, I.*, Mattio, T.G.*, Giacobini, E. and Richardson, J.S.
Dept. Pharmacol., So. Ill. Univ. Sch. Med., Springfield, IL 62708 USA

Neuromuscular junctions in the iris of aging (2-4 yrs) chicken show polymorphic signs of degeneration such as reduction and polymorphism of synaptic vesicles, increase of neurofilaments and mitochondria. Accumulations of cytoplasmic organelles and lysosomes are seen in the axoplasm of the nerve fiber. At later stages (5-9 yrs) the nerve ending is enveloped by Schwann cells infiltrating and partially filling the synaptic cleft. Quantitative changes in the ratio describing the relation V_{vv}/V_{vs} between volumes of terminals (V_{vv}=synaptic bouton volume fraction) and volumes of synaptic vesicles (V_{vv} = synaptic vesicles volume fraction) show a decrease from .4 to .2 between 4 month and 9 years. This indicates a progressive decrease in the volume occupied by synaptic vesicles and a possible functional deficit. We examined the ability of cholinergic synapses in the iris at various ages to take up the precursor ^{3}H-choline (Ch) and release the formed ^{3}H-acetylcholine (ACh) in response to high K^+ (115 mM) depolarization. We have observed that following release of ACh, exocytosis clearly prevails on endocytosis and a nearly total depletion of vesicles is present. Under acute conditions of stimulated release, aging terminals are still capable of an adequate depletion of ACh. However, under more strenuous conditions of multiple kinds of loading-reloading and release both Ch and phosphorylcholine are significantly depleted. These experiments point out for the first time a specific functional defect in the cholinergic synapse during aging.
(Supported by AFOSR Grants 81-9229; 83-0051, Nowatsky Eye Research Foundation and E.F. Pearson Foundation to E.G.)

A SPECIFIC FUNCTIONAL DEFECT OF PERIPHERAL CHOLINERGIC SYNAPSES DURING AGING

Giacobini, Ezio
So. Ill. Univ. Sch. Med., P.O. Box 3926, Springfield, IL 62708
217/785-2185

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THE ROLE OF A PRESYNAPTIC MUSCARINIC AUTORECEPTOR IN ACETYLCHOLINE RELEASE FROM RAT IRIS.

T.G. Mattio, E. Giacobini and J.S. Richardson. Department of Pharmacology, So. Ill. Univ. Sch. Med., Springfield, IL 62708 USA

In the albino rat iris we have demonstrated the presence of a muscarinic autoreceptor and elucidated its role after acetylcholinesterase (AChE) inhibition by diisopropylfluorophosphate (DFP). The electrically stimulated release of ACh (50 Hz, 20 mA, 5 ms square wave) in the rat iris was shown to be temperature, Na^+ and Ca^{++} dependent. Addition of 10^{-4}M and 10^{-5}M scopolamine in the superfusion buffer increased ACh release by 190 and 150%, respectively. The addition of 10^{-4}, 10^{-5} and 10^{-6}M DFP in the buffer sigificantly decreased the release of ACh and inhibited AChE activity by more than 90%. This inhibition of ACh release was totally reversed by scopolamine (10^{-6}M) indicating the involvement of a muscarinic autoreceptor. The accumulation of ACh in the synaptic cleft after DFP, results in muscarinic activation and a consequent feedback inhibition of ACh release. (Supported by Grant AFOSR-83-0051)

To be completed by presenting author:

Giacobini (Last name) E. (From) x Gac Iraulin

Address (for correspondence) Southern Illinois University School of Medicine;

P.O. Box 3926 Town Springfield, Illinois

Country 62708 USA Day-time telephone no. 217 Telex 785-2185

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Dynamics of Cholinergic Function Symp.
-Oglebay, W.Va. Oct. 30 thru Nov. 4, 1983-

AGING OF CHOLINERGIC SYNAPSES: FICTION OR REALITY?

Ezio Giacobini, Southern Illinois University School of Medicine, P.O. Box 3926, Springfield, Illinois 62708 USA

Combined neuropathological and biochemical evidence suggests that a primary degeneration of cholinergic axons projecting to the cortex, and a secondary reduction in number of cholinergic neurons may occur in specific subcortical nuclei (basal forebrain), during pathological aging in humans. The factors inducing such a selective loss in cholinergic function are not known. Quantitative analysis of neuronal population density and biochemistry show that neurons and synapses other than cholinergic may also be affected by the same aging process. Variable data have been reported with regard to the relationship between neuronal losses and cholinergic changes and to the magnitude of the reductions. In order to firmly establish a cholinergic hypothesis of senile dementia, we will first discuss relevant questions such as:

1. Are biochemical changes selectively localized to certain brain nuclei or are they distributed to all cholinergic synapses in the CNS?

2. Are changes related to the normal cerebral aging process, i.e. are they mechanisms of enzymatic adaptation or are they specific for senile dementia? How important is the age range of the controls? How important is the severity of the disease?

3. Which is the primary target for the chemical damage and the neuronal degeneration? Does the aging process involve both pre- and postsynaptic structures? Does the process involve cholinergic terminals firstly and perikarya secondly?

4. Are cholinergic neurons in the PNS and CNS equally affected?

5. Is there a relationship between the reduction in cholinergic cortical innervation and the pathogenesis of plaques?

In the second part of our presentation, a model of peripheral cholinergic aging, the iris, will be introduced. This model allows us to study major cholinergic parameters together with pupillary function. In humans, pupillary size constitutes a predictable marker of age-related pupillary function and senile miosis seems to contribute a reliable sign of aging of the cholinergic innervation of the eye. Observations will be presented which support the view that terminals of cholinergic neurons, particularly in the PNS, represents more vulnerable targets of aging process than cell bodies. Recent attempts to characterize the cholinergic damage to synaptic membrane function will be discussed.

Supported in part by AFOSR grant #83-0051 and Nowatski Eye Fund.

Dynamics of Cholinergic Function Symp.
Oct. 30 thru Nov. 4, 1983
Oglebay, W.Va.

EFFECTS OF DFP ON ACETYLCHOLINE METABOLISM AND RELEASE AND PUPILLARY FUNCTION IN THE RAT.

T.G. Mattio, J.S. Richardson and E. Giacobini, Southern Illinois University School of Medicine, P.O. Box 3926, Springfield, Illinois 62708 USA

The effects of acute topical administration of diisopropylphosphorufluoridate (DFP) on cholinergic biochemistry and ACh release were determined and correlated to pupillary function in the rat. DFP (5 ug) reduced acetylcholinesterase (AChE) activity to 36% at 1 min and to 8% after 5 min and remained decreased for up to 6 hrs. Pupillary area was normal at 1 min and by 3.5 to 4 min complete miosis occurred and no light reflex could be elicited for up to 6 hrs. Acetylcholine (ACh) levels were increased 34% at 1 min and by 5 min showed a 54% increase. This increase remained stable for 120 min after which it decreased to 28% at 6 hrs. Choline levels were decreased 22% at 5 min but recovered by 15 min and remained at control levels through all time points studied. The presence of a presynaptic-muscarinic receptor was demonstrated in the iris. The role of this receptor in inhibiting ACh release in the presence of DFP was also determined. DFP shows an inhibitory effect on ACh release which was blocked by scopolamine suggesting that it is mediated through a muscarinic receptor. The rat iris proved to be a good model for studying of AChE agents since biochemical findings are easily correlated to physiological effects on the pupil.

