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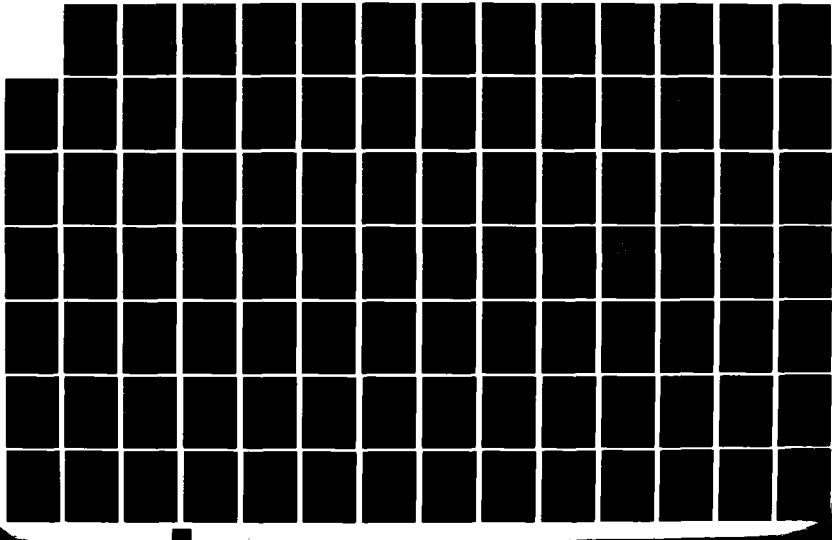

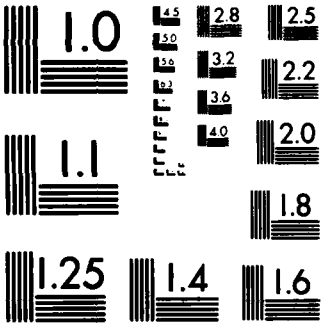

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Dynamics of Chlorinergic Function: Acetylcholine in Health Disease and Aging

Final Scientific Report

April 1984

Israel Hanin, Ph.D.

Supported by

U. S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701-5012

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20. ABSTRACT *(Continue on reverse side if necessary and identify by block number)*
Proceedings and Program for 1983 Conference on Dynamics of Cholinergic Function.

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FINAL SCIENTIFIC REPORT

Grant #: DAMD17-83-G-9534
Project Title: Dynamics of Cholinergic Function: Acetylcholine in Health, Disease and Aging.
Principal Investigator: Israel Hanin, Ph.D.

An international conference on the subject of "Dynamics of Cholinergic Function: Acetylcholine in Health, Disease and Aging" was convened October 31 through November 4, 1983, in Oglebay Park, West Virginia. The conference, sponsored by the Western Psychiatric Institute and Clinic (WPIC), was organized and chaired by Dr. Israel Hanin, Professor of Psychiatry at WPIC and the University of Pittsburgh School of Medicine.

The conference was attended by 155 scientists from all over the world. Countries represented by the participants included Belgium, Canada, France, Germany, Hungary, Israel, Italy, the Netherlands, Sweden, Switzerland, the United Kingdom and the United States (a comprehensive list of all the participants and their mailing addresses, is included as Appendix I of this report).

This was the fifth in a series of similar conferences, convened approximately every three years, each time under the organization and coordination of a different host, assisted by an international scientific committee consisting of individuals with acknowledged expertise in the cholinergic system. The distinguished scientific committee that assisted Dr. Hanin with the planning and coordination of this scientific program consisted of:

John P. Blass, M.D., Ph.D., Cornell University, USA
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Edith Heilbronn, Ph.D. University of Stockholm, Sweden
Bo Holmstedt, M.D., Karolinska Institutet, Sweden
Donald J. Jenden, M.D., University of California at Los Angeles, USA
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Giancarlo Pepeu, M.D., University of Florence, Italy

Earl Usdin, Ph.D., University of California at Irvine, USA
Victor P. Whittaker, M.D., Max Planck Institute, Germany.

In an attempt to encompass the current status of our knowledge in the field, the scientific program covered a variety of areas relevant to cholinergic mechanisms. Topics included:

1. Anatomy of Central Cholinergic Systems
2. Aging of Cholinergic Synapses
3. Biochemical Events at the Cholinergic Synapse
4. Factors in Presynaptic Cholinergic Function
5. Membrane Function and Cholinergic Action
6. Cholinesterases and their Reactivators
7. Clinical Aspects of Cholinergic Function
8. Cholinergic Mechanisms in Cardiac Function
9. Interaction of Acetylcholine with Other Transmitter Systems
10. Studies with AF64A
11. Neurotoxic Agents and False Transmitters
12. Precursor Availability and Acetylcholine Synthesis in vivo.

Enclosed with this report is a copy of the official Program of the conference, which provides further details regarding the scientific program, the various speakers, and other relevant items. The Program is denoted as Appendix II of this report.

Many of the subjects covered in the conference were chosen to reflect areas of potential interest to the US Army Medical Research and Development Command. For example, topics 1 and 2 dealt directly with issues of importance in identifying and localizing sites of cholinergic function in the brain, and the effect of normal aging on central cholinergic activity, respectively. Topics 3,4 and 5 dealt with biochemical and neurochemical events at the terminal site of the cholinergic nerve, as well as at the synaptic level. These topics addressed the issue of regulatory activity at the cholinergic nerve terminal, which is essential for the normal functioning of the cholinergic system. Topic 6 dealt with cholinesterases and with compounds which affect their function in vivo. The studies with AF64A (topic 10) reflected ongoing work in several laboratories using this neurotoxic agent as a novel tool for the selective destruction of cholinergic nerve terminals in vivo.
The remaining topics adressed the effect of external events (other neurotransmitters, various pharmacological agents,

neurotoxins) on the dynamics of cholinergic function in the intact animal.

The conference comprised a carefully selected mixture of poster presentations and formal talks. Individuals selected represented a cross section of academia, government and industry. Attempts were made, in the initial planning of the list of participants, to balance the group between young investigators who are starting their careers, and more experienced researchers who already are highly accomplished in the field. We believe that this combination contributed much to the success of the conference.

Enclosed with this report are abstracts of the various talks which were presented at the conference. These are identified as Appendix III to this report. The proceedings of the conference will ultimately be published by Plenum Press, under the title of "Dynamics of Cholinergic Function". Dr. Hanin is currently in the process of editing all of the manuscripts which were submitted by the participants for inclusion in this book.

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Appendix II
DAMD17-83-G-9534
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Conference on Dynamics of Cholinergic Function

PROGRAM

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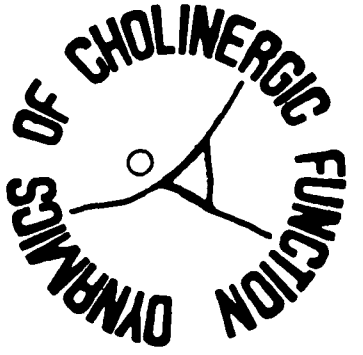
West Virginia

October 31 - November 4, 1983



CONFERENCE ON DYNAMICS OF CHOLINERGIC FUNCTION

WILSON LODGE
OGLEBAY PARK
WEST VIRGINIA

OCTOBER 31 - NOVEMBER 4, 1983

The proceedings of this conference will be published by Plenum Press.

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Donald J. Jenden, M.D. (USA)
Alexander G. Karczmar, M.D., Ph.D. (USA)
Giancarlo Pepeu, M.D. (ITALY)
Earl Usdin, Ph.D. (USA)
Victor P. Whittaker, M.D. (GERMANY)

LOCAL COMMITTEE:

Israel Hanin, Ph.D.
Toni Lieteau
Joyce O'Leary

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UCB Secteur Pharmaceutique

Unilever Research Laboratorium

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DYNAMICS OF CHOLINERGIC FUNCTION

Oglebay Park, West Virginia

October 31, - November 4, 1983

GENERAL PROGRAM

MONDAY, OCTOBER 31

9:00 a.m.	Registration	
2:00 p.m.	Session #1:	**Anatomy of Central Cholinergic Systems** *Co-chairpersons:* Edith McGeer, Ph.D. Giancarlo Pepeu, M.D.
Evening	Hoedown/Barbecue	

TUESDAY, NOVEMBER 1

8:30 a.m.	Session #2:	**Aging of Cholinergic Synapses** *Co-chairpersons:* Ezio Giacobini, M.D., Ph.D. Alan M. Goldberg, Ph.D.
2:00 p.m.	Poster #1 Biochemical Events at the Cholinergic Synapse (Receptors) Factors in Presynaptic Cholinergic Function Membrane Function and Cholinergic Action *Chairperson:* Donald J. Jenden, M.D.	
Evening	Special Evening Session: Bo Holmstedt, M.D. Frank C. MacIntosh, Ph.D.	

WEDNESDAY, NOVEMBER 2

8:30 a.m.	Session #3:	**Cholinesterases and Their Reactivators** *Co-chairpersons:* George P. Koelle, M.D., Ph.D. Peter G. Waser, M.D.
2:00 p.m.	Poster #2 Clinical Aspects of Cholinergic Function Cholinergic Mechanisms in Cardiac Function Interaction of Acetylcholine with Other Neurotransmitter Systems Studies with AF64A *Chairperson:* Israel Hanin, Ph.D.	
Evening	Free	

THURSDAY, NOVEMBER 3

8:30 a.m. Session #4: **Neurotoxic Agents and False Transmitters**

Co-chairpersons:
Abraham Fisher, Ph.D.
Edith Heilbronn, Ph.D.

2:00 p.m. Travel to Pittsburgh

Evening Banquet

FRIDAY, NOVEMBER 4

8:00 a.m. Session #5: **Precursor Availability on Acetylcholine Synthesis In Vivo**

Co-chairpersons:
John P. Blass, M.D., Ph.D.
Earl Usdin, Ph.D.

Overview and Summary:
Alexander G. Karczmar, M.D., Ph.D.

MONDAY P.M.

PRESENTATIONS*

SESSION #1:

ANATOMY OF CENTRAL CHOLINERGIC SYSTEMS

Co-chairpersons: Edith G. McGeer,
University of British Columbia, Vancouver, Columbia, CANADA

Giancarlo Pepeu,
University of Florence, Florence, ITALY

Larry L. Butcher, University of California at Los Angeles, Los Angeles, California, USA

"Cholinergic Systems in the Central Nervous System"

Edith G. McGeer, University of British Columbia, Vancouver, Columbia, CANADA

"Specific Aspects of Cholinergic Neuroanatomy"

Robert G. Struble, The Johns Hopkins University, Baltimore, Maryland, USA

"Cortical Cholinergic Innervation: Distribution and Source in Monkeys"

Giancarlo Pepeu, Florence University, Florence, ITALY

"Neurotransmitters that Act on Cholinergic Magnocellular Forebrain Nuclei Influence Cortical Acetylcholine Output"

Luigi Amaducci, University of Florence, Florence, ITALY
(See Sorbi in abstracts)

"Lateralization of Cholinergic and Energy Related Enzymes in Human Temporal Cortex"

Paul M. Salvaterra, City of Hope, Duarte, California, USA

"Biochemistry and Immunocytochemistry of Choline Acetyltransferase"

Annica B. Dahlstrom, University of Gőteborg, Gőteborg, SWEDEN

"Immunohistochemical Evidence for the Intra-Axonal Transport of nAChR-Like Material in Motor Neurons"

Lorenza Eder-Colli, CMU, Geneva, SWITZERLAND

"Immunological Approach to Cholinergic Transmission: Production of Monoclonal Antibodies Against Presynaptic Membranes Isolated from the Torpedo Electric Organ"

MONDAY P.M.

James K. Wamsley, University of Utah, Salt Lake City, UTAH, USA

"Autoradiographic Localization of Subtypes of Muscarinic Agonist and Antagonist Binding Sites: Alterations Following CNS Lesions"

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* The scientific program is listed according to the actual presenter of each communication. Please refer to the abstracts for a complete list of all investigators in each project.

TUESDAY A.M.

SESSION #2:

THE AGING OF CHOLINERGIC SYNAPSES

Co-chairpersons: Ezio Giacobini,
Southern Illinois University, Springfield, Illinois, USA

Alan M. Goldberg,
Johns Hopkins University, Baltimore, Maryland, USA

Ezio Giacobini, Southern Illinois University, Springfield, Illinois, USA

"Aging of Cholinergic Synapses: Fiction or Reality?"

Agneta Nordberg, Uppsala University, Uppsala, SWEDEN

"The Aging of Cholinergic Synapses: Ontogenesis of Cholinergic Receptors"

Ronald F. Mervis, Ohio State University, Columbus, Ohio, USA

"Influence of Diet on Mouse Brain Cholinergic Parameters: Effects of Strain and Age"

Dean O. Smith, University of Wisconsin, Madison, Wisconsin, USA

"Acetylcholine Content, Release, and Leakage at the Neuromuscular Junction of Mature Adult and Aged Rats"

Gary E. Gibson, Cornell Medical College, White Plains, New York, USA

"Interactions of Calcium Homeostasis, Acetylcholine Metabolism, Behavior and 3,4-Diaminopyridine During Aging"

Donald L. Price, Johns Hopkins University, Baltimore, Maryland, USA

"Basal Forebrain Cholinergic Systems in Primate Brain: Anatomical Organization and Role in the Pathology of Aging and Dementia"

Edythe D. London, Baltimore City Hospital, Baltimore, Maryland, USA

"Relations Between Choline Acetyltransferase and Muscarinic Binding in Aging and Alzheimer's Disease"

TUESDAY P.M.

POSTER #1:

Chairperson: Donald J. Jenden,
University of California, Center for the Health Sciences,
Los Angeles, California, USA

(Authors are listed in alphabetical order.)

BIOCHEMICAL EVENTS AT THE CHOLINERGIC SYNAPSE (RECEPTORS)

Tamas Bartfai, Arrhenius Laboratory, Stockholm, SWEDEN
(see Nordström in abstracts)

"In Vivo and In Vitro Studies on a Presynaptic Muscarinic Antagonist and Postsynaptic Agonist: BM-5"

Joan Heller Brown, University of California, San Diego, La Jolla, California, USA

"Differential Effects of Carbachol and Oxotremorine on Muscarinic Receptors, Cyclic AMP Formation, and Phosphoinositide Turnover in Chick Heart Cells"

Richard Dahlbom, Uppsala University, Uppsala, SWEDEN

"Stereoselectivity of Some Muscarinic and Antimuscarinic Agents Related to Oxotremorine"

Erik Danielsson, Arrhenius Laboratory, Stockholm, SWEDEN

Interactions of Alaproclate, a Selective 5HT Uptake Blocker, with Muscarinic Receptors: In Vivo and In Vitro Studies"

Eduardo D. P. DeRobertis, Facultad de Medicine, Buenos Aires, ARGENTINA

"Localization and Regulation of Muscarinic Receptors in Central Synapses"

Frederick J. Ehlert, University of California at Los Angeles, Los Angeles, California, USA

"The Interaction of Alkylating Derivatives of Oxotremorine with the Muscarinic Receptor"

J. N. Hawthorne, University of Nottingham Medical School, Nottingham, ENGLAND

"Polyphosphoinositide and Phosphoprotein Responses to Muscarinic Receptor Activation"

Edith Heilbronn, University of Stockholm, Sundbyberg, SWEDEN

"Studies on a cAMP-Dependent Protein Kinase Obtained from Nicotinic Receptor-Bearing Microsacs"

TUESDAY P.M.