Supported in part by AFOSR grant #83-0051 and Nowatski Eye Fund.

Review of Air Force Sponsored
Basic Research in Biomed. Sci.
Irvine, CA, 1983

EFFECTS OF DFP ON THE RELEASE OF ACETYLCHOLINE: ROLE OF A PRESYNAPTIC MUSCARINIC RECEPTOR.

Ezio Giacobini and Thomas Mattio
Southern Illinois University School of Medicine
P.O. Box 3926
Springfield, IL 62708 USA

The albino rat iris contains a dense plexus of cholinergic nerve terminals whose cell bodies are located in the ciliary ganglion. This structure is a good model for the study of cholinergic function due to its homogeneity. Following characterization of the high affinity choline (Ch) uptake system, the electrically stimulated release of acetylcholine (ACh) was studied. ACh pools were labelled by uptake of ^{3}H-Ch for 10 min (1 uM). The irises were then rinsed and put in a release chamber modified from Potashner (1978). After a 10 min wash the tissue was stimulated by a 50 Hz, 20 mA, 5 ms square wave for 1 min while being superfused by oxygenated Elliots B buffer. The perfusate was collected into scintillation vials, after which 2 ml of cocktail was added and the radioactivity released was determined by liquid scintillation counting. The tritium released was expressed as a percentage of the total tritium present in the tissue at the time of release. We demonstrated that the tritium released was 95-100% ^{3}H-ACh. The release of ACh was found to be Na^{+}, Ca^{++} and temperature dependent. The addition of scopolamine (10^{-9}-10^{-3}M) increased the release of ACh up to 190% while, the addition of choline (10^{-3}M) decreased the release of ACh. This decrease in release was reversed by the addition of 10^{-6}M scopolamine, demonstrating the presence of a presynaptic muscarinic receptor, as has been described in other tissues. The addition of the irreversible cholinesterase inhibitor diisopropyl fluorophosphate (DFP) (10^{-4}, 10^{-5}, 10^{-6}M) into the superfusion buffer resulted in a significant decrease in the stimulated release of ACh with esterase activity inhibited by more than 90%. DFP at 10^{-7} and 10^{-8}M inhibited esterase activity by 60 and 40%, respectively, but had no effect on the release of ACh. This decrease on the release of ACh was found to be totally reversible with the addition of 10^{-6}M scopolamine into the buffer. The decrease in release of ACh by DFP can be attributed to the accumulation of ACh in the synaptic cleft and consequently its agonistic effect on the presynaptic muscarinic receptor therefore decreasing release of ACh. The muscarinic antagonist scopolamine, at a concentration where itself does not affect release, was able to totally reverse this effect. This data demonstrates the presence of a presynaptic muscarinic receptor on cholinergic terminals in the rat iris and suggests a mechanism by which DFP decreases the release of ACh from cholinergic terminals. (Supported by Air Force grant #81-0229 and 83-0051.)

283.11 UTILIZATION OF CHOLINE TRANSPORTED BY SODIUM-DEPENDENT, HIGH-AFFINITY CHOLINE CARRIERS FOR ACETYLCHOLINE SYNTHESIS: COMPARISON OF RAT AND GUINEA-PIG FOREBRAIN SYNAPTOSOMES. B. J. Pyliotis, T. J. Carlton* and [illegible]*, Department of Pharmacology, University of Western Ontario, London, Ont., Canada. N6A 5C1.

Controversy exists over the role of choline transported into synaptosomes by sodium-dependent, high-affinity carriers in the synthesis of acetylcholine (ACh). Some of the observed differences could be due to species variability in the parameters measured and the mechanisms involved. Largely, studies have involved the use of rat and guinea-pig brain, species which are known to differ with respect to the molecular forms (pI) of choline acetyltransferase. In the present report, we compare the kinetics of choline transport and the conversion of ^{3}H-choline to ^{3}H-ACh in resting and K$^+$-depolarized synaptosomes prepared from rat and guinea-pig forebrain. Analysis of choline transport over the ^{3}H-choline concentration range 0.1 to 100 uM revealed typical biphasic kinetics with apparent Michaelis constants, K_m, of 2 and 109 uM and V_{max} of 14.3 and 304.6 pmol/mg protein/4 min for rat forebrain synaptosomes; kinetics of transport for choline into synaptosomes prepared from guinea-pig brain did not differ significantly. Following incubation of anticholinesterase-treated synaptosomes with 1 uM choline, conversion of ^{3}H-choline to ^{3}H-ACh was quantitated by preparative HPLC separation of the choline metabolites and liquid scintillation spectrometry. Velocity for choline uptake in rat brain synaptosomes (^{3}H-choline) (1 uM) was 20.2 pmol/mg protein/4 min of which 84% was transported by sodium-dependent processes; in comparison, choline transport velocity into guinea-pig synaptosomes was 18.7 pmol/mg protein/4 min, 79% of which was analysed in sodium-free (lithium substituted) medium. In regular K$^+$ (5 mM) medium, it was observed that 72% of ^{3}H-choline transported into rat brain synaptosomes by sodium-dependent processes was acetylated, while in guinea-pig only 57% of such choline was metabolized to ACh ($P<0.02$). Following K$^+$-induced depolarization, 74% of ^{3}H-choline transported by sodium-dependent mechanisms in rat synaptosomes was acetylated, however, in guinea-pig synaptosomes this parameter was significantly increased to 82%. The net increase in choline uptake following K$^+$-depolarization was 141.5% and 133% in rat and guinea-pig, respectively, relative to resting transport. Thus, in guinea-pig synaptosomes the percentage of choline transported via sodium-dependent carriers directed to ACh synthesis is increased by depolarization of the nerve terminal. These results suggest that there may be differences underlying the coupling of choline transport to the enzymatic acetylation reaction and the utilization of exogenous choline in the synthesis of ACh in synaptosomes from brain of rat and guinea-pig.
(Supported by the Medical Research Council of Canada).

283.12 EFFECTS OF DFP ON THE RELEASE OF ACETYLCHOLINE: ROLE OF A PRESYNAPTIC MUSCARINIC RECEPTOR. T.G. Hollis*, E. Giacobini, J.S. Richardson*, (SPON: C. Su). Dept. Pharmacology, Southern Illinois University School of Medicine, Springfield, IL 62708

The albino rat iris contains a dense plexus of cholinergic nerve terminals whose cell bodies are located in the ciliary ganglion. This structure is a good model for the study of cholinergic function due to its homogeneity. Following characterization of the high affinity choline (Ch) uptake system, the electrically stimulated release of acetylcholine (ACh) was studied. ACh pools were labelled by uptake of ^{3}H-Ch for 10 min (1 uM). The irises were then rinsed and put in a release chamber modified from Patterson (1978). After a 10 min wash the tissue was stimulated by a 50 Hz, 20 mA, 5 ms square wave for 1 min while being superfused by oxygenated Tillotts B buffer. The perfusate was collected into scintillation vials, after which 2 ml of cocktail was added and the radioactivity released was determined by liquid scintillation counting. The tritium released was expressed as a percentage of the total tritium present in the tissue at the time of release. It demonstrated that the tritium released was 95-100% ^{3}H-ACh. The release of ACh was found to be Na$^+$, Ca^{++} and temperature dependent. The addition of scopolamine (10^{-4}-10^{-5}M) increased the release of ACh up to 190% while, the addition of choline (10^{-3}M) decreased the release of ACh. This decrease in release was reversed by the addition of 10^{-6}M scopolamine, demonstrating the presence of a presynaptic muscarinic receptor, as has been described in other tissues. The addition of the irreversible cholinesterase inhibitor diisopropyl fluorophosphate (DFP) (10^{-4}, 10^{-5}, 10^{-6}M) into the superfusion buffer resulted in a significant decrease in the stimulated release of ACh with esterase activity inhibited by more than 90%. DFP at 10^{-7} and 10^{-8}M inhibited esterase activity by 60 and 45%, respectively, but had no effect on the release of ACh. This decrease in the release of ACh was found to be totally reversible with the addition of 10^{-6}M scopolamine into the buffer. The decrease in release of ACh by DFP can be attributed to the accumulation of ACh in the synaptic cleft and consequently its agonistic effect on the presynaptic muscarinic receptor therefore decreasing release of ACh. The muscarinic antagonist scopolamine, at a concentration where itself does not affect release, was able to totally reverse this effect. This data demonstrates the presence of a presynaptic muscarinic receptor on cholinergic terminals in the rat iris and suggests a mechanism by which DFP decreases the release of ACh from cholinergic terminals. (Supported by Air Force grant #81-0229 and 83-0051.)

283.13 SECRETION OF ^{3}H-ACETYLCHOLINE FROM GUINEA-PIG ILEUM MYENTERIC PLEXUS IS ENHANCED BY INHIBITORS OF PHOSPHODIESTERASE. P. Ahlquist* and L. Sellstrom*, (SPON: P. Greengard). Division of Experimental Medicine, National Defence Research Institute, S-901 32 Umeå, Sweden.

The secretion of acetylcholine (ACh) is regulated by presynaptic muscarinic feedback inhibition. The possible involvement of endogenous cyclic nucleotides in this control was investigated using two inhibitors of phosphodiesterase. The ACh stores of the cholinergic nerves of the myenteric plexus of the guinea-pig ileum longitudinal muscle preparation were labelled with ^{3}H-choline. The preparation was mounted in an organ chamber and superfused with Tyrode solution containing hemicholinium-3 (10^{-5} M) and eserine (10^{-5} M). Stimulation was with trains of 150 shocks (0.5 ms, 120 V) at a low frequency (0.5 Hz). The results are expressed as the evoked fractional secretion of total ^{3}H-ACh. Addition of 3-isobutyl-1-methylxanthine (IBMX, 2.25 mM) enhanced the evoked secretion of ^{3}H-ACh by 99 ± 28 % (n=6, $p<0.001$). From the effects of IBMX (1-5 mM), no/1 the concentration yielding half-maximal enhancement (K_m) was determined to be 2.0 mM. The maximal increase over the control level at infinitely high concentration of IBMX (V_{max}) was estimated to be 190 %. Furthermore, the effects of IBMX (1 or 2 mM) were not altered by atropine (10^{-7} M). A structurally different inhibitor of phosphodiesterase, SQ 20,006, also slightly enhanced the ^{3}H-ACh secretion but within a very narrow concentration range. The secretion was enhanced by 40-110% by SQ 20,006 (0.3-0.5 mM). Above this range the secretion was enhanced drastically, about 10-fold, and was probably not related to the inhibition of phosphodiesterase. The results suggest that endogenous cyclic nucleotides are not involved in muscarinic "autoinhibition" of ^{3}H-ACh secretion in guinea-pig ileum myenteric plexus. However, it is conceivable that adenosine 3',5'-cyclic monophosphate may be involved in the enhancement of evoked ^{3}H-ACh secretion caused by activation of other receptors.

283.14 ACTIVATION OF ACETYLCHOLINE SYNTHESIS IN THE ABSENCE OF RELEASE. DEPENDENCE ON SODIUM, CALCIUM AND THE SODIUM PUMP. R.J. Birks, Department of Physiology, McGill University, Montreal, Canada H3G 1Y6.

Following a 15 min inhibition of the sodium pump in the cat superior cervical ganglion by perfusion with K-free Locke solution, a 10 min recovery in normal Locke produced a 51% increase in acetylcholine stores. The increase in stores occurred without increase in acetylcholine release. Thus this procedure of pump inhibition followed by recovery selectively activates acetylcholine synthesis. The increase in stores, which occurred entirely during the 10 min recovery period in which the sodium pump was reactivated, represents a rate of synthesis of acetylcholine of 5.1% of stores per min: equal to the maximum rate that can be achieved during high frequency preganglionic nerve stimulation. The increase was not affected by substituting isethionate for chloride in the perfusion fluids. It was prevented by reducing sodium to 25 mM in the K-free Locke and also prevented by omitting calcium from the perfusion fluids. It is concluded that the selective activation of acetylcholine synthesis following the pause in sodium pumping was a direct result of an increased sodium pump rate and an increase in internal calcium in the nerve terminals. It is proposed that similar ionic events produced by repetitive nerve impulses likewise activate acetylcholine synthesis independently of release of transmitter or depletion of stores.

TEMPERATURE ACCLIMATION MODIFIES THE CHLORIDE CONDUCTANCE OF GREEN SUNFISH MUSCLE FIBERS. M. G. Klein* (SPONSOR: C. L. Prosser). Department of Physiology and Biophysics, University of Illinois, Urbana, IL 61801

The passive electrical properties of skeletal muscle fibers from Green sunfish (Lepomis cyanellus) have been determined from cable analyses and rapid ion-substitution experiments. In sunfish acclimated to 25°C the resting chloride ion conductance, gCl, is larger than the potassium conductance, gK. Mean (±SE) values are 555 ±68 $\mu S/cm^2$ for gCl and 92 ±12 $\mu S/cm^2$ for gK (measured at T = 25°C, N = 7 fibers). Membrane capacitance, C_m, is 5.5 ±0.3 $\mu F/cm^2$. In sunfish acclimated to 7°C gCl is significantly reduced to 75 ±9 $\mu S/cm^2$ while gK is 65 ±6 μ/cm^2. C_2 is reduced to 3.9 ±0.5 $\mu F/cm^2$ (T = 7°C, N = 7). The Q_{10} of acclimation is 3.0 for gCl and 1.1 for gK. In both 25°- and 7°-acclimated sunfish the acute effect of temperature exhibited Q_{10} of 1.7 for gCl and 1.2 for gK over 5 to 30°C. Temperature acclimation appears to involve a reorganization of the chloride conductance pathway. Evidence is: i) The change in gCl occurs over a time course of 9-14 days. Changes in C_m develop in 5-9 days. ii) Membrane selectivity sequences to foreign anions are not the same in 25°- and 7°-acclimated sunfish. iii) Current-voltage relations measured with the three-microelectrode method show constant field rectification in 25°-sunfish, but are linear in 7°-sunfish. The fall in gCl in the cold appears to increase membrane excitability by reducing the magnitude of the passive shunt. (Supported by NSF PCM 79-14186).