Heinz Kilbinger, University of Mainz, Mainz, GERMANY

"Facilitation and Inhibition by Muscarinic Agonists of Acetylcholine Release from Peripheral Nerves"

Christer Larsson, Uppsala University, Uppsala, SWEDEN

"Characterization of ^{3}H-Nicotine Binding in Rodent Brain and Comparison with the Binding of Other Labeled Nicotinic Ligands"

Konrad Loffelholz, University of Mainz, Mainz, GERMANY

"Muscarinic Receptor Activation Increases Efflux of Choline from Isolated Heart and Rat Cortex In Vivo"

Mario Marchi, Institute of Pharmacology and Pharmacognosy, Genova, ITALY

"Presynaptic Muscarinic Receptors" Changes of Sensitivity During Long Term Drug Treatment"

Henry G. Mautner, Tufts University, Boston, Massachusetts, USA

"The Cholinergic Ligand Binding Material of Axonal Membranes"

Bjorn Ringdahl, University of California at Los Angeles, Los Angeles, California, USA

"Affinity and Efficacy of Oxotremorine Analogs at Ileal Muscarinic Receptors"

Rochelle D. Schwartz, National Institute of Mental Health, Bethesda, Maryland, USA

"Nicotinic Cholinergic Receptors Labeled by ^{3}H-Acetylcholine in Brain: Characterization, Localization and In Vivo Regulation"

Anders Sundwall, Research and Development KabiVitrum, Stockholm, SWEDEN

"Receptor Mediated Regulation of Uptake, Biotransformation and Release of Choline and Metabolites in Slices and P_2 Fractions from Different Mouse Brain Regions In Vitro"

E. Sylvester Vizi, Hungarian Academy of Sciences, Budapest, HUNGARY

"Presynaptic Receptors Modulating Acetylcholine Release"

Henry I. Yamamura, University of Arizona, Tucson, Arizona, USA

"Differential Light Microscopic Autoradiographic Localization of Muscarinic Cholinergic Receptors in the Brainstem and Spinal Cord of the Rat Using Radiolabeled Pirenzepine"

TUESDAY P.M.

FACTORS IN PRESYNAPTIC CHOLINERGIC FUNCTION

Michael Adler, U.S. Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, Maryland, USA

"Effects of DFP on Synaptic Transmission in Neuroblastoma-Myotube Co-Cultures"

Denes V. Agoston, Max-Planck-Institute für Biophysikalische Chemie, Gottingen, F. R. GERMANY

"Separation of Recycling and Reserve Synaptic Vesicles from Cholinergic Nerve Terminals of the Myenteric Plexus of Guinea-Pig Ileum"

Elias Aizenman, Johns Hopkins University, Baltimore, Maryland, USA

"Axonal Transport and Pharmacological Properties of ^{125}I-α Bungarotoxin Binding Sites in Rat Sciatic Nerve"

Lorenzo Beani, University of Ferrara, Ferrara, ITALY

"A Comparison Between Endogenous Acetylcholine Release and ^{3}H-Choline Outflow from Guinea-Pig Brain Slices"

George G. Bierkamper, University of Nevada, Reno, Nevada, USA

"Do Motor Neurons Contain Functional Presynaptic Cholinergic Autoreceptors?"

Alan Boyne, University of Baltimore, Baltimore, Maryland, USA

"Quick Freezing Preserves a Large Unstable Vesicle Population in Torpedine Electric Organ Nerve Terminals"

Clark A. Briggs, City of Hope, Duarte, California, USA

"Long Term Potentiation of Cholinergic Synaptic Transmission in a Sympathetic Ganglion"

Paul T. Carroll, Texas Tech University, Lubbock, Texas, USA

"Depolarization-Induced Hydrolysis of Cytoplasmic ACh in Mouse Brain Tissue"

Silvana Consolo, Mario Negri Institute of Research, Milan, ITALY

"Acetylcholine and Choline Content in Fast and Slow Muscle of the Rat: Effect of Drugs"

Jack R. Cooper, Yale University School of Medicine, New Haven, Connecticut, USA

"Presynaptic Modulation of Acetylcholine Release"

TUESDAY P.M.

Ilze Ducis, Max-Planck-Institut für Biophysikalische Chemie, Gottingen, F. R. GERMANY

"Tentative Indentification of the Choline Transporter in Cholinergic Presynaptic Plasma Membrane Preparations from Torpedo Electric Organ"

Ezio Giacobini, Southern Illinois University, Springfield, Illinois, USA (see Mattio in abstracts)

"Effects of DFP on Acetylcholine Metabolism and Release and Pupillary Function in the Rat"

Johan Häggblad, University of Stockholm, Sundbyberg, SWEDEN

"Acetylcholine Release from Rat Diaphragm: A Search for Release Regulatory Mechanisms"

Bruce Howard, University of California at Los Angeles Medical School, Los Angeles, California, USA

"Acetylcholine Release from PC12, A Clonal Cell Line"

Jean-Claude Maire, Massachusetts Institute of Technology, Cambridge, Massachusetts, USA

"Effects of Exogenous Choline on Acetylcholine Release from Slices of Rat Striatum"

R. Massarelli, Center for Neurochemistry, Strasbourg, FRANCE

"Efflux of Choline from Neurons and Glia in Culture"

Peter C. Molenaar, Leiden University Medical Center, Leiden, the NETHERLANDS

"Evoked Release of Acetylcholine at the Motor Endplate"

John J. O'Neill, Temple University School of Medicine, Philadelphia, Pennsylvania, USA

"Inhibition of Acetylcholine Synthesis In Vitro"

Rob L. Polak, Medical Biological Laboratory, TNO, Rijsvijk, the NETHERLANDS

"Resting Release of Acetylcholine at the Motor Endplate"

Roy D. Schwarz, Warner Lambert/Parke-Davis Company, Ann Arbor, Michigan, USA

"Control of the Release of ^{3}H-Acetylcholine from Rat Hippocampal Slices by Aminopyridines and Phencyclidine"

Janusz B. Suszkiw, University of Cincinnati, Cincinnati, Ohio, USA

"Relationship Between Calcium Entry and ACh Release in K+-Stimulated Rat Brain Synaptosomes"

Klaus Wachtler, Institut für Zoologie der Tierärztlichen Hochschule, Hanover, WEST GERMANY

"Cholinergic Neurones in the Nervous System of Ancient Chordates"

MEMBRANE FUNCTION AND CHOLINERGIC ACTION

Yves Dunant, Department of Pharmacology, CMU, Geneva, SWITZERLAND

"Presynaptic Changes Accompanying Transmission of a Single Nerve Impulse. An Interdisciplinary Approach Using Rapid-Freezing"

Peter Kasa, Central Research Laboratory Medical University, Szeged, HUNGARY

"The Role of Glia Cells in Neuronal Acetylcholine Synthesis"

Robert Manaranche, C.N.R.S., Gif-sur-Yvette, FRANCE

"Biochemical and Ultrastructural Studies of Acetylcholine Release Induced by Glycera Convoluta Neurotoxin"

Nicholas Morel, C.N.R.S., Gif-sur-Yvette, FRANCE

"Characterization of Plasma Membrane Proteins of Cholinergic Synaptosomes"

Guillermo Pilar, University of Connecticut, Storrs, Connecticut, USA

"Target Tissue Influences on Cholinergic Development of Parasympathetic Motor Neurons"

Jack C. Waymire, University of Texas Medical School, Houston, Texas, USA

"Cholinergic Regulation of Catecholamine Biosynthesis in Adrenal Medullary Cells Through Multiple Site Phosphorylation of Tyrosine Hydroxylase"

SPECIAL EVENING SESSION

Bo Holmstedt, Karolinska Institutet, Stockholm, SWEDEN

"Solanaceous Anticholinergic Compounds: Atropine and Scopolamine"

Frank C. MacIntosh, McGill University, Drummond, Montreal, CANADA

"Cholinergic Physiology: Retrospect and Prospect"

WEDNESDAY A.M.

SESSION #3:

CHOLINESTERASES AND THEIR REACTIVATORS

Co-chairpersons: George P. Koelle,
University of Pennsylvania Medical School,
Philadelphia, Pennsylvania, USA

Peter G. Waser,
University of Zurich, Zurich, SWITZERLAND

Ladislav Tauc, Laboratoire de Neurobiologie Cellulaire, Gif-sur-Yvette, FRANCE

"Molecular Relationship Between Acetylcholinesterase and Acetylcholine Receptors"

Edson X. Albuquerque, University of Maryland School of Medicine, Baltimore, Maryland, USA

"The Interaction of Anticholinesterase Agents with the Astro Receptor Complex"

Jean Massoulie, Ecole Normale Superieure, Paris, FRANCE

"Polymorphism of Acetylcholinesterase: Possible Insertion of the Various Molecular Forms in Cellular Structures"

George P. Koelle, University of Pennsylvania Medical School, Philadelphia, Pennsylvania, USA

"An Endogenous Neurotrophic Factor for the Maintenance of AChE and BuChE in the Preganglionically Denervated Superior Cervical Ganglion of the Cat"

Wolf-D. Dettbarn, Vanderbilt University, Nashville, Tennessee, USA

"Acetylcholinesterase and the Maintenance of Neuromuscular Structure and Function"

Bo Karlen, University of Stockholm, Stockholm, SWEDEN

"Influence of Cholinesterase Inhibitors on Acetylcholine Turnover in Mouse Brain"

Ing. K. Ho, University of Mississippi Medical Center, Jackson, Mississippi, USA

"Striatal Dopamine γ-Aminobutyric Acid-Acetylcholine Interaction in Organophosphate-Induced Neurotoxicity"

Tsung-Ming Shih, U.S. Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, Maryland, USA

"Cholinergic Effects of HI-6 in Soman Poisoning"

WEDNESDAY A.M.

Ingrid Nordgren, Karolinska Institutet, Stockholm, SWEDEN

"Succinylcholine – A Method of Determination, Distribution and Elimination"

Peter G. Waser, University of Zurich, Zurich, SWITZERLAND

"Pharmacokinetics of ^{14}C-Sarin"

WEDNESDAY P.M.

POSTER #2:

Chairperson: Israel Hanin,
University of Pittsburgh School of Medicine,
Pittsburgh, Pennsylvania, USA

(Authors are listed in alphabetical order.)

CLINICAL ASPECTS OF CHOLINERGIC FUNCTION

Sten-Magnus Aquilonius, University Hospital, Uppsala, SWEDEN

"Cholinergic Mechanisms in Spinal Cord and Muscle: Changes in Ameotrophic Lateral Sclerosis (ALS)"

Volker Bigl, Karl-Marse University, Leipzig, GERMANY

"Cholinergic Hypofunction and Dementia: Relation of Neuronal Loss in Different Parts of the Nucleus Basalis to Cortical Neuropathological Changes"

John Blass, Burke Rehabilitation Center, White Plains, New York, USA

"Red Cell Choline in Dementias"

David M. Bowen, University of London, Queen Square, London, ENGLAND

"Reduced Acetylcholine Synthesis in Alzheimer's Disease is a Clinically Relevant Change"

James N. Davis, Duke University Medical Center, Durham, North Carolina, USA

"Peripheral Sympathetic Nerves Replace Central Cholinergic Nerves After Injury in the Adult Mammalian Brain"

Kenneth L. Davis, Veterans Administration Medical Center, Bronx, New York, USA

"Cholinergic Mechanisms in Alzheimer's Disease"

Edward F. Domino, Lafayette Clinic, Detroit, Michigan, USA

"Plasma and Red Blood Cell Choline in Aging: Rats, Monkeys and Man"

Sven-Åke Eckernas, University Hospital, Uppsala, SWEDEN

"The Use of Positron Emission Tomography (PET) for the Evaluation of Regional Choline Metabolism in the Brain"

Pierre Etienne, Douglas Hospital Research Center, Verdun, Quebec, CANADA

"Pattern of Cell Loss in Basal Forebrain Structures of Early and Late Onset Alzheimer Cases"

WEDNESDAY P.M.

Elliot S. Gershon, National Institute of Mental Health, Bethesda, Maryland, USA

"Increased Density of Muscarinic Receptors on Fibroblasts Associated with Vulnerability to Affective Disorders"

Eitan Friedman, New York University Medical Center, New York, New York, USA (see Lerer in abstracts)

"Cortical Cholinergic Hypofunction and Behavioral Impairment Produced by Basal Forebrain Lesions in the Rat"

J. Christian Gillin, University of California, San Diego, California, USA (see Kaufman in abstracts)

"Muscarinic Binding with QNB in Frontal Cortex, Hypothalamus, and Pons is Similar in Suicides and Controls"

David S. Janowsky, University of California, San Diego, La Jolla, California, USA

"Central Acetylcholine and Stress Induced Cardiovascular, Neuroendocrine and Behavioral Changes"

Alan G. Mallinger, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, USA

"Membrane Transport of Choline by Human Erythrocytes: Relationship to Intracellular Choline Content"

K. Martin, University of Cambridge, Cambridge, ENGLAND

"Factors That Determine the Effects of Lithium on the Choline Transport Systems in Erythrocytes"

S. Mykita, Center for Neurochemistry, Strasbourg, FRANCE

"Effect of CDP-Choline on Hypocapnic Neurones"

Felicita Pedata, Florence University, Florence, ITALY

"Effects of Nootropic Drugs on Brain Cholinergic Mechanisms: Biochemical and Behavioral Investigations"

Michael J. Pontecorvo, Lederle Laboratories, Pearl River, New York, USA

"Cholinergic Dysfunction and Memory: Implications for the Development of Animal Models of Aging and Dementia"

S. Craig Risch, University of California at San Diego, School of Medicine, La Jolla, California, USA

"Muscarinic Supersensitivity of Anterior Pituitary ACTH and β-Endorphin Release in Major Depressive Illness"

WEDNESDAY P.M.

Kathleen A. Sherman, New York University Medical Center, New York, New York, USA

"Red Blood Cell Choline: Predictor of Response to Acetylcholine Precursor Therapy?"

Natraj Sitaram, Lafayette Clinic, Detroit, Michigan, USA

"Cholinergic REM Induction as a Marker of Endogenous Depression"

Michael Stanley, Lafayette Clinic, Detroit, Michigan, USA

"Cholinergic Receptor Binding in the Frontal Cortex of Suicide Victims"

CHOLINERGIC MECHANISMS IN PHYSIOLOGIC FUNCTION

Henry Brezenoff, New Jersey Medical School, Newark, New Jersey, USA

"Brain Acetylcholine in Hypertension and Behavior: Studies Using N-(4-Diethylamino-2-Butynyl)-Succinimide"

Oliver M. Brown, State University of New York, Syracuse, New York, USA

"Parasympathetic Innervation of the Heart: Acetylcholine Turnover In Vivo"

Jerry J. Buccafusco, Medical College of Georgia, Augusta, Georgia, USA

"Inhibition of Brain Acetylcholine Biosynthesis by Clonidine and Methyldopa: Relevance to Hypertensive Disease"

John D. Catravas, Medical College of Georgia, Augusta, Georgia, USA

"Acetylcholine-Prostanoid Interaction in the Pulmonary Circulation"

Matthew N. Levy, Mount Sinai Medical Center, Cleveland, Ohio, USA

"Tendency for Repetitive Vagal Activity to Synchronize Cardiac Pacemaker Cells"

Paul Martin, Mount Sinai Medical Center, Cleveland, Ohio, USA

"Phasic Dependent Effects of Brief Vagal Stimuli on AV Conduction and Atrial Contractile Force"

WEDNESDAY P.M.