EFFECT OF DFP ON ACETYLCHOLINE METABOLISM IN THE RAT IRIS. T. G. Mattio, E. Giacobini and J. S. Richardson. Department of Pharmacology, Southern Illinois University School of Medicine, Springfield, IL 62708.

The iris contains cholinergic nerve endings whose cell bodies are located in the ciliary ganglion. This makes this structure a good model of nerve terminal function free from contamination by cell body and glia effects. Following the characterization of the uptake system for choline (Ch) and the release of acetylcholine (ACh) in the isolated rat iris we have studied, the effect of the increase in ACh concentration following local administration of the irreversible cholinesterase inhibitor diisopropyl fluorophosphate (DFP). At the various times after the topical administration of 0.1% DFP in sesame oil onto the corneal surface, the rats were sacrificed and the irises were removed. Pupil diameter was measured, ACh as well as Ch levels were determined and acetylcholinesterase (AChE) activity measured in segments of the same iris. One minute after DFP, no changes were found in pupil diameter and ACh levels, but AChE activity was decreased by 65%. At 5 minutes, pupil diameter was reduced by 60% (and remained at this level for the duration of the experiment), Ch by 30%, AChE by 92%, and ACh was increased by 38%. At 15 minutes ACh was increased by 28%, and Ch was still reduced (10%) but continued to recover reaching control levels at 60 minutes. Acetylcholine levels were still increased at 60 and 120 minutes. AChE activity was still inhibited 86% and 74% at 60 and 120 minutes, respectively. Our results show that in peripheral cholinergic terminals, in spite of the continual inhibition of AChE activity and the functional pupillary paralysis following a single exposure to antiChE agents, ACh and Ch tend to return toward normal levels. (Supported by Grant AFOSR-81-0229 to E.G.)

A

ENHANCEMENT BY GM_1 GANGLIOSIDE TREATMENT OF ACETYLCHOLINESTERASE AND CHOLINE ACETYLTRANSFERASE RESPONSE IN RAT HIPPOCAMPUS FOLLOWING LESION OF THE ENTORHINAL CORTEX

Barbara Oderfeld-Nowak, Maria Jezierska*, Jolanta Ulas*, Katarzyna Mitros* and Małgorzata Skup*, Nencki Institute of Experimental Biology, Polish Academy of Sciences, 3 Pasteur Street, 02093 Warsaw, Poland.

The reinnervation response of cholinergic fibres of the hippocampal formation to ablation of the entorhinal cortex is well known. This response can be further potentiated by the administration of GM_1 monosialoganglioside. The entorhinal cortex was removed unilaterally by aspiration and the rats were allowed to survive for 21 days. The rats were daily injected with buffer or with GM_1 ganglioside (5 and 30 mg/kg i.m. respectively), purchased from FIDIA Res. Labs, Italy. The biochemical analyses were performed on the dorsal parts of the ipsilateral hippocampus (the contralateral part was used as control) taken in toto or on microdissected fascia dentata, regio superior and regio inferior. In animals treated with GM_1 there was a dose-dependent increase of CAT activity. 20 and 35% increase with 5 and 30 mg/kg when compared with the contralateral unlesioned side. The same treatment did not cause any significant change of GAD activity indicating the selectivity of the ganglioside effect on this model lesion.

B

EFFECT OF DFP ON ACETYLCHOLINE METABOLISM IN THE RAT IRIS.

Rettig, T.G.*, Gioggini, E. and J.S. Richardson*. Dept. Pharm., Southern Ill. Univ. School of Medicine, Springfield, IL 62708

The iris contains cholinergic nerve endings whose cell bodies are located in the ciliary ganglion. This makes this structure a good model of nerve terminal function free from contamination by cell body and glia effects. Following the characterization of the uptake system for choline (Ch) and the release of acetylcholine (ACh) in the isolated rat iris we have studied, the effect of the increase in ACh concentration following local administration of the irreversible cholinesterase inhibitor diisopropyl fluorophosphonate (DFP). At various times after the topical administration of 0.1% DFP in sesame oil onto the corneal surface, the rats were sacrificed and the irises were removed. Pupil diameter was measured, ACh as well as Ch levels were determined and acetylcholinesterase (AChE) activity measured in segments of the same iris. One minute after DFP, no changes were found in pupil diameter and ACh levels, but AChE activity was decreased by 65%. At 5 minutes, pupil diameter was reduced by 60% (and remained at this level for the duration of the experiment), Ch by 30%, AChE by 92%, and ACh was increased by 38%. At 15 minutes ACh was increased by 28%, and Ch was still reduced (10%) but continued to recover reaching control levels at 60 minutes. Acetylcholine levels were still increased at 60 and 120 minutes. AChE activity was still inhibited 86% and 74% at 60 and 120 minutes, respectively. Our results show that in peripheral cholinergic terminals, in spite of the continual inhibition of AChE activity and the functional pupillary paralysis following a single exposure to antiChE agents, ACh and Ch tend to return toward normal levels. (Supported by GRANT AFOSR-81-0229 to E.G.)

C

CHOLINE ACETYLTRANSFERASE CONTENTS IN SINGLE SPINAL MOTOR NEURONS FROM SEVEN SPECIES OF VERTEBRATES. Takashi Kato and Yoshiya I. Murashima.* Dept.,Biochem.,Inst.Brain Res., Univ.Tokyo Faculty.Med.,Tokyo 113, Japan.

Single cell bodies (0.25-5.75 ng dry weight) of motor neurons were isolated from freeze-dried sections of fresh spinal cords of vertebrates as shown in the Table below. Human samples (0.95-3.42 ng) were also isolated from spinal cords obtained at autopsy. Choline acetyltransferase activities of these single neurons were determined by measuring acetyl-CoA formation in the reverse reaction by use of an enzymatic amplification reaction, CoA cycling. Rat neurons had the highest activity and the cold-blooded animals showed about one-tenth of the activities of the warm-blooded animals. The specific activities on a dry weight basis were widely distributed among individual neurons from each species (see S.D. in Table). Although human neurons were obtained under different morbid and postmortem conditions, their activities were very low and of the similar order of magnitude as those of neurons from cold-blooded animals: 1) male, 70 yrs: 48.4±46.5 (15) [4 h delay to autopsy, lung cancer]; 2) female, 43 yrs: 36.2±30.6 (11) [11 h ,uterus cancer]; 3) female, 11 yrs: 23.2±18.5 (13) [3.5 h, cardiovascular malformation]. In a model experiment, the enzyme was degenerated 50 % in mouse brains 11 h after death. Thus, the low activities are thought to be one of the characteristics of human neurons.