INTERACTION OF ACh WITH OTHER NEUROTRANSMITTER SYSTEMS

William T. Blaker, National Institute of Mental Health, St. Elizabeths Hospital, Washington, D.C., USA

"$GABA_A$ vs. $GABA_B$ Modulation of Septal-Hippocampal Interconnections"

Darwin L. Cheney, Ciba-Geigy Corporation, Summit, New Jersey, USA

"Septal Gabaergic Neurons: Localization and Possible Involvement in the Septal-Hippocampal Feedback Loop"

Nae J. Dun, Loyola University Medical Center, Maywood, Illinois, USA

"Serotonin and its Role in Modulation of Cholinergic Transmission in Prevertebral Ganglia"

Britta Hedlund, Yale University School of Medicine, New Haven, Connecticut, USA

"Vasoactive Intestinal Polypeptide (VIP) – Muscarinic Cholinergic Interactions"

Herbert Ladinsky, Mario Negri Institute of Research, Milan, ITALY

"Regulation of Cholinergic Activity in the Rat Striatum by the Corticostriatal Pathway"

B. V. Rama Sastry, Vanderbilt University School of Medicine, Nashville, Tennessee, USA

"Regulation of Acetylcholine Release from Rodent Cerebrum by Presynaptic Receptors, Methionine Enkephalin and Substance P"

Susan E. Robinson, Medical College of Virginia, Richmond, Virginia, USA

"Contribution of the Dorsal Noradrenergic Bundle to the Effect of Amphetamine on Acetylcholine Turnover"

Bernard Scatton, Synthelabo-L.E.R.S., Bagneux, FRANCE

"Excitatory Amino-Acid Influence on Striatal Cholinergic Transmission"

Paul L. Wood, Douglas Hospital Research Center, Verdun, Quebec, CANADA

"Substantia Innominata-Cortical Cholinergic Pathway: Regulatory Afferents"

WEDNESDAY P.M.

INTERACTION OF ACh WITH OTHER NEUROTRANSMITTER SYSTEMS

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Paul L. Wood, Douglas Hospital Research Center, Verdun, Quebec, CANADA

"Substantia Innominata-Cortical Cholinergic Pathway: Regulatory Afferents"

WEDNESDAY P.M.

Richard E. Zigmond, Harvard Medical School, Boston, Massachusetts, USA

"Cholinergic and Peptidergic Regulation of Ganglionic Tyrosine Hydroxylase Activity"

STUDIES WITH AF64A

Fiorella Casamenti, Florence University, Florence, ITALY

"Biochemical and Behavioral Effects of AF64A in the Rat"

M. R. Kozlowski, Pfizer Central Research, Groton, Connecticut, USA

"Histochemical and Biochemical Effects of the Injection of AF64A into the Nucleus Basalis of Meynert"

Thomas Walsh, National Institute of Environmental Health Services, Research Triangle Park, North Carolina, USA

"AF64A Produces Long-Term Learning and Memory Impairments in the Rat"

THURSDAY A.M.

SESSION #4:

NEUROTOXIC AGENTS AND FALSE TRANSMITTERS

Co-chairpersons: Abraham Fisher,
Israel Institute for Biological Research,
Ness-Ziona, ISRAEL

Edith Heilbronn,
University of Stockholm, Sundbyberg, SWEDEN

Brian Collier, McGill University, Drummond, Montreal, CANADA

"Synthesis, Storage and Release of Choline Analogue Esters"

Charles R. Mantione, National Institute of Mental Health, Bethesda, Maryland, USA

"Possible Mechanisms Involved in the Presynaptic Cholinotoxicity Due to AF64A In Vivo"

E. H. Colhoun, University of Western Ontario, London, Ontario, CANADA

"Neurotoxic Effects of Nitrogen Mustard Analogues of Choline"

Donald J. Jenden, University of California, Los Angeles, California, USA

"Pharmacological Properties of Some Alkylating Oxotremorine Analogs"

Stanley Parsons, University of California, Santa Barbara, Santa Barbara, California, USA

"New Pharmacological Tools to Study Acetylcholine Storage in Nerve Terminals"

Michael J. Dowdall, Queen's Medical Center, Nottingham, ENGLAND

"Phospholipase Neurotoxins as Probes for the Presynaptic Cholinergic Membrane in Torpedo"

Maurice Israel, C.N.R.S., Gif-sur-Yvette, FRANCE

"A Reconstituted Presynaptic Membrane Equipped with Protein Structures which Permit the Calcium Dependent Release of Acetylcholine"

FRIDAY A.M.

SESSION #5:

PRECURSOR AVAILABILITY ON ACh SYNTHESIS IN VIVO

Co-chairpersons: John P. Blass,
Burke Rehabilitation Center, White Plains, New York, USA

Earl Usdin,
University of California at Irvine, Irvine, California, USA

Richard S. Jope, University of Alabama, Birmingham, Alabama, USA

"Biochemical Effects of Lecithin Administration"

Steven H. Zeisel, Boston University School of Medicine, Boston, Massachusetts, USA

"Factors which Influence the Availability of Choline to Brain"

Lynn Wecker, Louisiana State University Medical Center, New Orleans, Louisiana, USA

"The Utilization of Supplemental Choline by Brain"

R. Jane Rylett, University of Western Ontario, London, Ontario, CANADA

"Mechanisms of Acetylcholine Synthesis: Coupling with Choline Transport"

Judith Richter, Indiana University Medical Center, Indianapolis, Indiana, USA

"Choline Uptake in the Hippocampus: Indirect Inhibition of Septal-Hippocampal Cholinergic Neurons by Barbiturates"

Frans Flentge, Academic Hospital, Groningen, the NETHERLANDS

"Effects of the Combined Treatment of Rats with Fluphenazine and Choline or Lecithin on the Striatal Cholinergic and Dopaminergic System"

Israel Hanin, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, USA

"Manipulation of Dietary Choline and Sleep EEG Profile in Rats"

Renato Corradetti, Florence University, Florence, ITALY

"Choline Fluxes to and from the Rat Cerebral Cortex Studied with the 'Cup Technique' In Vivo"

OVERVIEW AND SUMMARY: Alexander G. Karczmar
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Appendix III
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Conference on Dynamics of Cholinergic Function

ABSTRACTS

Wilson Lodge at Oglebay Park
West Virginia

October 31 - November 4, 1983

EFFECT OF DFP ON SYNAPTIC TRANSMISSION IN NEUROBLASTOMA-MYOTUBE CO-CULTURES:

Michael Adler, Tony Chang, Robert Foster, Donald Maxwell, Gregory Mark, and John Glenn, Neurotoxicology Branch, U.S. Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, Maryland 21010

The actions of the irreversible cholinesterase inhibitor, diisopropylfluorophosphate (DFP), were investigated on spontaneous miniature synaptic potentials (MSPs) in co-cultures of G8-1 myotubes and NG108-15 neuroblastoma x glioma cells. Functional nicotinic cholinergic synapses were generated by adding ~50,000 NG108-15 cells to 35 mm culture dishes containing 10-12 day old G8-1 myotubes. Co-cultures were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% horse serum, 0.01 mM hypoxanthine, 0.016 mM thymidine and 1.0 mM dibutyryl cyclic-AMP. For intracellular recordings, the culture dishes were transferred to the heated stage (35°C) of a phase contrast microscope (200x) and impaled with 3M KCl filled microelectrodes (20-35 Megohm). Myotubes in contact with NG108-15 neurites or soma were examined for the presence of spontaneous MSPs in DMEM or DMEM containing DFP. Under control conditions, the percent of myotubes with MSPs increased from <3% after 1 day in co-culture to ~30% after 5 days of co-culture and remained near this level for the next 2 weeks. The MSPs were usually biphasic, consisting of a depolarizing phase that resembled miniature endplate potentials of vertebrate neuromuscular junctions, followed by a smaller hyperpolarizing component. The hyperpolarization reversed at ~ -75 mV and was insensitive to changes in extracellular Cl^-, suggesting that it is carried by K^+ ions. The MSP frequency ranged from 2 to 48/min (mean ± SEM = 17.2 ± 2.1/min, n=9 myotubes); the higher frequencies were generally associated with myotubes having multiple nerve contacts. The G8-1 acetylcholinesterase activity was extremely low in myoblasts (0.03 nmole ^{14}C-acetylcholine/min/mg protein) and in myotube cultures exposed for < 5 days to horse serum. The acetylcholinesterase activity gradually increased thereafter and reached maximal values of 1.1 nmole ^{14}C-acetylcholine/min/mg protein 10-12 days after the onset of fusion. To assess the role of cholinesterase on synaptic transmission, high concentrations of DFP (0.1 - 1.0 mM) were added to co-cultures exhibiting maximal muscle cholinesterase activity followed by extensive washing. In the presence of 0.1 mM DFP, the MSP frequency was reduced by almost 50% and the MSP amplitude decreased by 35%. However, the MSP time course was not significantly altered. Raising the DFP concentration to 1.0 mM caused further reductions in the MSP frequency and amplitude. The MSPs recovered to control values within 5-10 min of perfusion in drug-free solution. Further washing for up to 50 min caused no additional changes in the MSP amplitude or configuration. DFP at concentrations between 0.01 mM and 1.0 mM reduced the amplitude of focal microiontophoretic acetylcholine (ACh) potentials with an IC_{50} of 0.22 mM. This block showed no apparent voltage dependence between +50 and -100 mV. In the same concentration range, DFP depressed the slope and maxima of the steady-state ACh dose-response relationship, indicating a noncompetitive blockade. With prolonged incubation (~30 min), DFP concentrations $\geq$ 0.25 mM also enhanced the desensitization rate of the muscle membrane to ACh. DFP had little effect on either NG108-15 or G8-1 cells alone or in co-culture with regard to 1) action potential generation, and 2) passive electrical properties.

The results indicate that DFP blocks synaptic transmission by interfering with nicotinic receptor activation. The absence of marked potentiation in the MSP amplitude and decay following washout of excess DFP suggests that the myotube cholinesterase activity may be insufficient to influence the ACh lifetime. Alternatively, since the NG108-15/G8-1 synapses lack junctional folds, diffusion of ACh may be sufficiently rapid to terminate transmitter action.

SEPARATION OF RECYCLING AND RESERVE SYNAPTIC VESICLES FROM CHOLINERGIC NERVE TERMINALS OF THE MYENTERIC PLEXUS OF GUINEA-PIG ILEUM: Denes V. Agoston, Joseph W. Kosh, Panagiotis E. Giompres and Victor P. Whittaker, Abteilung Neurochemie, Max-Planck-Institut für biophysikalische Chemie, Postfach 2841, D-3400 Göttingen, F R Germany.

Acetylcholine-rich synaptic vesicles were isolated from myenteric plexus-longitudinal muscle strips derived from the guinea-pig ileum by the method of Dowe, Kilbinger and Whittaker (J. Neurochem. 35, 993-1003, 1980) using either unstimulated preparations or preparations field stimulated at 1 Hz for 20-30 min (Paton and Zar, J. Physiol. 194, 13-33, 1968) using pulses of 1 msec duration and 10 V/cm intensity. The organ contained either deuterated (d_4) choline (50 uM) or [^{3}H]acetate (2 uCi.ml^{-1}). d_4 acetylcholine was measured by gas chromatography-mass spectrometry. As with Torpedo electromotor cholinergic vesicle preparations made under similar conditions the distribution of newly synthesized (d_4 or [^{3}H]) acetylcholine in the zonal gradient from stimulated preparations was not identical with that of endogenous (d_0, [^{1}H]) acetylcholine, but corresponded to a subpopulation of denser vesicles (equivalent to the VP_2 fraction from Torpedo; Giompres, Zimmermann and Whittaker, Neuroscience 6, 775-785, 1981, and references contained therein) that had preferentially taken up newly synthesized transmitter. The density difference between the reserve (VP_1) and recycling (VP_2) vesicles was less than that observed in Torpedo but this smaller difference can be accounted for theoretically by the difference in size between the vesicles of the two tissues.

At rest, a lesser incorporation of labelled acetylcholine into the vesicle fraction was observed, and the peaks of endogenous and newly synthesized acetylcholine coincided. Stimulation in the absence of label followed by addition of label did not lead to incorporation of labelled acetylcholine, suggesting that the synthesis and storage of acetylcholine in this preparation and its recovery from stimulation is much more rapid than in Torpedo.

AXONAL TRANSPORT AND PHARMACOLOGICAL PROPERTIES OF ^{125}I-αBUNGAROTOXIN BINDING SITES IN RAT SCIATIC NERVE.

Elias Aizenman, William R. Millington, Marco A. Zarbin, George G. Bierkamper*, and Michael J. Kuhar. The Johns Hopkins University, Baltimore, MD. 21205, and The University of Nevada, Reno, NV. 89557*.

The specific objective of this study was to carry out an autoradiographic investigation that would demonstrate the presence and axonal transport, as well as determine the pharmacological properties, of ^{125}I-αBungarotoxin binding sites in peripheral nerves. The results of this project hope to provide basic information toward resolving the ongoing controversy surrounding the existence of functional cholinergic receptors in motoneuron terminals and sensory nerves.

Optimal binding conditions were determined in whole brain 8um frozen sections. These conditions were then used to label binding sites in ligated sciatic nerves (8um longitudinal frozen sections). Sections were preincubated (20 min, R.T.) in 50 mM PBS with or without displacer, then incubated (60 min, R.T.) in 2 nM ^{125}I-αBungarotoxin (200 Ci/mmol) plus or minus displacer, and washed in cold (4°C) buffer (15 min). Autoradiograms were produced after a 4-week exposure period on photographic emulsion-coated cover-slips apposed to the sections. Quantification of autoradiograms was performed by grain counting.

We observed that binding sites accumulate at both sides of the ligature in a time dependent fashion. A saturation analysis of the receptor-toxin interaction gave approximate dissociation constants (K_D) and receptor concentration (B_{max}) values of, respectively, 1.28 nM and 184 fmol/mg protein for proximally accumulating sites, and 0.55 nM and 121 fmol/mg protein for distal sites (12 hr. ligation). Displacement curves generated the following inhibition constants (K_I) in the reaction of the binding site with the toxin (for proximal and distal sites, respectively, in uM): 0.39 and 0.13, curare; 0.78 and 0.65, nicotine; 31.22 and 12.94, decamethonium; >300 and >300, atropine.

These observations suggest that:

(1) ^{125}I-αBungarotoxin binding sites are bidirectionally transported in the rat sciatic nerve;
(2) anterogradely and retrogradely transported sites have similar pharmacological properties;
(3) binding patterns have "nicotinic" characteristics; and,
(4) the binding site-toxin interaction is not "irreversible" as it is with muscle receptors.

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INTERACTION OF ANTICHOLINESTERASE AGENTS WITH THE ACETYLCHOLINE RECEPTOR COMPLEX: E.X. Albuquerque, K.-P. Shaw, A. Akaike, D. Rickett[1] and Y. Aracava. University of Maryland, School of Medicine, Baltimore, & [1] USAMRDC, Ft. Detrick, Maryland, U.S.A.