	[mol./s per cell /h ± S.D.]		
Cat	221	± 133	(15)[a]
Rabbit	152	± 83	(20)
Rat	273	± 164	(20)
Hen	161	± 101	(16)
Bullfrog	35.9	± 20.9	(15)
Yellowtail[b]	20.5	± 17.3	(16)

[a] Numbers of samples assayed in parentheses; [b] S.quinqueradiata

D

QUANTITATION OF ACETYLCHOLINE BY CHEMILUMINESCENCE. APPLICATION TO RELEASE FROM RAT HEMIDIAPHRAGM.

Jonas Hagglad*, Mikael Eriksson* and Edith Hellbronn Unit of Neurochemistry and Neurotoxicology, University of Stockholm, S-172 46 Sundbyberg, Sweden.

The chemiluminescence method for acetylcholine (ACh) quantitation according to Israel and Lesbats was modified to fit analysis of amounts of ACh released from the hemidiaphragm of the rat. Oxidants were not used. Instead a purification step was introduced, as mammalian tissues release substances that quench the light reaction. ACh was precipitated with potassium periodide (KI_3), the precipitate was dissolved in ether. ACh was extracted from the ether by 10^{-4} M HCl. The aqueous phase was assayed for ACh by chemiluminescence. Tritiated ACh was used as internal standard. The experiments were performed in the presence of 1 µM TTX with sarin as an anticholinesterase. Release of ACh from hemidiaphragms of the rat, quantitated by the reported method, compares well with values found by others (basal, 3.5 mM K^+ : 0.5 ± 0.08 pmoles/min x hemidiaphragm and evoked, 50 mM K^+ : 1.9 ± 0.23 pmoles/min x hemidiaphragm).

This work was supported by the Swedish Medical Research Council, B83-13X-03907-11, and the Swedish Council for planning and cooperation of research.

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fects of drugs on uptake and release in rat iris parasympathetic neurons.
Richardson, J.S., Mattio*, T.G., and Giacobini, E.
U Conn Biolch Sci, Storrs CT 06268, U Sask Pharmacol Saskatoon S7N 0W0

The isolated iris of the rat accumulates choline (Ch) by high finity (K_D = 6.6 nM) and low affinity (K_D = 100 nM) processes. The take of 1 μM Ch at 37°C is inhibited in a dose-dependent manner by cholinium (IC_{50} is 70 μM). Hemicholinium (IC_{50} is 1 nM) and by substitution of equi-molar lithium for the sodium in the buffer (IC_{50} when lithium has placed 60% of the sodium). At 0°C, Ch uptake is reduced by over 85% and at 0°C uptake is not sensitive to hemicholinium. This suggests that a high affinity process is active transport into cholinergic nerve terminals while the low affinity process may be diffusion into other tissues. The uptake of 1 μM Ch at 37° is inhibited by 1 mM ouabain and is reduced in a dose dependent manner by the irreversible anticholinesterase diisopropylfluorophosphate (DFP) and by physostigmine, two anticholinesterase with different properties. Hexamethonium, an anticholinesterase lacking agonist activity, has a biphasic effect on Ch uptake being increased by 1 and 10 nM concentrations but not affected by .1 or 1000 nM concentrations. The release of labeled acetylcholine (ACh) induced by electrical stimulation of the iris, increases as stimulation frequency is increased up to 5 Hz. In the absence of an anticholinesterase inhibitor, the radioactivity in the perfusate was about 80% ACh. The release of ACh evoked by electrical stimulation (2 ms, 10 Hz, 50 mA, 35°C) was reduced by cholinergic, beta, and the norepinephrine uptake blockers imipramine and amitriptyline. ACh release was increased in a dose-dependent way by scopolamine. These results are consistent with the existance on the parasympathetic nerve terminal of presynaptic muscarinic receptors and muscarinic autoreceptors that inhibit the release of ACh.
Supported in part by grant NS18958-01-1209 to Dr. Giacobini.

114.

MONOCLONAL ANTIBODIES TO CHOLINE ACETYLTRANSFERASE FROM RAT BRAIN
Crawford, G.*, Salvaterra, P.M.
City of Hope Research Institute, Duarte, CA 91010

We have recently described the production and partial characterization of five monoclonal antibodies reacting with rat brain choline acetyltransferase. Antibodies purified from ascites fluids were found to recognize a cluster of determinants restricted to a small portion of the enzyme surface. (Crawford et al, PNAS in press). One of the antibodies recognizes a determinant not destroyed by glutaraldehyde fixation and is presently being applied in a number of immunocytochemical investigations of choline acetyltransferase distribution in rat brain. Titration curves of the antibodies have been analyzed by Scatchard's method. Each antibody displays a single affinity for ChAT in the range of K_{as} 10^6 to 10^9 M^{-1}. Cross reaction studies indicate that several antibodies react with enzyme present in other rodent brains, such as mouse and guinea pig, primate brain enzyme from baboon and human as well as enzyme from human placenta. One of the antibodies also cross reacts with enzyme from chicken brain, frog spinal cord, and Aplysia ganglion. The determinants thus appear to be not only clustered in a small region of the enzyme surface but also highly conserved. (Supported by NS 18858).

115 PLASTICITY OF NICOTINIC SYNAPTIC TRANSMISSION
Briggs, C.A.; I.M. Brown; D.A. McAfee
City of Hope Research Institute Duarte, CA 91010

Previous studies have demonstrated long term potentiation (LTP) of synaptic transmission in the superior cervical ganglion of the rat (Brown and McAfee, 1982, Science 215:1411-1413). We obtained these results by incubating the ganglion in curare to reduce excitability, and then repetitively stimulated the preganglionic nerve at 20 Hz for only 20 seconds. While measuring the compound action potential in response to single preganglionic stimuli once every minute, we observed a 2-fold increase in the response amplitude which decayed as a double exponential with time constants 1-3 minutes (PTP) and 30-230 minutes (LTP). These findings, based on extracellular measurements, have now been confirmed using intracellular techniques.

In 21 of 41 cells, stimulation of the preganglionic nerve at 20 Hz for 20 sec induced an increase in the nicotinic excitatory postsynaptic potential (EPSP) or an increase in the ability of synaptic stimulation to generate an action potential in the postsynaptic neuron. The EPSP's were frequently obscured by synaptically driven action potentials which appeared after the tetanic stimulation. These effects lasted for 30 minutes to several hours, as long as the recording could be maintained. The potentiation was not accompanied by measurable changes in resting membrane potential or input resistance. Direct nonsynaptic stimulation of the postsynaptic neuron (20 Hz for 20 sec) failed to induce any increase in synaptic transmission in cells. Thus, LTP in the ganglion appears to be due to an increase in the efficacy of nicotinic synaptic transmission. We have hypothesized that LTP is accompanied by an increase in acetylcholine release but this awaits direct measurement.
Supported NSF BNS-12414.