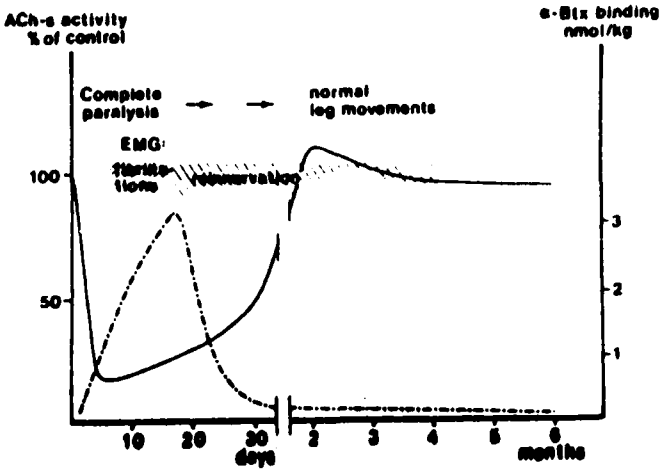
A group of anticholinesterase agents have been studied to define their actions as cholinesterase inhibitors and also to disclose other possible direct interactions with the acetylcholine receptor-ionic channel complex (AChR). In vivo as well as in vitro studies have been conducted with the organophosphate agents, soman and sarin, and also with neostigmine, physostigmine (PHY) and pyridostigmine (PYR). Pretreatment with either PHY or PYR appears to be most effective against lethal concentrations of soman and/or sarin in rats. Voltage clamp, patch clamp, and noise analysis studies have been performed in rat myoballs and interossal muscles of the frog toe. These studies disclosed that soman, sarin, neostigmine and PYR have weak agonistic properties, i.e., they activate the AChR and they also induce receptor desensitization. For example, neostigmine activates ionic channels with low opening frequency but with conductance (about 15 pS) and lifetime similar to that of ACh. PYR on the other hand activated channels with very low conductance (9-10 pS). However, neostigmine had significant agonistic effect at much lower concentration (20-50 μM) than PYR (100-200 μM) and as the concentration was raised this effect was combined with a receptor desensitization. In contrast, however, PHY (10-50 μM) did not have agonistic effect but acted mostly as an open channel blocker. The drug caused a marked decrease in channel lifetime but did not change channel conductance. The quaternary derivative of PHY also caused shortening of channel lifetime but only when it was applied inside the patch micropipet, that is, when the drug was able to reach the receptor from its outer surface. In contrast to quaternary PHY, PYR which is a quaternary compound, was able to cause its effects on the AChR whether it was applied via the bath or via the pipet using on the myoball or inside-out conditions. In addition, recordings of endplate currents (EPC) showed that quaternary PHY but not tertiary PHY at concentrations of 100 μM caused a double exponential decay of the EPC current recorded at positive holding potentials and at negative potential caused a markedly shortened, single exponential EPC decay phase. In conclusion, the anticholinesterase agents studied in addition to blocking cholinesterase, affect the AChR so that PYR and neostigmine are both weak agonists but with different potency, while the PHY, whether quaternary or tertiary, blocks the ionic channel of the nicotinic receptor primarily in open conformation. (Supported by U.S. Army Medical Research and Development Command Contract DAMD 17-81-1279.)

CHOLINERGIC MECHANISMS IN SPINAL CORD AND MUSCLE: CHANGES IN AMYOTROPHIC LATERAL SCLEROSIS (ALS): Sten-Magnus Aquilonius, Håkan Askmark and Per-Göran Gillberg, Department of Neurology, University Hospital, S-751 85 Uppsala, SWEDEN

Spinal cord: Choline acetyltransferase (ChAT) was assayed in small tissue samples punched out from cryosections of human, bovine, cat and rat spinal cords. Distribution patterns were similar in the different species with high activity in the ventrolateral part of the ventral horn and in the ventral root region. In the cord from man and cow high ChAT activity was demonstrated in the apical part of the dorsal horn. In ALS ChAT reductions were not restricted to the motor neuron area alone and the high ChAT activity area in the dorsal horn was also considerably reduced.
In autoradiographs of human and rat spinal cords very high ^{3}H-QNB binding was depicted in the motor neuron area and in the apical part of the dorsal horn. In this region of primary afferents muscarinic receptors may take part in the regulation of pain.

Muscle: The aim is to evaluate the potential applications of ChAT analysis and receptor quantitation for the purposes of diagnosis in clinical muscle biopsies. Acetylcholine (ACh) synthesizing activity and binding of nicotinic ligands (α-Btx, d-TC) were studied in different rat hind limb muscles during denervation and reinnervation (schematic illustration of results in figure). Further, changes in extrajunctional receptors have been visualized and quantitated by computer assisted analysis of in vitro ^{3}H-Btx autoradiographs of whole muscles.

Application of the aforementioned methods in biopsies from human biceps and leg muscles has so far been rather unsuccessful due to a high unspecific ACh synthesizing activity. As a guide for end-plate rich biopsies the topographical localization of end-plates in whole human muscles has been determined by staining large cryosections for AChE.



Schematic illustration of ACh synthesizing activity (——), ^{3}H-α-Btx binding (—·—·) and EMG in rat peroneus longus muscle following cryolesion of the sciatic nerve.

PURIFICATION OF RAT BRAIN BUTYRYLCHOLINESTERASE. PRODUCTION OF SPECIFIC IMMUNE SERUM AND IMMUNOLOGICAL LOCALIZATION OF THE ENZYME IN ADULT RAT CEREBELLUM : Françoise Barth and M. Said Ghandour, Centre de Neurochimie du CNRS, 5 rue Blaise Pascal, 67084 STRASBOURG Cedex, FRANCE.

Eighty per cent of adult rat brain butyrylcholinesterase (E.C.3.1.1.8.) is membrane-bound. An enzymatic preparation, enriched in activity about 700 fold, was obtained by solubilization at pH 4 with Triton X-100, followed by ammonium sulphate precipitation, ultrafiltration-dialysis and chromatography on carboxymethyl cellulose. ^{3}H-DFP, a specific cholinesterase ligand was used for identifying the constituent polypeptides of the butyrylcholinesterase molecule. The material corresponding to two of the bands was used to raise antisera in rabbits. The antisera obtained gave a single immunoprecipitate line and precipitated the brain butyrylcholinesterase. Immunological identity between soluble and membrane-bound butyrylcholinesterases was demonstrated by these antisera.
The cellular localization of BuChE in rat cerebellum was determined by immunofluorescence with the immune sera. The antisera specifically stains only glial cells.

DO MOTOR NEURONS CONTAIN FUNCTIONAL PRESYNAPTIC CHOLINERGIC AUTORECEPTORS?

George G. Bierkamper*, Elias Aizenman, William R. Millington, *University of Nevada School of Medicine, Reno, NV 89557 and The Johns Hopkins University, Baltimore, MD 21205, USA.

Presynaptic cholinergic autoreceptors may exist on motor nerve terminals and act to modulate acetylcholine (ACh) release in a negative feedback manner. This hypothesis has been the focus of recent investigations in our laboratory with the aims of proving the existence of the receptor, determining its pharmacologic characteristics, and demonstrating that it can alter ACh release. Binding studies with ^{125}I-alpha bungarotoxin (a-BGT) in the sciatic nerve provide evidence that a-BGT binding sites are transported axonally and that nicotinic drugs are very effective displacers, while atropine is not. These studies are presented elsewhere in this conference by Aizenman et al. Thus, it appears that the receptor is of the nicotinic type. Functionally, it is presumed that nicotinic agonists would decrease release and antagonists would increase release. The vascular perfused rat phrenic nerve-hemidiaphragm preparation (VPH) has been used to test the effects of agonists and antagonists on the release of ACh during nerve stimulation (10Hz). HEPES-buffered perfusion medium contained neostigmine (10^{-5}M) and choline chloride (10uM). ACh was measured by radioenzymatic assay. A single infusion of a-BGT (50ug), a nicotinic receptor antagonist, increased stimulated ACh release by approximately 70% one hr after infusion. d-tubocurarine was expected to increase ACh release, however, a significant decrease in release was observed. Two nicotinic agonists, nicotine (10^{-5}M) and carbachol (10^{-5}M) appeared to reduce ACh release by approximately 12% and 25%, respectively. Intracellular recordings from the VPH revealed a significant decrease in endplate potential amplitude of the 2nd, 3rd, and 4th events of trains of 5 stimuli (50Hz; 20ms apart) when carbachol was pressure injected through a micropipette onto the endplate region. These preliminary studies suggest that nicotinic ACh autoreceptors exist on motor nerve terminals and function in a negative feedback manner such that diminished synaptic cleft levels of ACh up regulate release; excessive cleft levels would down regulate the amount of ACh released. The mechanisms of control of this regulation (membrane changes; ACh synthesis; choline uptake?) and the temporal aspects of regulation have yet to be identified.

CHOLINERGIC HYPOFUNCTION AND DEMENTIA: RELATION OF NEURONAL LOSS IN DIFFERENT PARTS OF THE NUCLEUS BASALIS TO CORTICAL NEUROPATHOLOGICAL CHANGES: Volker Bigl[1], Thomas Arendt[1], Albert Tennstedt[2], and Alexander Arendt[3], Karl Marx University Leipzig, Paul Flechsig Institute for Brain Research, Dept. Neurochemistry (1) and Institute of Pathology (3). (2) Neuropathology Dept., District Hospital for Neurology and Psychiatry, Mühlhausen-Pfafferode, German Democratic Republic.

The severe reduction of cholinergic neurons in the nucleus basalis (NbM) in senile and presenile dementia (SDAT) as well as other forms of dementia is now well established. These neurons provide for most if not all of the cholinergic afferents to the cerebral cortex and the hippocampal formation, and their reduction in SDAT is reflected in a severe deficiency of cortical presynaptic parameters. This cholinergic cortical projection is topographically well organized, and the cholinergic input to the different cortical areas is provided by a relatively discrete population of NbM neurons.

From a neuropathological point of view neuritic plaques and tangles are the predominant diagnostic feature in SDAT. Nature and mechanisms of formation of the neuritic plaques are, however, still poorly understood. The accumulation or preservation of AChE activity within the plaques has led to the suggestion that they are remnants of degenerated cholinergic terminals.

In order to study the possible relationship between the loss of cholinergic afferents to the cortex and the development of plaques in more detail we performed quantitative counts of neuritic plaques in different cortical areas and related them to the neuronal loss in the different parts of the NbM. The cases for this study were selected post mortem on the basis of clinical history and neuropathological diagnosis. Total number of nucleolated large NbM neurons was counted in the different subdivisions of the NbM in every tenth section (20 um, cresyl violett stain) throuout the entire length of the cell population. The volume of the NbM was determined by planimetry. Sections of hippocampus, frontal (area 8 and 11), parietal (area 7), temporal (area 20), and occipital (area 17) cortex were stained by the Braunmühl technique and the neuritic plaques counted.

The individual cases show large variations in the overall neuronal loss in the NbM, the total number of neuritic plaques in the cortex as well as in the distribution of plaques in different cortical areas. In some cases significant differences in plaque counts between the two hemispheres were detected. One case each with predominantly affected occipito-temporal, temporal, occipital and frontal cortex, and hippocampus was selected for this study. As reported previously loss of cholinergic neurons in SDAT is markedly different in the different subpopulations of the NbM. Taking into account the topography of this cholinergic pathway as studied in rat and monkey, the distribution of neuritic plaques in different cortical areas seems to match the loss of those NbM neurons which innervate the predominantly affected cortical areas.

$GABA_A$ VS. $GABA_B$ MODULATION OF SEPTAL-HIPPOCAMPAL INTERCONNECTIONS:

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Previous studies in this laboratory have demonstrated a GABAergic modulation of the cholinergic septal-hippocampal pathway, in that, for example, intraseptal injection of the $GABA_A$ agonist muscimol into the rat decrease the turnover rate of acetylcholine (TR_{ACh}) in the hippocampus in a dose-dependent manner. This is in keeping with histochemical evidence of a substantial GABAergic innervation of the septal nuclei. On the other hand, numerous behavioral studies have shown cholinergic components in these limbic structures to be involved in response inhibition, disruption of which leads to innappropriate responding during extinction of a food-reinforced lever press response. The present study was thus undertaken to correlate the behavior with GABAergic modulation of the cholinergic septal-hippocampal system. We shall differentiate the behavioral effects associated with GABAergic inhibition of the septal-hippocampal cholinergic pathway from those associated with substance P inhibition of the pathway. In addition we will show that $GABA_A$ receptor activation in the septum produces effects different from those of $GABA_B$ receptor activation.

Generally, the experimental protocol involved implantation of chronic septal cannulae into rats, followed by several training sessions on a continuous reinforcement schedule. On the day of the experiment, rats were intraseptally injected with the appropriate drug and placed in the operant chamber 15 minutes later. Five minutes of continuous reinforcement was followed by 10 minutes of non-reinforcement (extinction). Immediately afterwards, deuterated phosphorylcholine was infused via the lateral tail vein for the determination of the TR_{ACh}.

Doses of muscimol (.3 to 3 nmoles) which decreased the hippocampal TR_{ACh} also increased the response rate during extinction. Higher doses (10-30 nmoles) further decreased the TR_{ACh} in the hippocampus, decreased the TR_{ACh} in the cortex, and were accompanied by sedation. Intraseptal bicuculline, a $GABA_A$ antagonist, in equivalent doses had the opposite behavioral effect, i.e. a general decrease in responding. In addition preliminary experiments have shown that intraseptal beta-endorphin, which decreases the hippocampal TR_{ACh} via an activation of GABAergic interneurons (i.e. in a bicuculline-reversible manner), has a behavioral effect similar to that of muscimol. Conversely, intraseptal substance P, which decreases the hippocampal TR_{ACh} independently of a GABAergic mechanism, has no behavioral effect in this paradigm. All these results indicate that a $GABA_A$ type modulation of the cholinergic projections from the septum to the hippocampus may be an important influence on extinction.

Intraseptal baclofen, a $GABA_B$ agonist, has behavioral effects similar to muscimol, is 5-10 times more potent but fails to change hippocampal TR_{ACh}. Three to five weeks after bilateral injection of kainic acid into the hippocampus, there is a significant loss of bicuculline-insensitive GABA binding ($GABA_B$ sites) in the septum, but no loss of baclofen-insensitive GABA binding ($GABA_A$ sites). This loss is presumably due to the degeneration of the glutamatergic hippocampal pyramidal cells which have terminals in the lateral septum. We hypothesize that both muscimol and baclofen exert their behavioral effects by decreasing the functional output of the hippocampus. The action of muscimol includes an inhibition of the stimulatory cholinergic input to the hippocampus, whereas baclofen acts as a presynaptic inhibitor of hippocampal efferents which terminate in the septum. This interpretation is in agreement with the results of others indicating baclofen to be a presynaptic inhibitor of excitatory amino acid release.

RED CELL CHOLINE IN DEMENTIAS: John Blass, Israel Hanin*, Laurie Barclay, Ursala Kopp*, and Michael Reding, Cornell Univ. Medical College, White Plains, NY, 10605, and the *Univ. of Pittsburgh School of Medicine, Pittsburgh, PA, 15213, USA.

Abnormal elevations of red cell choline have been reported in a lengthening list of neuropsychiatric disorders, including (in three brief communications) Alzheimer's disease. We, therefore, did a prospective study of 105 persons in a dementia clinic, measuring red cell/plasma choline ratios to allow for dietary variations.

Patients were classified clinically in White Plains before choline analyses were done. The diagnosis of dementia requires evidence of global cognitive impairment of more than three month's duration in a clear sensorium. Alzheimer's disease (DAT) was diagnosed in 50 patients who had the typical insidious onset, progressive course, and no other adequate explanation for the dementia; they included four in whom dementia developed on a background of Parkinson's disease. The 20 patients with multi-infarct dementia (MID) had a relatively sudden onset, stuttering downhill course, and evidence of vascular disease with modified Hachinski scores of four or more. Nine patients with "mixed dementia" had characteristics of both DAT and MID. In ten others, specific causes such as chronic schizophrenia or syphilis were adequate to account for the symptoms. The 16 intellectually intact subjects were normal on cognitive and behavioral tests used, although some had other types of nervous system dysfunction. The DAT and non-DAT groups were adequately matched for age (70 ± 1 vs 74 ± 2) and sex (33/50 vs 25/55 female), and the degree of cognitive and behavioral impairment was similar in DAT and non-DAT demented patients.

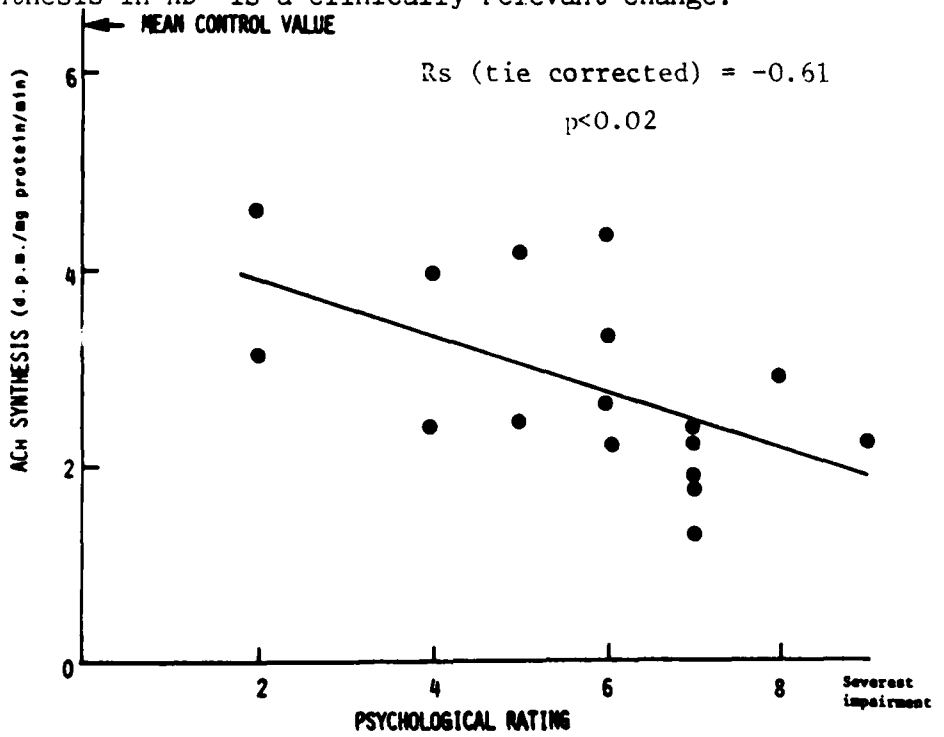
Choline measurements were done in Pittsburgh, without knowledge of the clinical classifications of the patients, on samples which had been promptly deproteinized and acidified to ensure stable, reliable choline values.

The ratio of choline in red cells to plasma was higher in DAT than non-DAT subjects (3.8 ± 0.5 vs 2.0 ± 0.2, $p<0.001$). Values for the different non-DAT groups varied from 1.7 to 2.1. Elevations above 2.8 occured in some patients in all groups, however, although more frequently in DAT than non-DAT (23/50 vs 6/55). Elevation of red cell choline is not a specific, let alone a diagnostic finding in DAT.

The tendency for red cell choline to be elevated in DAT is, however, part of an increasing body of evidence that Alzheimer's disease manifests itself systemically as well as in the nervous system.

REDUCED ACETYLCHOLINE (ACh) SYNTHESIS IN ALZHEIMER'S DISEASE (AD) IS A CLINICALLY RELEVANT CHANGE: David M.Bowen*, David Neary**, Neil R.Sims* and Julie S.Snowden**, *Institute of Neurology, London and **Department of Neurology, Royal Infirmary, Manchester, U.K. (N.R.Sims is now at Burke Rehabilitation Center, White Plains, New York, U.S.A).

AD patients in the presenium were drawn from a larger series of young individuals, with progressive dementia and cortical atrophy in whom the precise clinical diagnosis had been uncertain. The general aim of this collaborative investigation has been to provide a diagnostic classification of this fatal group of disorders and to unearth potentially reversible changes which might be drug responsive. A psychological rating of magnitude of dementia from 0-9 was obtained on the basis of patients performance on a variety of mental tests. Disorders of language, perceptuo-spatial function and memory were each rated from 0-3, in terms of perceived extent of impairment (absent, mild, moderate or severe), and the overall psychological rating represented a cumulation of these three assessments. Fresh neocortex from the non-dominant middle temporal gyrus was obtained at diagnostic craniotomy. It was processed to yield tissue prisms for measuring ACh synthesis by incorporation of [U-^{14}C] glucose into [^{14}C] ACh[1]. Incubations were carried out in a high concentration of choline (2 mM) and K^+ (31 mM). The figure shows that a significant relationship (Spearman's Rank Correlation Test) exists between severity of dementia and [^{14}C] ACh synthesis. All samples showed the histological hallmarks of AD. The amount of radioactive transmitter formed is thought to be a genuine measure of the mass of ACh synthesized[1] so the present results demonstrate for the first time that reduced ACh synthesis in AD[2] is a clinically relevant change.



1. Sims NR, Bowen DM, Allen SJ, Smith CCT, Neary D, Thomas DJ & Davison AN. (1983) J.Neurochem. 40, 503-509.
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QUICK FREEZING PRESERVES A LARGE UNSTABLE VESICLE POPULATION IN TORPEDINE ELECTRIC ORGAN NERVE TERMINALS. Alan F. Boyne and Thomas E. Phillips, Department of Pharmacology and Experimental Therapeutics, University of Maryland, Baltimore, Md 21201 and Department of Pharmacology, Northwestern Medical School, Chicago, Ill 60611.

The elegant application of the quick freezing technique to neuromuscular junctions by Drs. John Heuser and Thomas Reese and their collaborators has put an end to the era of reliance on aldehyde fixations in the correlation of ultrastructure and quantitative details of electrophysiology. We have attempted to apply the same technique to Torpedine electric organ nerve terminals in the hope of resolving some of the biochemical controversies associated with that sytem.

We have quick frozen isolated stacks of Narcine brasiliensis electric organ from unstimulated fish, using a commercially available, liquid nitrogen based, bounce-free, Quick freezing system and have made the following observations.
(1). There is a large, heretofore unacknowledged population of synaptic vesicles attached to the presynaptic side of the nerve terminal membrane. In Narcine b., 15 to 20% of the vesicle population occupy 30% of the available presynaptic locations.
(2). Vesicles only attach to areas of the nerve terminal which are overlaid with basal lamina; this requirement correlates with a lack of vesicle attachments on the 'back' of the terminals, which face the intercellular space
(3). In recent freezings of juvenile (7day) specimens of Torpedo omata, we have found that almost all of the vesicles in the small terminals are in attachment sites on the presynaptic face.

We suggest the following conclusions:
(i) Membrane-attached vesicles are the immediate source of the 'metabolically active' vesicle population which develops in slowly stimulated electric organ.
(ii) They are also the' membrane operators' or 'vesigates' postulated in different laboratories to explain biochemical data.

The loss of these vesicles in aldehyde fixation is variable, being minimized in a fixation medium containing 90 mM Na oxalate. This implies that the presence of traces of Ca in more conventional media contributes to their presumed exocytotic discharge. Their preferential location in these unstable sites in developing terminals suggests that previous arguements that quantal release begins before vesicles appear may need revision.

We further suggest that selective Ca uptake by these vesicles, which would be exposed to to the maximal concentrations of Ca entering during nerve terminal depolarization, could drive selective ACh turnover in this specific population, thereby assuring the preferential release of newly synthesized neurotransmitter.

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BRAIN ACETYLCHOLINE IN HYPERTENSION AND BEHAVIOR: STUDIES USING N-(4-DIETHYL-AMINO-2-BUTYNYL)-SUCCINIMIDE: Henry E. Brezenoff, Norman Hymowitz, Rachel Giuliano and Willie Mae Coram. University of Medicine and Dentistry of New Jersey, Newark, New Jersey 07103

N-(4-diethylamino-2-butynyl)-succinimide (DKJ-21) is reported to be a relatively selective antagonist of muscarinic receptors in the central nervous system (Dahlbom et.al., Life Sci. 5, 1625, 1966). We have used this compound to extend our studies of the role of brain acetylcholine (ACh) in hypertension and behavior.

In the mature unanesthetized spontaneous hypertensive rat (SHR), intracerebroventricular injection of hemicholinium-3 or i.v. administration of either atropine (0.5-5 mg/kg) or DKJ-21 (3-25) mg/kg) caused dose-dependent falls in blood pressure. When monitored non-invasively by tail cuff occlusion, blood pressure remained depressed for more than 24 hours. Antihypertensive doses of these drugs inhibited the centrally mediated pressor response to physostigmine but did not inhibit the pressor responses to noradrenaline or angiotensin. Atropine, but not DKJ-21, blocked the vasodepressor effect of ACh injected i.v. Intracerebroventricular injections of DKJ-21 also reduced blood pressure in the SHR. In contrast, neither atropine nor DKJ-21 caused a significant hypotensive response in unanesthetized normotensive rats.

Experiments have been conducted in rats trained to pressure a lever on a fixed-interval 50-sec schedule of food reenforcement. Physostigmine inhibits responding in a dose dependent manner. Likewise, both atropine and methylatropine inhibit responding in doses above 0.1 mg/kg. The effects of the antimuscarinic drugs appear to be related to xerostomia, since partially chewed food pellets were found in the food bin and excreta trays. Neither atropine nor methylatropine were able to reverse the inhibitory effects of physostigmine. In contrast, DKJ-21 (40-60 mg/kg prevented the inhibitory effects of physostigmine, while doses as high as 120 mg/kg did not alter responding when given alone.

LONG TERM POTENTIATION OF CHOLINERGIC SYNAPTIC TRANSMISSION IN A SYMPATHETIC GANGLION: Clark A. Briggs, Richard E. McCaman and Donald A. McAfee, Div. Neurosciences, Beckman Research Inst. of the City of Hope, Duarte, CA 91010.

In the rat superior cervical ganglion, the efficacy of nicotinic synaptic transmission is increased for hours following brief tetanic stimulation of the preganglionic nerve at 20 Hz for 10-20 seconds (T.H. Brown and D.A. McAfee (1982) Science 215:1411; C.A. Briggs, et al. (1983) Am. Soc. Neurochem. 14: abs. 115). Postganglionic compound action potentials and nicotinic postsynaptic potentials were increased during this long-term potentiation (LTP) in the absence of correlated changes in postsynaptic membrane potential or resistance. Therefore, we have tested the hypothesis that LTP results from an increase in release of acetylcholine (ACh) from preganglionic terminals. The ACh content of the ganglion and the release of ACh into the bathing medium was measured by the choline kinase - ^{32}P-ATP assay (R.E. McCaman and J. Stetzler (1977) J. Neurochem. 28:669).

For the measurement of ACh content, homologous pairs of ganglia were superfused in vitro at 22°-24°C with oxygenated Locke's solution containing 10 uM choline. Initially, control measurements were made in which neither member of the pair was stimulated. The ACh content of single non-stimulated ganglia averaged 1.4 ± 0.1 pmol/ug protein (mean ± SEM, n = 12). The difference between members of unstimulated pairs was 0.2 ± 0.1 pmol/ug protein (n = 6), with each difference considered a positive number. The ACh content was not increased by stimulating one member of a pair at (a) 20 Hz for 20 seconds followed by a 5 min rest period or (b) 20 Hz for 60 min followed by a 17 min rest period. The differences between stimulated minus unstimulated paired control ganglia for (a) were 0.2 ± 0.1 pmol/ug protein (n=6) and for (b) were 0.01 ± 0.1 pmol/ug protein (n=6). We conclude that rat ganglia do not show an increase in ACh content following stimulation.

For measurement of ACh release each ganglion was bathed in a 70 ul chamber suitable for electrophysiological measurements at 22°-24°C in oxygenated Locke solution containing 20 uM eserine, 2 uM atropine, and 100 uM d-tubocurarine or 200 uM hexamethonium. Samples for ACh assay were obtained by withdrawing 50 ul of the bath and replacing it with 50 ul of fresh medium once every five minutes. ACh release was evoked by stimulating the preganglionic nerve at 0.2 Hz. After an initial 25 min, control ACh release was stable at 0.61 ± 0.02 to 2.10 ± 0.07 pmol/5 min in different experiments. As expected, ACh release was greatly increased during a preganglionic tetanus (20 Hz, 20 sec) sufficient to induce LTP. In addition, ACh release was increased to a lesser extent following the tetany by an average of 0.4 pmol/5 min or 31% in five experiments. The potentiation of ACh release and of compound action potential amplitude showed little or no decay from 10 min to 60 min following the tetanus. Basal release, or that in the absence of preganglionic stimulation, averaged 0.30 ± 0.03 pmol/5 min in three experiments. Stimulation at 20 Hz for 20 sec did not alter subsequent basal release. Therefore, tetanic stimulation increased evoked release with little or no contribution from spontaneous release. Similarly, ACh released during the tetanus cleared the ganglia during the first ten minutes of the post-tetanic period.

Thus, in the rat superior cervical ganglion, a single 20 Hz, 20 sec tetanus results in prolonged increase in the amount of ACh released per preganglionic impulse, without measureably increasing ACh content. We propose that this presynaptic mechanism underlies long-term potentiation.

This work was supported by American Heart Association fellowship 766 F1-1, PHS grant NS 18857, and NSF Grant BNS 12414.

DIFFERENTIAL EFFECTS OF CARBACHOL AND OXOTREMORINE ON MUSCARINIC RECEPTORS, CYCLIC AMP FORMATION, AND PHOSPHOINOSITIDE TURNOVER IN CHICK HEART CELLS: Joan Heller Brown and Susan L. Brown, University of California, San Diego, La Jolla, California 92093, USA

We have studied two biochemical responses elicited by cholinergic agonists in embryonic chick heart cells, inhibition of catecholamine-stimulated cyclic AMP formation and stimulation of phosphoinositide hydrolysis. Our objective was to determine whether the same receptor mediates these two responses. Both responses are activated by carbamylcholine (carbachol) and the effect of carbachol on either response is blocked by atropine but not by d-tubocurare. Pirenzepine also inhibits the carbachol-stimulated cyclic AMP and phosphoinositide responses. Antagonist dose response curves suggest that the muscarinic receptors mediating the two responses do not differ in their affinities for antagonists. There is, however, a marked difference in the agonist dose-response relationships for the two responses: the carbachol concentration necessary for half-maximal inhibition of cyclic AMP formation is 2×10^{-7} M while 2×10^{-5} M carbachol is necessary to half-maximally stimulate phosphoinositide hydrolysis. The specific muscarinic agonist oxotremorine also inhibits cyclic AMP formation, and is about 10 times more potent than carbachol in this regard. In contrast phosphoinositide hydrolysis is not stimulated by oxotremorine. Oxotremorine apparently interacts with the receptors mediating phosphoinositide hydrolysis, because it causes a concentration-dependent blockade of the response to carbachol. Thus oxotremorine behaves as an antagonist, binding but not causing the receptor changes leading to the phosphoinositide response. One explanation for these data is as follows: carbachol and oxotremorine both induce or recognize a state of the receptor with high agonist affinity and it is this receptor state that mediates the cyclic AMP response; only carbachol induces the low-affinity state of the receptor necessary for phosphoinositide hydrolysis. The two receptor states do not differ in antagonist affinities.