116 L(-)SULPIRIDE ENHANCEMENT OF STRIATAL ACETYLCHOLINE RELEASE IN VITRO
Lee, H.K. and Czarnecki, E.
Dept. of Neurology, Henry Ford Hospital, Detroit, MI 48202

The effect of L(-)sulpiride, a specific dopamine-2(D-2:non-adenylate cyclase-linked)receptor antagonist virtually devoid of muscarinic activity (SPEDDING et al 1979), on the release of acetylcholine(ACh) from rat striatal tissue slices was examined. Male Sprague-Dawley rats(250-350 Gm) were decapitated and striatal slices were prepared. Slices were gassed(100% O_2) continuously during a 20 min pre-incubation in Krebs-phosphate buffer containing glucose (5mM) and choline (50μM), and were immediately aliquoted to oxygenated tubes containing no drug(control) or L(-)sulpiride(10^{-5},10^{-4} M) in either 5 or 30mM K^+ Krebs-phosphate(glucose,5mM;choline,50μM; pargyline,-0.2mM) for a further 10 min. ACh in incubation medium was measured immediately by a radioreceptor method (IKEDA et al,Life Sci.31:347-54, 1982). Tissue protein(0.5-0.8mg) was measured by the biuret method.

Incubation condition	Control	L(-)Sulpiride 10^{-5}M	L(-)Sulpiride 10^{-4}M	L(-)Sulpiride 10^{-3}M
5mM K^+	452 ± 32(4)	497 ± 27(3)	482 ± 18(3)	506 ± 25(3)
30mM K^+	601 ± 25(4)*	926 ± 22(3)**	978 ± 22(3)**	746 ± 20(3)*†

Each value is the mean(± sem) ACh released/mg protein/10 min);SEM of the sample number in parentheses, each based on triplicate assays. (*)and(**) indicate $P<.01$ or $<.001$, respectively, vs. appropriate control values. (†) indicates a $p<.05$ difference between 30 and 5mM K^+ control conditions.

These data show a significant enhancement of ACh release under depolarizing(30mM K^+) conditions and a lack of effect of L(-)sulpiride on 5mM K^+ (basal) ACh release from rat striatum. The results suggest that D-2 [illegible] receptors are important in mediating DA control of striatal ACh release, and emphasize that stimulated transmitter release must be [illegible] L(-)sulpiride's effect to be apparent. [Supported by the Fund

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221.13 EFFECTS OF DFP AND OTHER DRUGS ON CHOLINERGIC NERVE FUNCTION IN THE RAT IRIS. J.S. Richardson, T.G. Hutton*, M.L. Bernstein-Goral*, and E. Giacobini, Lab. of Neuropsychopharmacology, Dept of Biobehavioral Sciences, Univ. of Connecticut, Storrs, CT 06268.

The analysis of acetylcholine (ACh) levels, metabolism and release, as well as the release of choline, were performed on segments of rat iris to investigate the mechanisms involved in the response of the iris to acute and chronic cholinesterase inhibition. At various times after the topical administration of 0.1% DFP in sesame oil to the corneal surface, the rats were decapitated and the irises were removed. Pupil diameter was measured and ACh levels and cholinesterase activity were determined in each iris following extraction of remaining free DFP with chloroform. No changes were detected 1 min. after the DFP, but by 5 min., pupil diameter was reduced by 50%, esterase activity was reduced by 90%, and ACh was increased by 600%. There were no further changes 30 min. after the DFP, but by 60 min., ACh had returned to control levels even though esterase activity was still inhibited by 90% and pupil diameter was still reduced by over 50%.

Choline is taken up by the rat iris by a low affinity process (K_m=66.6 μM) and by a high affinity active transport system (K_m=1.16 μM) that is temperature sensitive, sodium dependent, and is blocked in a dose dependent manner by hemicholinium-3 at 10 nM or greater concentrations. Choline uptake is also reduced by millimolar concentrations of scopolamine and cocaine.

Electrical stimulation by 20 mA, 5 msec, 100Hz nearly square waves of isolated rat iris preparations by incubation at 37°C in Elliott's B buffer with tritiated choline, evokes a 1- to 2-fold increase in the release of tritium over the spontaneous release during pre-stimulation baseline. Scopolamine and DFP alter the release profile with 10 nM scopolamine increasing evoked release. 1 μM scopolamine increases spontaneous release, and 1 μM DFP reduces both spontaneous and evoked release. These results are consistent with the existence of presynaptic muscarinic receptors that control the release of ACh from cholinergic nerve terminals in the rat iris.

Supported by Grant AFOSR-81-0229 to Dr Ezio Giacobini.

221.14 SUBCELLULAR DISTRIBUTION OF CHOLINE KINASE ACTIV
...LOCALIZATION: EVIDENCE FOR MEMBRANE ASSOCIATED ENZY
...and L. Becker, Dept. Pharmacology, Louisiana Sta
...Medical Center, New Orleans, LA 70112.

Evidence indicates that the enzyme choline ki
which catalyzes the conversion of choline to pho
localized to the cytosolic fraction of rat brain
support for the presence of membrane associated
Our interest in the relationships among choline
transport and utilization for the synthesis of PC
and acetylcholine led us to reinvestigate the sub
bution of this enzyme in rat striata. Enzyme act
cytosol from the crude mitochondrial fraction wa
initial wet weight/hr, as compared to the activi
from the purified synaptosomal fraction which wa
initial wet weight/hr. When the crude mitochondr
was fractionated, 67% o. the total enzyme activi
the cytosol, while the osmotically shocked membra
of activity. However, when membranes were subjec
tion in Triton X-100, significant enzyme activity
1.58 umoles/g initial wet weight/hr (29% of the
the crude mitochondrial fraction). Since Triton
roots membranes, a less severe detergent
mine how tightly the enzyme was bound to the mem
of the membrane fraction with 1.5 M NaCl revealed
1.44 umoles/g initial wet weight/hr, similar to
total membrane solubilization. When the salt trea
recentrifuged, 58% of the membrane associated act
leased into the supernatant while the remaining
present in the pellet. The amount of choline kina
was a function of ionic strength and was linear t
1.5 M NaCl. Kinetic analyses indicated that the
in the cytosolic, Triton-treated and salt release
0.74, 0.68 and 0.53 mM, respectively. Results in
choline kinase activity is associated with both t
membrane fractions from rat striata. The ability
kinase activity in the presence of a high ionic s
evidence to suggest that the enzyme is bound to t
through an ionic interaction and not by a covalen
hydrophobic interactions. Although the specific
membrane associated enzyme has not, as yet, been
suggest that it may play a regulatory role in ref
flux of choline as choline enters nerve terminals
synthesis of phosphorylcholine and acetylcholine.
USPHS grants NS(NS-02278 and NIMH-)3443.)