We used radioligand binding studies with [^{3}H]QNB to study muscarinic receptors on intact chick heart cells. There is a homogeneous population of [^{3}H]QNB binding sites with an affinity of 12 pM. Pirenzepine also binds to all receptor sites with a single apparent affinity. Agonist competition experiments demonstrate differences in the binding of carbachol and oxotremorine. The carbachol-[^{3}H]QNB competition curve is biphasic and the data are best fit by a model in which 60% of the sites have high and 40% have low agonist affinity. The oxotremorine competition curve is monophasic, suggesting that all of the sites have the same high affinity for oxotremorine. These data support the notion that carbachol causes formation of a low-affinity receptor state that is necessary for the phosphoinositide response and is not formed in the presence of oxotremorine. There are alternative interpretations of the data, including the possibility that the low-affinity state is a desensitized form of the receptor, and that a single receptor state could mediate both responses if the efficiency of receptor-effector coupling differed for these responses. Regardless of which interpretation is correct our findings demonstrate that the muscarinic receptor which couples to adenylate cyclase and that which couples to the phosphoinositide response are distinguished by their sensitivity to agonists and responsiveness to oxotremorine. The use of these "selective" agonists should provide a means for elucidating the role of receptor-mediated change in cyclic nucleotide and phosphoinositide metabolism in subsequent cellular events.

PARASYMPATHETIC INNERVATION OF THE HEART: ACETYLCHOLINE TURNOVER IN VIVO. Oliver M. Brown, Joseph J. Salata and Lynne A. Graziani, State University of New York-Upstate Medical Center, Syracuse, New York, 13210, USA.

In an effort to better describe the parasympathetic innervation of the heart we have employed a direct chemical approach which allows for the characterization of the dynamics of cardiac acetylcholine (ACh). We report a method and description of in vivo choline (Ch) uptake and ACh synthesis in rat heart. Deuterium labeled Ch was pulse injected (i.v.) into rats anesthetized with chloral hydrate and pentobarbital. The animals were sacrificed at various times following injection and their hearts were removed and homogenized. Samples were extracted and analyzed for labeled and unlabeled Ch and ACh by pyrolysis mass fragmentography (PMF). From these data we calculated the specific activities of Ch and ACh, rate constants for ACh and turnover rates of ACh. Rat hearts were found to accumulate deuterated-Ch rapidly and efficiently such that 35% of total heart Ch was labeled at 30 sec. From this maximum, the specific activity of Ch decayed in two phases. There was an initial rapid fall from 30 sec to 2 min with a half-time of 2.2 min, and a more gradual decline between 2 and 60 min with a half-time of 28.2 min. This slower decay was described by the regression line, log y = -0.0107x -0.677. A portion of the labeled Ch was synthesized by the heart into deuterated-ACh. The specific activity of ACh rose to a maximum of about 0.11 at 1 min and declined thereafter. Between 2 and 60 min the decline had a half-time of 28.8 min (log y = -0.0106x -1.030). Thus, following an initial equilibration period of approximately 2 min, activities of Ch and ACh decayed in a parallel manner, with near identical half-times and regression slopes. It appears that we have a precursor-product relationship that follows steady-state kinetics. Using a kinetic model with steady-state and open-system assumptions, we calculated the in vivo turnover rate of ACh in rat heart to be 0.144 nmol/g/min. These results indicate that the total pool of ACh in the in vivo rat heart turns over approximately once every hour.

Some preliminary experiments were performed to study the effects of electrical stimulation of the vagus nerves on ACh synthesis in the in vivo rat heart. Deuterated-Ch was injected into 30 rats as described above, at time zero. At 2 min 15 rats received pulse train electrical stimulation to both cervical vagus nerves (controls were not stimulated). At 5 min all rats were sacrificed and their hearts were dissected (in situ) into two areas: a) right atrium + S.A. node (the area richest in ACh), and b) the remainder of the heart. The pulse train stimulation allowed us to entrain the heart rate; which we did to a rate that was 75% of the resting (pre-stimulation) rate. Vagal stimulation resulted in an increase in the specific activity of ACh in both areas examined: the right atrium + S.A. node increased from .17 to .25 with electrical stimulation, the remainder from .059 to .076. These preliminary results indicate that the present model is responsive to perturbations in ACh turnover. And that the cardiac deceleration resulting from vagal stimulation is accompanied by an increase in the in vivo synthesis rate of ACh.

INHIBITION OF BRAIN ACETYLCHOLINE (ACH) BIOSYNTHESIS BY CLONIDINE AND METHYLDOPA: RELEVANCE TO HYPERTENSIVE DISEASE: Jerry J. Buccafusco, Medical College of Georgia and Veterans Administration Medical Center, Augusta, Georgia 30912, USA.

Clonidine (C) and methyldopa (M) are clinically employed antihypertensive drugs having a central mechanism of action. While both drugs act through stimulation of central alpha adrenergic receptors, substantial evidence exists which indicates that central catecholaminergic neurons are not required for C and M to lower blood pressure. Our recent experiments in normotensive rats and in mice have demonstrated that C can produce marked inhibition of the biosynthesis of regional brain ACh. The purpose of this study was to determine whether C and M could inhibit the biosynthesis of brain ACh in a model of human hypertension, the spontaneously hypertensive rat (SHR). These animals were surgically prepared with chronic indwelling arterial and venous catheters and an intracerebroventricular (icv.) cannula guide. At the time of the experiment arterial pressure was recorded from freely-moving SHR. The biosynthesis of brain ACh was estimated by measuring the formation of ^{3}H-ACh following icv. injection of 20μ Ci of ^{3}H-choline. Endogenous ACh also was measured and specific activity-time curves for regional brain ACh obtained for control SHR and for SHR pretreated with M (200 mg/kg,iv.). Calculation of relative turnover rates revealed a 34-54% reduction by M with the greatest effect occuring in the medulla oblongata and hypothalamus. This inhibition of brain ACh synthesis was accompanied by a significant fall in arterial pressure of 53/28 mmHg. C (30μ g/kg, iv.) elicited a time dependent fall in arterial pressure of 18/16 and 27/22 mmHg by 10 and 100 min after injection, respectively. This was associated with a time-dependent reduction in brain ACh synthesis where in the most sensitive regions, the caudal hypothalamus and the pons the formation of ^{3}H-ACh was reduced 10% and 21% at the 10 min point and 44% at the 100 min point, respectively. At the 100 min time point significant inhibition of ^{3}H-ACh formation also was observed for the rostral hypothalamus (40%), medulla oblongata (45%), thalamus-septum (28%) and midbrain (30%). The striatum, which initially exhibited the greatest cholinergic activity, was not similarly affected by clonidine. In the last series of experiments the specific activity of brain ACh was measured 2 min following icv. ^{3}H-choline in SHR and in normotensive control animals. For the brain regions thus far examined ACh synthesis was significantly increased by 56% and 50%, respectively, in rostral and caudal hypothalamus. No difference was observed for cortex or hippocampus. These findings indicate that experimental hypertension is associated with enhanced cholinergic activity in certain brain regions perhaps localized to the hypothalamus or lower brainstem. The ability of C and M to reduce this exaggerated cholinergic activity in SHR to levels observed for the normotensive animal suggests that these agents, at clinically relevant doses, produce their antihypertensive effects though a central "anticholinergic" action. The fact that hypertension is produced in man following chemical stimulation of brain cholinergic receptors may point to similar conclusions concerning some forms of hypertensive disease.

CHOLINERGIC SYSTEMS IN THE CENTRAL NERVOUS SYSTEM: Larry L. Butcher and Nancy J. Woolf, Department of Psychology and Brain Research Institute, University of California, Los Angeles, California, 90024, USA.

Using a combined procedure that permits the visualization on the same tissue section of (1) retrogradely and anterogradely transported neuronal labels, (2) immunohistochemically demonstrated choline-O-acetyltransferase [polyclonal and monoclonal antibodies], (3) acetylcholinesterase [pharmacohistochemical regimen], and (4) Nissl substance and myelin, we have mapped the distribution and projection patterns of putative cholinergic neurons in the central nervous system of the rat. Such neurons evince two organizational schemata: those that are intrinsically organized, local circuit cells and those that are projection neurons. Local circuit neurons are found in the caudate-putamen complex, nucleus accumbens, olfactory tubercle, and probably also the cerebral cortex and hippocampus. Five separable, but not necessarily mutually exclusive, systems of presumed cholinergic projection neurons have been ascertained: (1) the basal forebrain cholinergic system [components: medial septal nucleus, nuclei of the vertical and horizontal limbs of the diagonal band, ventral pallidum/lateral preoptic area, nucleus preopticus magnocellularis, bed nucleus of the stria terminalis, nucleus basalis, and nucleus of the ansa lenticularis] projecting widely to the cerebral cortex, hippocampus, olfactory bulbs, amygdala, and brainstem, particularly the habenular nuclei and interpeduncular nucleus, (2) the pedunculo-pontine complex and laterodorsal tegmental nucleus projecting to the thalamus, habenula, interpeduncular nucleus, pretectal nuclei, tectum, and possibly also the globus pallidus, entopeduncular nucleus, subthalamic nucleus, lateral hypothalamus, and basal forebrain cholinergic complex, (3) the brainstem reticular formation that putativley provides, in part, cholinergic afferents to the spinal cord, (4) the various somatic and parasympathetic cell bodies of cranial nerves III-VII and IX-XII, and (5) various somata in the spinal cord, including prominently the α- and γ-motor neurons of the ventral horn and the preganglionic sympathetic neurons of the intermediolateral cell column at thoracic and lumbar levels. No doubt additional cholinergic systems and refinements in the descriptions of existing cellular aggregates and pathways will be ascertained and and delimited as more data become available.

Despite the fact that the neuroanatomy of central cholinergic systems is becoming increasingly understood, there is presently a relative paucity of information concerning their physiology. Nonetheless, various experimental findings suggest that some of these systems are involved in neuronal processes operating at the highest levels of brain function and integration, including learning and memory, sleep and wakefulness, and motor behavior.

DEPOLARIZATION INDUCED HYDROLYSIS OF CYTOPLASMIC ACh IN MOUSE BRAIN: Paul T. Carroll, Department of Pharmacology, Texas Tech Univ. Health Science Center, Lubbock, Texas 79430.

Recent reports suggest that neither depolarization of motor nerve terminals nor depolarization of brain tissue stimulates the Ca^{2+} independent, spontaneous release of ACh (Katz & Miledi, 1981; Meyer & Cooper, 1981; Gibson & Peterson, 1981). In both preparations, this form of ACh release appears to occur from the cytoplasm (Katz & Miledi, 1977; Carroll & Aspry, 1980; Carroll, 1983). To ascertain why depolarization fails to stimulate the Ca^{2+} independent, spontaneous release of ACh, minces of mouse forebrain were incubated in a Krebs solution without added Ca^{2+} and 0.1mM EGTA and the effects of elevated K^+ and veratridine on the subcellular levels of ACh determined. Both agents significantly reduced the level of cytoplasmic (S_3) ACh without changing the ACh content of the vesicular fraction (P_3) or stimulating ACh release into the medium. Upon addition of Ca^{2+}, 2 changes occurred, a stimulation of ACh release and a significant reduction in the level of P_3 ACh. The choline formed from the veratridine-induced but not the K^+-induced hydrolysis of S_3 ACh was transferred to the P_3 fraction. Pretreatment of minces with "penetrating" AChE inhibitors enabled both depolarizing agents to augment the Ca^{2+} independent, spontaneous release of ACh, possibly by inhibiting an intraterminal form of AChE responsible for the depolarization-induced breakdown of S_3 ACh. To determine whether the substrates produced by the hydrolysis of S_3 ACh might be used for the formation of ACh released from the vesicular fraction, minces were initially pretreated in a Ca^{2+} free Krebs or a Ca^{2+} free 35 mM K^+ Krebs, the latter pretreatment being used to selectively lower the ACh content of the S_3 fraction and make less available for hydrolysis during subsequent depolarization. Both sets of pretreated minces were then incubated in Krebs with or without veratridine and the veratridine-induced release of ACh determined as a function of pretreatment (Veratridine was used to depolarize brain tissue rather than elevated K^+ because the choline formed from veratridine--induced hydrolysis of cytoplasmic ACh is transferred to the P_3 fraction whereas the choline formed from the K^+ induced hydrolysis of cytoplasmic ACh is not). The results indicated that a selective loss of S_3 ACh prior to depolarization reduced the amount of ACh released by veratridine during a 10 min period of incubation but not during a 5 min period.

In summary, depolarization of brain tissue with veratridine or elevated K^+ appears to hydrolyze cytoplasmic ACh rather than stimulate its release. However, if the intraterminal form of AChE is sufficiently inhibited prior to depolarization, then depolarization of brain tissue with these agents will stimulate the Ca^{2+} independent, spontaneous release of ACh. It is suggested that the intraterminal ACh hydrolysis caused by depolarization may serve a supplementary role to extraterminal ACh hydrolysis in providing substrate for the formation and release of ACh from vesicles (supported in part by NSF grant BNS 8117975).

BIOCHEMICAL AND BEHAVIORAL EFFECTS OF AF64A IN THE RAT: Fiorella Casamenti, Laura Bracco, Felicita Pedata and Giancarlo Pepeu, University of Florence, Dept. of Pharmacology, 50134 Florence, Italy.

Ethylcholine mustard aziridinium ion (AF64A) is a selective neurotoxin for the cholinergic neurons. In this study some of its neurochemical and behavioral effects were investigated in the rat, 7 and 20 days after administration.
Male wistar rats (140-150 g body weight) were injected i.c.v with 32nmol in 10 ul of AF64A. Shortly after injection the rats showed hyperexitability and abnormal posture, within a week they lost 20-30% of their body weight and the mortality was between 30 and 50%. In the surviving rats showing no gross neurological symptoms ChAT activity and high affinity choline uptake (HACU) in the cerebral cortex, hippocampus and striatum, as well as ACh release from the cerebral cortex were measured. Seven days after injection ChAT activity showed a 46% decrease in the hippocampus, 30% in the striatum and 26% in the cerebral cortex. Only a small, statistically not significant decrease in HACU was found in the hippocampus and 20% decrease in the cerebral cortex. No difference was detected in ACh release from the cerebral cortex between rats treated with AF64A and its solvent, nor was the stimulatory effect of amphetamine (1 mg/kg i.p.) on ACh release affected by AF64A treatment. Twenty days after injection the rats had recovered their body weight, but ChAT activity still showed a 41% decrease in the hippocampus; at the same time, its levels were normal in the cerebral cortex and striatum. A 27% reduction in hippocampal HACU was also found. These rats, tested in a one trial, passive-avoidance conditioned response 3hs before sacrifice, revealed an impairment of the response, since retest latency was 93$\pm$ 3 sec in the vehicle treated (n=40) and 56$\pm$ 7 sec in the AF64A treated animals (n=21 $P<0.001$). In conclusion, i.c.v. administration of AF64A produces a long lasting decrease in the presynaptic cholinergic markers only in the hippocampus, while the alterations in cortical and striatal cholinergic mechanisms are rapidly reversible. The hippocampal cholinergic deficit appears to be associated with a cognitive impairment.
This work was supported by CNR grant n 8202043.04

ACETYLCHOLINE - PROSTANOID INTERACTION IN THE PULMONARY CIRCULATION: John D. Catravas, Jerry J. Buccafusco and Hassan El-Kashef, Medical College of Georgia, Augusta, GA, 30912, USA.