221.15 STIMULATION OF LOCAL CEREBRAL GLUCOSE UTILIZATION IN THE RAT VISUAL SYSTEM BY PHYSOSTIGMINE. E. D. London and * Ehn
Laboratory of Neurosciences, National Institute on Aging, Gerontology Research Center, Baltimore City Hospitals, Baltimore, MD 21224.

Two previous studies have used the functional mapping technique of Sokoloff et al. (J. Neurochem. 28: 897, 1977) to examine the effects of physostigmine (PHY), a reversible carbamate acetylcholinesterase inhibitor, on the regional uptake of [C-14]2-deoxy-D-glucose ([C-14]DG) by the rat brain (Nelson, S.R., et al., Brain res. 152: 246, 1978; Friedland, R.P. and Reibach, A.C. Soc. Neurosci. Abs. 3: 494, 1981). In both of these studies, neostigmine produced no effects on [C-14]DG uptake, but PHY increased uptake in the superficial layer of the superior colliculus. In order to extend these findings, we examined PHY effects on local cerebral glucose utilization (LCGU) throughout the visual system of the rat brain, and compared PHY effects on LCGU with effects of the muscarinic agonist, oxotremorine (OXO).

Three month old male Fischer-344 rats were used for this study. Each rat was given one of the following treatments: control (saline, 1 ml/kg, i.p.), PHY (0.1 or 1 mg/kg of PHY salicylate, i.p., 20 min after atropine methylbromide, 1 mg/kg, s.c.), PHY (1 mg/kg, 20 min after scopolamine HBr, 1 mg/kg, i.p.). Saline and PHY were injected 20 min before [C-14]DG. OXO was injected 2 min before [C-14]DG. LCGU was determined as described by Sokoloff et al. (J. Neurochem. 28: 897, 1977).

At dose 0.1 mg/kg of PHY produced no significant effects. 1 mg/kg of PHY dramatically stimulated LCGU in the superficial layer of the superior colliculus (122%), the nucleus of the optic tract of the pretectal area (126%), and the following components of the accessory visual system: the superior (49%), lateral (59%), and medial terminal (123%) nuclei, and the inferior fasciculus (150%). PHY did not significantly affect LCGU in the visual cortex or in the dorsal nucleus of the lateral geniculate body. Scopolamine did not significantly antagonize the stimulatory effects of PHY in components of the visual system.

OXO increased LCGU in the superficial layer of the superior colliculus (73%) and in the nucleus of the optic tract (57%) and produced nonsignificant increases in some components of the accessory visual system. All OXO effects on LCGU were blocked by prior treatment with scopolamine. These results indicate that cholinergic drugs can influence function in the rat visual system. The fact the PHY effects on LCGU are not antagonized by scopolamine indicates that they result from nicotinic rather than muscarinic actions of the drug. A nicotinic rather than muscarinic action in the accessory visual system is further supported by the relative lack of effect of OXO in these brain regions.

221.16 CHOLINERGIC DEPOLARIZATIONS OF CENTRAL NORADRENE
T. W. Egan,* J. T. Williams* and R. A. Nicoll (SPO
Neuropharmacology Laboratory, Department of Nutri
Science, M.I.T., Cambridge, MA 02139.

There is a high concentration of cholinergic mu
sites on locus coeruleus (LC) neurons, and iontop
application of ACh increases the firing rate of t
recorded with extracellular electrodes in vivo.
recordings were made from neurons of rat LC in a
prep. Drugs were applied either by superfusion o
from a fine tipped pipette above the slice surfac
with acetylcholine (ACh) depolarized LC neurons
associated with an increase in input resistance.
ejection of ACh (pipette concentration 100 mM - 1
ms at 5 - 20 nA) caused a biphasic depolarizatio
early component of rapid onset and decay (duratio
a slower, longer lasting component (duration 10 -
depolarizations, were both potentiated by physost
and persisted in Ca^{++}-free high-Mg^{++} solutions. T
was blocked by hexamethonium (100 μM), the slow c
blocked by hyoscine. The antagonism by hyoscine
depolarization was competitive with a pA_2 of 8.5
5). The results indicate that ACh has both nicot
muscarinic actions on brainstem noradrenergic neu

MECHANISMS OF CHOLINE UPTAKE AND ACETYLCHOLINE RELEASE IN PERIPHERAL CHOLINERGIC SYNAPSES.

Giacobini, E., Richardson, J.S. and Mattio, T.G., Laboratory of Neuropsychopharmacology, Department of Biobehavioral Sciences, University of Connecticut, Storrs, CT 06268, USA

A new procedure allowing to perform a multiple set of microanalyses of ACh (acetylcholine) metabolism and release, as well as of Ch (choline) uptake, has been applied to segments of single rat irises. The characteristics of the high and low affinity Ch uptake system which have been previously described by us for the developing and aging avian iris (Marchi et al., Dev.Neurosci.3,185,1980 & Brain Res.195,423,1980) have now been determined for the adult rat iris as well. As in the chicken, the rat iris exhibits two distinct Ch uptake systems. One component, a Na^+ dependent, temperature sensitive, high affinity system (K_m= 1.37 µM) which is blocked by ouabain and hemicholinium, is most likely confined to cholinergic nerve terminals. A second component, probably localized in the iris muscle cells, is Na^+ independent and shows low affinity (K_m= 433.3 µM). Only the high affinity component is reduced by µM concentrations of scopolamine and DFP. Electrical stimulation of the isolated iris by 20 mA, 5 msec 100 Hz nearly square waves causes a 200% increase in the outflow of radioactivity following incubation with (^{3}H)Ch in the presence of scopolamine. Scopolamine and DFP alter the release profile with 10 nM scopolamine increasing the evoked release, 1 µM scopolamine increasing spontaneous release, while 1 µM DFP reduces both the spontaneous and evoked release. These results are consistent with the existence of presynaptic muscarinic autoreceptors that control the release of ACh from the cholinergic terminals in the rat iris.

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...r. Soc. Neurochem., 13th Ann. ...
Grossinger, NY, March 14-19, 1982.