The pulmonary and systemic vascular beds differ in their response to several vasoactive substances. Acetylcholine (ACh) is a potent systemic vasodilator, however its effects on the pulmonary circulation are not clear. Utilizing the isolated rabbit lung preparation perfused *in situ* under constant flow (92 ± 3 ml/min) with a physiologic salt solution, we studied the effects of graded concentrations of ACh (10^{-10} - 10^{-4} M) in the recirculating perfusate on pulmonary arterial pressure (PAP) and pulmonary vascular resistance (PVR). The lungs were ventilated to 15 cm water with 97% O_2 - 3% CO_2. ACh (10^{-8} - $4x10^{-7}$ M) produced a dose-dependent increase in PAP and PVR which required up to 20 min to return to pre-drug levels. Physostigmine (10^{-5} M) maintained the maximal PAP response to ACh for the duration of the experiment (up to two hours). Higher concentrations of ACh produced rapid and sustained pulmonary hypertension which resulted in irreversible edema. Increasing the resting pulmonary arterial vascular tone by pre-treating with KCl (10 mM) or phenylephrine (0.1 mM) failed to affect the pressor actions of ACh. Pre-treatment or post-treatment with atropine (10^{-5} M) completely prevented or reversed the pressor effects of ACh, respectively. There was a significant delay (up to 20 min), however, in the reversal of the ACh-induced pulmonary hypertension by atropine, suggesting the existence of additional mediator(s) of the pressor actions of ACh in the pulmonary circulation. Histamine receptor (H_1 and H_2) or angiotensin II receptor blockade had no effect on the PAP actions of ACh, however pre-treatment with the prostaglandin synthesis (cyclo-oxygenase) inhibitor indomethacin (10^{-5} M), completely abolished the PAP response to ACh up to concentrations of 10^{-4} M. In experiments where airway pressure was not regulated, it rose signifcantly in response to increasing concentrations of ACh, reflecting the ability of ACh to stimulate tracheo-bronchial smooth muscle contraction. ACh-induced increases in airway pressure were unaffected by pre-treatement with indomethacin (10^{-5} M) but were totally inhibited by pre-treatment with atropine (10^{-5} M). In anesthetized, open-chested rabbits, infusion of ACh (20 ug/kg/min) into the jugular vein, *in vivo*, produced a simultaneous increase in PAP and a decrease in systemic arterial pressure. Pretreatment with indomethacin (1 mg, iv) completely abolished the PAP but not the systemic arterial response to ACh, whereas pretreatment with atropine (1 mg/kg, iv) completely prevented changes in both PAP and systemic pressure. We conclude that the muscarinic receptor-mediated PAP response, but not the airway or systemic arterial pressure response to ACh requires the release of a prostanoid.

SEPTAL GABAERGIC NEURONS: LOCALIZATION AND POSSIBLE INVOLVEMENT IN THE SEPTAL-HIPPOCAMPAL FEEDBACK LOOP: Darwin L. Cheney, Ciba-Geigy Corporation, Pharmaceuticals Division, Neuroscience Research, Summit, New Jersey, 07901, USA, and P. Panula, University of Helsinki, Department of Anatomy, Helsinki, Finland.

Much has been learned both histologically and biochemically about the major connections of the distinct areas of the septal complex, the functional interaction with the hippocampus and the chemical character of some of these pathways. The cholinergic septal-hippocampal pathway serves as a well-defined link between these two important structures of the limbic system.

It has been shown that cholinergic neurons, regulated by GABAergic interneurons, originate in the medial septum and diagonal band and project to area CA_3 of the hippocampus where they impinge on glutamatergic pyramidal cells. The glutamatergic neurons send collaterals back to the lateral septum where they functionally regulate another set of GABAergic neurons. Morphological evidence suggests that there are two different GABAergic neuronal systems operating in the septum: a population of small cells in the lateral septal nucleus and a group of large cells in the medial septum and diagonal band.

Antisera against L-glutamate decarboxylase (GAD), the synthetic enzyme for gamma-aminobutyric acid (GABA) have been used to locate GABAergic neurons and nerve terminals in the septal complex of the rat by using the peroxidase-antiperoxidase method. Varying densities of immunoreactive terminals are observed in saline treated rats but nerve cell bodies are only demonstrated after intraventricular or intraseptal injections of colchicine. Small and medium-sized GAD-positive neurons are found in lateral septal nuclei, the largest number of these cells being in the pars dorsalis, and in the bed nucleus of the stria terminalis. Large GAD-immunoreactive neurons are located in the medial septal nucleus and in the diagonal band the GAD-positive cell bodies are distributed similarly to cholinergic neurons.

These results provide direct morphological evidence for the presence of neurons capable of synthesizing GABA in septal nuclei. Moreover, it may be postulated the population of small cells in the lateral septal nucleus is associated with the glutamate neuron terminals projecting from the hippocampus, whereas the group of large cells in the medial septum and diagonal band is associated with the cell bodies and dendrites of cholinergic neurons projecting to the hippocampus.

NEUROTOXIC EFFECTS OF NITROGEN MUSTARD ANALOGUES OF CHOLINE: E.Howard Colhoun, Department of Pharmacology and Toxicology, Faculty of Medicine, The University of Western Ontario, London, Canada, N6A 5C1.

The prototype compound for our studies on cholinergic neurotoxic agents is the aziridinium ion (Az) isomer of methyl-2-acetoxyethyl-2'-chloroethylamine (acetylcholine mustard; AChM Az). AChM was first synthesized by Hanby and Rydon (1947), but Jackson (1972),studying the pharmacology of β-haloalkylamines, showed clearly the significance of the formation of the Az ion for biological activity. Although capable of interacting with cholinergic receptors, there is controversy over whether alkylation takes place; the time-dependent inhibition of acetylcholinesterase (AChE) may be irreversible. The conditions under which the Az forms will be described since this reaction is fundamental to the biological activity of the chloroethylamines used as neurotoxins; in our laboratory these include choline mustard aziridinium ion (ChM Az), monoethylcholine mustard aziridinium ion (MEChM Az), acetylmonoethylcholine mustard aziridinium ion (AMEChM Az) and ethoxycholine mustard aziridinium ion (EChM Az). ChM Az and MEChM Az (=AF64A) are obtained by alkaline hydrolysis of the acetylated parent compounds, AChM Az and AMEChM Az. Toxicity studies in mice showed that AChM Az, AMEChM Az and EChM Az were cholinergic agonists with characteristic acetylcholine (ACh)-like effects. The order of potency was AChM Az > AMEChM Az > EChM Az. Only EChM Az produced a secondary delayed lethal effect occurring in mice surviving the acute phase of toxicity; the mechanism of this toxicity has not been established. ChM Az and MEChM Az produced a hemicholinium-like toxicity in mice indicating an action at the presynaptic cholinergic nerve ending. Subsequent experiments with the rat phrenic nerve-diaphragm preparation and rat forebrain synaptosomes confirmed the presynaptic action of these compounds. In vivo studies on the anaesthetized rat showed an effect on phrenic nerve discharge from the CNS, as well as paralysis of the diaphragm. The mechanisms of action of ChM Az and MEChM Az in particular were studied on rat brain synaptosomes. The results showed potent, selective and irreversible inhibition of high-affinity choline transport, with ChM Az being twice as potent. AChM Az and EChM Az were poor inhibitors of high-affinity choline uptake. Further studies using the diaphragm preparation showed a time-dependent, irreversible presynaptic blockade of neuromuscular transmission with order of potency being ChM Az > MEChM Az > AChM Az (with eserine) > EChM Az. The N-ethyl substituent on MEChM-Az did not produce qualitative differences when compared with ChM Az but some quantitative differences were apparent; parameters such as the affinity for a site may not be altered by the ethyl group, but the reactivity of the Az may be changed with MEChM Az being unable to form complex bonds with nucleophilic groups as readily. The possible molecular interactions of ChM Az at the cholinergic nerve terminal have been studied in greater detail. This analogue was found to be both an inhibitor (irreversible at high concentrations and long exposure times) of choline acetyltransferase (ChAT), as well as a substrate for the enzyme indicating the possibility for the production of a false transmitter molecule should ChM Az gain access to the presynaptic terminal. Injection of ChM Az into lateral ventricles of rat brain caused decreases in high-affinity choline transport and ChAT activity in hippocampus, cortex and striatum at 7 days following a single dose (20 nmoles). Lower doses injected into medial septum caused lesions selectively to the hippocampus. Like MEChM Az (=AF64A), ChM Az may be an apparent 'selective cholinotoxin' for animal model studies of cholinergic hypofunction. In summary, we have shown that the toxicity of the nitrogen mustard analogues of choline studied in our laboratory can be attributed to actions on the cholinergic nervous system; the delayed toxicity produced by EChM Az in mice may be an exception. (Supported by MRC,GRCO and CGRS Canada)

SYNTHESIS, STORAGE AND RELEASE OF CHOLINE ANALOGUE ESTERS: Brian Collier, McGill University, 3655 Drummond, Montreal H3G 1Y6, Canada.

We have attempted to use analogues of choline to increase our understanding of the processes associated with acetylcholine synthesis, storage and release in cholinergic nerve endings of mammalian tissue. Initially, we studied ethyl analogues of choline. These enter cholinergic nerve endings of cerebral cortex or of preganglionic fibers by transport via the choline uptake system; part of accumulated analogue is acetylated and ester, but not unchanged analogue, can be released by stimuli that normally release acetylcholine. The subcellular distribution of these false esters was only slightly different from that of acetylcholine. Similar results pertain to studies with choline analogues with extended amino alcohol chain, such as homocholine. We then prepared ethyl derivatives of homocholine. The mono- and di-ethyl compounds, but not the tri-ethyl one, can be transported by the choline transport mechanism. Both of the compounds that are transported are acetylated, though somewhat less well than homocholine. The acetyl ester of diethylhomocholine localizes rather poorly to synaptic vesicles, most of the false ester being recovered with cytoplasmic components. The acetyldiethylhomocholine was not clearly released by stimuli that release acetylcholine. Thus the structural specificity of storage and release mechanisms is evident, and seems to be similar.

The synthesis of acetylcholine is accelerated by stimuli that release transmitter. So too is that of acetyldiethylhomocholine. Thus, releasability of ester is not required for ester synthesis to be regulated. Stimuli that normally release transmitter enhance choline transport activity and, thus, increases the precursor delivered into the nerve terminal. This change is not a sufficient stimulus for the activation of ester synthesis, and it will be concluded that acetylcholine synthesis is controlled at some step distal to precursor delivery. The effect of compound AH 5183 on stimulus-induced increase of ester synthesis will be presented with the expectation that S. Parsons can interpret it.

(The work is sustained by M.R.C. (Canada) funds; the talk will be sustained by the hope of an ale from L.W. funds).

ACETYLCHOLINE AND CHOLINE CONTENT IN FAST AND SLOW MUSCLE OF THE RAT: EFFECT OF DRUGS: Silvana Consolo, Maurizio Romano, Cristina Scozzesi and Herbert Ladinsky, Cholinergic Neuropharmacology, Mario Negri Institute for Pharmacological Research, Milan, Italy 20157.

A sensitive and specific radioenzymatic assay is described for the determination of ACh in skeletal muscle, an ACh-poor but choline-rich tissue. Muscle is frozen in liquid nitrogen and pulverized. ACh and choline are extracted into acetone-formic acid mixture and the ACh is seperated from choline by paper electrophoresis. Traces of choline are further quantitatively removed by phosphorylation. The ACh is hydrolyzed and the resultant choline is enzymatically acetylated with tritiated acetylcoenzyme A of high specific activity and counted after extraction into toluene based phosphor by liquid-liquid cation exchange chromatography. The lower sensitivity of the method is about 4 pmoles ACh. Excess amounts of phosphorylcholine, monomethylaminoethanol, dimethylaminoethanol, carnitine and acetylcarnitine do not interfere with the assay.

The ACh contents of 3 microwaved rat skeletal muscles were (in pmoles/muscle): extensor digitorum longus (EDL), 41.8±2.7; soleus, 35.4±2.7; gastrocnemius, 168.0±6.5. The choline contents were (nmoles/muscle): 2.29±0.1; 2.09±0.2; 24.17±1.1, in the 3 muscles, respectively. Freshly excised but not microwaved EDL contained 20% lower ACh content and 79% higher choline content. EDL ACh content was decreased by 79% and 86% at 10 and 30 days after sectioning the sciatic nerve.

The level of EDL ACh is proteic. It was decreased by the i.m. injection of hemicholinium-3, 1.5 mg/kg, 60 min by about 20% and was increased by procaine, 15-60 mg/kg, i.m., 15 min in a dose dependent manner up to 100%. Eserine, 1 mg/kg, and naloxone, 5 mg/kg, were without effect.

PRESYNAPTIC MODULATION OF ACETYLCHOLINE RELEASE: Jack R. Cooper, James H. Reese and Houngsup Park, Department of Pharmacology, Yale Univ. School of Med. New Haven, CT 96510 USA

Modulation of synaptic transmission, both pre and postsynaptic, appears to be a logical explanation for behavioral changes so we have begun to explore this possibility, focusing on presynaptic modulation. Since the guinea pig ileum myenteric plexus-longitudinal muscle preparation has been a model tissue for decades to study this phenomenon, we have developed a synaptosomal preparation from this tissue which is preloaded with [^{3}H]choline in order to generate [^{3}H]ACh. Using nicotinic stimulation to evoke release and screening a variety of agents for their ability to affect the release of [^{3}H]ACh, we have identified, in addition to an excitatory nicotinic receptor, an inhibitory muscarinic receptor, inhibitory adrenergic receptor (α_2 type) and inhibitory purinergic receptor (A_1 or R_1 type). When ACh release is evoked by KCl or veratridine, minimal modulation is noted with these agents. No modulation occurs when release is evoked with 8-bromocyclic AMP or forskolin. In the synaptosomal preparation, morphine, enkephalins, somatostatin, VIP, and CCK-8 exhibit no modulatory activity. In the intact myenteric plexus-longitudinal muscle that is electrically stimulated, enkephalins and GABA are inhibitory, CCK-8 is stimulatory, and neurotensin gave a biphasic effect. That the purinergic receptor is physiologically relevant is suggested by the observation that when adenosine deaminase is added to either the synaptsomal or intact preparation in order to destroy endogenous adenosine, ACh release is markedly increased.