I COLCHICINE AND VINBLASTINE DO NOT AFFECT THE FRAGMENTATION OF TUBULIN WITH THROMBIN AND CATHEPSIN D

Ramay-Schwartz, M., Bracco, F., Graf, L., and Laitha, A.
Center for Neurochemistry, Ward's Island, New York 10035

Tubulin is a heterodimer composed of two subunits (α and β) with molecular weights of 55,000 and 53,000 respectively. Tubulin has one high-affinity binding site for colchicine and two for vinblastine, both of which are different from the colchicine site. Colchicine and vinblastine inhibit microtubule polymerization; they also inhibit the reaction of some SH-groups in tubulin with alkylating reagents. We found that cytoplasmic soluble tubulin is rapidly broken down by brain cathepsin D and thrombin, whereas membrane-bound tubulin is strongly resistant to cathepsin D, but is broken down (although at a much lower rate) by thrombin. It was therefore of interest to investigate whether colchicine and vinblastine would interfere with the breakdown of tubulin by thrombin or cathepsin D, proteinases with widely different substrate specificities. Thrombin breaks down the two chains of tubulin with different rates (α chain is much more sensitive than β), and it seems to split a few selective bonds, generating 6 well-defined products. Cathepsin D hydrolyzes the protein without the appearance of any major detectable electrophoretic components, suggesting a less selective cleavage mechanism. The time course of tubulin digestion with thrombin and cathepsin D and the characteristic degradation pattern with thrombin were not changed in the presence of colchicine or vinblastine or both. These data suggest that colchicine and vinblastine binding sites of tubulin are not the sites for hydrolysis by these proteinases (or any other proteinases) and also that the interaction of tubulin with the drugs does not result in an overall conformational change of the protein to alter its susceptibility to proteolytic action. The stability of membrane-bound tubulin is thus due to protection of other than vinblastine or colchicine sites.

THE MYOTROPHIC PROTEIN SCIATIN IS A TRANSFERRIN-LIKE POLYPEPTIDE

Markelonis, G.J., Bradshaw, R.A. and Oh, T.H.
Univ. Maryland Sch. Med., Balto., Md. and Washington Univ. Sch. Med., St. Louis, Mo.

Sciatin, an acidic glycoprotein from chicken sciatic nerves, has myotrophic effects on avian skeletal muscle cells in culture. As sciatin was found to have certain structural similarities to transferrin, we have further investigated the physicochemical characteristics of sciatin in order to determine the relationship between these two proteins. Sciatin was found to be strikingly similar to ovotransferrin in amino acid composition. In addition, amino acid sequence analysis revealed that sciatin and ovotransferrin had identical amino-terminal sequences for at least the first 20 amino acid residues (NH2-Ala-Pro-Pro-Lys-Ser-Val-Ile-Arg-Trp-Cys-Thr-Ile-Ser-Ser-Pro-Glu-Gln-Lys-Lys-Cys-). Chicken ovotransferrin, but not human serum transferrin, crossreacted with rabbit anti-sciatin antibodies on rocket immunoelectrophoresis and double immunodiffusion in agar. In addition, in the presence of bicarbonate, sciatin bound approximately 2 mols of ferrous iron/mol protein. Using the purification procedure developed for sciatin, we purified a protein from chicken serum which crossreacted with anti-sciatin serum, migrated at a position identical to that of sciatin or ovotransferrin on two-dimensional gel electrophoresis, had an amino composition very similar to ovotransferrin and sciatin, and had myotrophic effects on cultured muscle cells. From these data, we conclude that sciatin is a growth-promoting polypeptide closely related structurally to transferrin.

Supported by grants from the NIH (NS 16076-G.J.M.; NS 15013-T.H.O.; NS 10229-R.A.B. and AM 13362-R.A.B.) and the MDA (T.H.O.).

333 UPTAKE AND RELEASE IN CHOLINERGIC NERVE TERMINALS OF THE RAT IRIS.

Richardson, J.S., Mattie, T.G.*, and Giacobini, E.
Pharmacol USASK Saskatoon SK S7N0W0 & Biobehav Sci UCONN Storrs CT 06268

Prior to an investigation of the acute and chronic effects of drugs inhibiting cholinesterase on the biochemical parameters of uptake, release and metabolism in cholinergic neurons innervating the eye, the uptake of choline and the release of acetylcholine were characterized in the isolated iris of the rat. The iris contains nerve endings whose cell bodies are located in autonomic ganglia. This makes the iris a good model for the study of nerve terminal function relatively free from contamination by cell body and glial effects.

In the choline uptake studies, the irises were each preincubated in .5 ml Elliot's B buffer for 5 min at 37°C, followed by the addition of .5ml of carrier choline containing a tracer amount of tritiated choline. The incubation was continued for 5 min at 37°C. The uptake of choline by the rat iris is linear over 6 concentrations of choline ranging from 0.1 uM to 10 uM. The linear regression correlation coefficient is 0.99. The uptake of 1 uM choline by the iris is reduced by over 50% ($p > .001$) in low sodium buffer, and is blocked in a dose dependent manner by 0.01 uM (no effect) to 100 uM (32% reduction $p > .001$) ouabain. Compared to uptake at 37°C, choline uptake is reduced by 68% ($p > .001$) at 20°C, and by 85% ($p > .001$) at 0°C. In the release studies, the iris was preloaded with 1 uM choline plus tracer tritiated choline, placed in a stimulation chamber and perfused with Elliot's B buffer at 37°C at a rate of 1 ml per min. Electrical stimulation of the iris by 20 mA, 5 msec, 100Hz nearly square waves, caused an almost 2fold increase in the outflow of radioactivity. The identity of this radioactivity and the effects of drugs on uptake and release are being established.

(Supported by Grant AFOSR-81-0229 to E.G.)

334 SEROTONIN STORAGE: IDENTIFICATION OF AN ESSENTIAL SULFHYDRYL GROUP AND BICARBONATE BINDING SITE IN SBP. Tamir, H., Bernath, J.*, Muller, F.S., and Liu, K.P.* Division of Neurosci., N.Y.S. Psych. Inst. and Dept. of Psychiatry, Columbia Univ. Coll. of P&S, New York, N.Y. 10032.

Serotonergic neurons as well as paraneurons contain a specific serotonin binding protein (SBP) involved in the amine storage mechanism. In addition to storage, a major property of this protein is to protect 5-HT from oxidation by MAO. For example, while free amine (2×10^{-5}M) is oxidized to the extent of 60%, bound amine (to 50 μg SBP) is not oxidized by mitochondria (0.5 mg Pr/ml, 37°, 30 min). In order to better understand the mechanism regulating serotonin concentration in the cell we are studying factors that affect binding to SBP. We have shown that rat brain SBP has essential SH groups that are protected from chemical modification when serotonin is bound. We report now that when the protected -SH group(s) were exposed and labelled with ^{14}C-NEME, the protein labeled at its binding site comigrated on acrylamide gel with SBP-^{3}H-5-HT$_4$ complex (NH$_2$-SBE) indicating that the labelled protein and the binding protein are identical. We have shown that binding of the amine to the protein is greatly enhanced by Fe^{++} and phosphate. We now find that bicarbonate is 3 to 4 fold more effective than phosphate. Enhancement due to an increase in number of binding sites and not in affinity. Using atomic absorption analysis we find that bicarbonate and phosphate increased the amount of iron bound to the protein by 60 and 30 fold respectively. Enhancement by bicarbonate may therefore be due to increase in the number of Fe^{++} binding sites forming -S-Fe-S- complexes (each binding up to 4 molecules of the amine). These data support that oxidizing agents may impair storage of serotonin in vesicles. Moreover, a decrease in CO_2 production in the cell due either to impaired carbohydrate oxidation, hypoxia, hypoglycemia or thiamine deficiency may result in increased turnover of the amine. Supported by NSF-Grant 09335.