With respect to biochemical mechanisms underlying modulation, preliminary experiments with synaptosomes preloaded with ^{32}Pi showed no specific phosphorylated protein band on gel electrophoresis following the addition of either depolarizing or modulating agents.

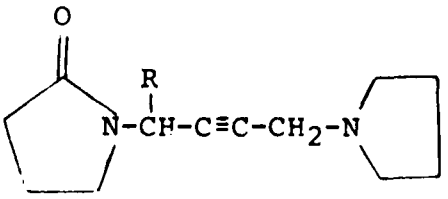
CHOLINE FLUXES TO AND FROM THE RAT CEREBRAL CORTEX STUDIED WITH THE "CUP TECHNIQUE" IN VIVO: Renato Corradetti, Rudolf Brehm*, Konrad Löffelholz* and Giancarlo Pepeu, University of Florence, Italy and * University of Mainz, DBR, Departments of Pharmacology.

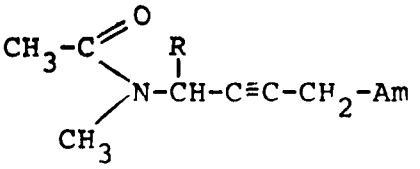
The "cup technique" has been found to be a valuable tool for studying the efflux of endogenous choline (Ch) from mammalian cerebral cortex (Corradetti et al., J.P.E.T. in press). Since the cup is placed on the cerebral cortex after removing the dura mater, the solution filling the cup has to be regarded as an enlargement of the extracellular space. By means of this technique we estimated that the rate of free Ch efflux from the cortex into the cup was 60 pmol/min /cm^2, resulting in a concentration of 0.33 µM in 10 min. Locally applied muscarinic agonists increased this rate, suggesting that acetylcholine stimulates mobilization of cellular Ch. However among the alternative sources of Ch, plasma deserves particular consideration. Indeed blood concentration of Ch (10 µM) is higher than that of the cup (0 to 0.33 µM), and surgery-induced lesions of the vessels could cause Ch efflux.Furthermore since the cerebral microcirculation is regulated by muscarinic mechanisms, changes in vessel permeability could bring about Ch efflux increase during muscarinic agents application. We therefore investigated the contribution of plasma Ch to Ch efflux from rat cortex. Perspex cups were placed on both the emispheres (dura removed) of urethane anaesthetized male Wistar rats. 100 µCi Methyl [^{3}H]-Ch chloride (NEN, 80 Ci/mmol) or 50 µCi [^{14}C]Carboxyl-inuline (NEN, 2.6 mCi/g), in saline, were infused in the tail vein (0.09 ml/min). Ringer solution (0.3 ml, 37°C) was replaced in the cup every two min and radioactivity measured. At the end of the infusion (10 min) the plasma levels of radioactivity were estimated. ^{14}C-inuline infusions provided evidence for the integrity of the vessels since minimal amounts were found in the cup (27 nM) in comparison with a plasma concentration of 134 µM. During ^{3}H-Ch infusions the efflux from the blood into the cup was 13 pmol/ min/cm^2. Locally applied muscarinic agonists (bethanechol 500 µM or eserine 300 µM) as well as increasing cup Ch concentration (10 µM) or cooling the hemisphere to 20°C in the presence of eserine, were unable to change the efflux of ^{3}H-Ch. Conversely, topically applied penicilline (4%) triplicated the efflux of ^{3}H-Ch from plasma into the cup. We conclude that less than 1/4 of the free Ch found in the cup under normal conditions originates from the plasma, and that the increase observed during the application of muscarinic agonists cannot be related to changes in vessel permeability. Hence this increase reflects mobilization of free Ch from cortical cells.

Supported by CNR grant n 82.02043.04 .

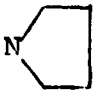
STEREOSELECTIVITY OF SOME MUSCARINIC AND ANTIMUSCARINIC AGENTS RELATED TO OXOTREMORINE: Richard Dahlbom and Bahram Resul, Department of Organic Pharmaceutical Chemistry, Biomedical Center, Uppsala University, Box 574, S-751 23 Uppsala, Sweden, Björn Ringdahl and Donald J. Jenden, Department of Pharmacology, School of Medicine, University of California, Los Angeles, California 90024, U.S.A.

The optical isomers of some alkyl-substituted analogs of the central muscarinic agent oxotremorine (1) and a congener (5) were prepared and tested for tremorogenic and tremorolytic activity in mice and for muscarinic and antimuscarinic activity on the isolated guinea pig ileum. In mice the compounds are tremorolytic or tremorogenic agents. On the isolated guinea pig ileum they behave as muscarinic agonists, partial agonists or competitive antagonists. The R-enantiomers were more potent than the S-isomers both in vivo and in vitro regardless of whether the compounds were agonists, partial agonists, or antagonists. The close structural similarity between agonists and antagonists make it seem probable that in the oxotremorine series agonists and antagonists interact with a common receptor site, in contrast to classical muscarinic antagonists which are believed to bind also to accessory receptor areas located close to the agonist binding site.





	R
1	H
2	CH_3
3	C_2H_5
4	C_3H_7

	R	Am
5	H	
6	CH_3	
7	CH_3	$N(CH_3)_2$
8	CH_3	$\overset{+}{N}(CH_3)_3$

IMMUNOHISTOCHEMICAL EVIDENCE FOR THE INTRA-AXONAL TRANSPORT OF nAChR-LIKE MATERIAL IN MOTOR NEURONS: Annica B. Dahlström[1], Serney Bööj[1], Ann-Gret Dahllöf[1], Sarah Fuchs[3], Edith Heilbronn[2] and Johan Holmdal[2], Inst. of Neurobiology, Univ. of Göteborg[1], Sweden, Neurochem. Dept., Univ. of Stockholm[2], Sweden, and Dept. of Chem. Immun., Weizmann Inst.[3], Rehovot, Israel.

Various reports in the literature indicate the presence of presynaptic nAChR sites at the motor endplate. Such presynaptic nAChR molecules are less likely to originate from the muscle cell, but rather have a neuronal source. We therefore investigated the possibility that nAChR may be present intraneuronally and undergo axonal transport in the motor nerve.

Sprague-Dawley rats were used. The sciatic nerves were ligated at the sciatic notch bilaterally 0-24 h before perfusion with 4% formaldehyde in PBS. In some animals the nerves were cut at the knee level 1-7 d before the ligation. Dissected nerves and spinal cord specimens (L2-L3) were postfixed, rinsed in PBS with 5% sucrose, cryostatsectioned and incubated for indirect immunofluorescence with the following antisera: rabbit anti-nAChR (isolated from Torpedo), human myastenia gravis IgG (containing IgG to nAChR), and monoclonals 5.14 and 5.5.6 against nAChR. FITC-labelled α-bungarotoxin was also used for incubation.

Material which cross-reacted with all four antisera was found to accumulate proximal, but also distal, to a ligature. In nerves with a previous axotomy 3-7 d earlier, the amount proximal to the collection ligature was increased as compared to control. The motor perikarya of the L1-L3 region contained moderate (with rabbit-anti-nAChR antiserum) to weak (with monoclonals 5.14 and 5.5.6) immunofluorescence.

The results indicate that material which cross-reacts with four different antisera containing antibodies to nAChR protein is located in motor perikarya and is transported distally in motor axons. Retrograde transport also occurs. It is possible that the observed material, not yet chemically identified, is related to the presynaptic nAChR sites in the motor endplate.

Supported by grants from the Swedish MRC (2207), Ake Wibergs Foundation, National Society for Trafik- och Polioskadade.

INTERACTIONS OF ALAPROCLATE, A SELECTIVE 5HT-UPTAKE BLOCKER, WITH MUSCARINIC RECEPTORS; IN VIVO AND IN VITRO STUDIES: Erik Danielsson[1], Anders Undén[1], Öie Nordström[1], Sven-Ove Ögren[2] and Tamas Bartfai[1], University of Stockholm, Arrhenius Laboratory, 106 91 Stockholm[1], and Asta Läkemedel AB, 151 85 Södertälje, Sweden[2]

Alaproclate (10-60 mg/kg) injected i.p. into mice 30 min prior to s.c. injection of oxotremorine (100-300µg/kg) or of physostigmine (200 µg/kg) potentiated and prolonged the resulting tremor in a dose-dependent manner. Although alaproclate is a selective 5-HT uptake blocker, pretreatment with the 5-HT receptor antagonist metergoline (1 mg/kg) did not prevent its effect on the oxotremorine-induced tremor. Atropine (1 mg/kg) fully inhibited the tremor caused by oxotremorine or physostigmine both in the presence and absence of alaproclate.

Among the muscarinic responses to oxotremorine which were potentiated by alaproclate are also hypothermia and salivation.

Alaproclate does not affect the acetylcholinesterase activity, the uptake of ^{3}H choline or its conversion into ^{3}H acetylcholine.

Alaproclate is a weak inhibitor of the muscarinic antagonist (^{3}H-4-N--methyl piperidinyl benzilate) binding (Ki ≈ 40 µM). Alaproclate inhibits the high affinity agonist binding to membranes from striatum. Alaproclate was not effective in changing the GTP effect on muscarinic ligand binding to membranes from rat heart.

The muscarinic receptor mediated increase in cyclic GMP levels in the rat hippocampus was partially inhibited by alaproclate (10 µM).

Thus the postsynaptic muscarinic responses that were studied up till today were unaffected or slightly inhibited by alaproclate and not giving any molecular explanation of the in vivo potentiation of the muscarinic agonist: oxotremorine evoked responses.

There is preliminary evidence for an effect of alaproclate on the release of acetylcholine.

PERIPHERAL SYMPATHETIC NERVES REPLACE CENTRAL CHOLINERGIC NERVES AFTER INJURY IN THE ADULT MAMMALIAN BRAIN: James N. Davis, Keith A. Crutcher and Roger Madison, Neurology Research Laboratory, VA Medical Center and Departments of Medicine (Neurology) and Pharmacology, Duke University Medical Center, Durham, North Carolina, 27705, U.S.A.

The adult mammalian brain is capable of vigerous neuronal rearrangements in response to injury. We have been interested in an unusual rearrangement in which sympathetic nerve fibers normally innervating blood vessels on the brain surface appear within the central nervous system.

Sympathetic ingrowth is found in the rat hippocampal formation after loss of the cholinergic septohippocampal pathway. Lesions of other known afferents to the hippocampus did not elicit sympathetic ingrowth, while partial lesions of the septal nucleus caused ingrowth only in the areas of hippocampus without cholinergic input. Sympathetic fibers in the hippocampus come to lie inside the blood brain barrier.

This replacement of central cholinergic neurons by peripheral noradrenergic neurons occurs when any of a number of basal forebrain cholinergic neurons are injured. Lesion of the substantia innominata (nucleus basalis) produced sympathetic ingrowth in the neocortex while lesions of the cholinergic septohabenular tract were associated with sympathetic fiber ingrowth in the medial habenula.

Sympathetic ingrowth appears to be initiated by a target factor produced in the hippocampal formation. The best hypothesis to explain current data is that this factor is normally transported away by septohippocampal fibers. After injury the factor accumulates in the hippocampal formation initiating sympathetic ingrowth. Sympathetic fibers may not be capable of removing the factor since ingrowth continues to increase for at least a year after septal lesions.

To date sympathetic ingrowth has been identified in two rodent species. A preliminary study of one monkey in our lab suggests that ingrowth may happen in primates. If sympathetic ingrowth occurs in humans it may play an important role in the functional deficit associated with Alzheimer's Disease, head injury and stroke. With a better understanding of the factors that regulate sympathetic ingrowth, it may be possible to predict pharmacological approaches to enhance recovery of function in brain injured or demented patients.

CHOLINERGIC MECHANISMS IN ALZHEIMER'S DISEASE: Kenneth L. Davis, M.D., Richard C. Mohs, Ph.D., Bonnie M. Davis, M.D., Blaine S. Greenwald, M.D., Celeste A. Johns, M.D., and Thomas B. Horvath, M.D., Dept. of Psychiatry, Veterans Administration Medical Center, Bronx, New York and Depts. of Psychiatry and Pharmacology, Mt. Sinai School of Medicine, New York, NY

Neurochemical studies indicating that cholinergic neurons are selectively lost in patients with Alzheimer's disease (AD) suggest that cholinomimetic drugs might benefit these patients and that markers for central cholinergic activity would be useful in selecting patients for cholinergic therapy. The present studies investigated possible CSF and neuroendocrine markers for cholinergic activity and tested the effects of physostigmine, administered intravenously and orally on symptoms of AD. CSF concentrations of ACh correlated with the degree of cognitive impairment in a sample of carefully diagnosed patients with AD but metabolites of other neurotransmitters were not related to cognitive state; this indicates that CSF ACh is a valid measure of cholinergic degeneration. Mean plasma cortisol concentrations were higher in patients with AD than in controls and correlated inversely with CSF MHPG; since anticholinergic drugs suppress cortisol this finding indicates that cortisol dysregulation may be a marker of abnormalities in other neurotransmitter systems. Intravenous physostigmine significantly and reliably enhanced memory in 13 of 16 patients tested but the dose producing the improvement varied among patients. Oral physostigmine decreased overall symptom severity in a reliable way in 6 of 9 patients tested. The extent of improvement was correlated with the increase in mean cortisol secretion produced by physostigmine, suggesting that the drug improved behavior and cognition only to the extent that it had a specific central cholinomimetic effect. All improvements due to physostigmine were modest and all patients who improved remained severely demented. Furthermore, these studies emphasize that clinical trials of potential cholinomimetic drugs for AD are most likely to yield useful results if biological markers for neurotransmitter activity are combined with careful symptom assessments.

ACETYLCHOLINESTERASE AND THE MAINTENANCE OF NEUROMUSCULAR STRUCTURE AND FUNCTION: Wolf-D. Dettbarn and Karl E. Misulis, Vanderbilt University, School of Medicine, Nashville, Tennessee 37212

Acetylcholinesterase (AChE), a constituent of the membranes at the neuromuscular junction, plays an essential role in the removal of acetylcholine (ACh) from the synaptic cleft by hydrolyzing ACh to choline and acetic acid. Inhibition of AChE profoundly modified neuromuscular transmission. This was shown by electrophysiological analyses, by studies of the response of the muscle to nerve stimulation and by observations on muscular activity in the intact animal in the absence of applied nerve stimulation.

Acute administration of AChE inhibitors produced a myopathy characterized by initial focal changes in the subsynaptic area and adjacent muscle area and spread so that by 12 hours a complete but localized necrosis had developed within the affected fibers. Increased spontaneous transmitter release, a reduction in quantum content, spontaneous and evoked antidromic action potentials were seen within the initial two hours of drug application. Inhibition of the externally localized molecular weight form of AChE, such as the 16S form, was critical for the necrosis.

A number of lines of evidence indicate that these effects were due, at least in large part, to the inactivation of AChE. 2-PAM prevented the electrophysiological effects and protected against the development of the myopathy when given within 30 minutes of AChE inhibitor administration in vivo. With progressive delay in 2-PAM administration this protection was less effective. Reversible AChE inhibitors such as prostigmine and physostigmine also caused muscle fiber necrosis, but if given in low concentrations actually protected against the necrosis. Nc necrotizing effect on denervated hemidiaphragm muscle was seen. Secondary events which may cause muscle necrosis may be explained by an increased net influx of Ca++ into the muscle fiber; an event triggered either by an AChE inhibition induced neurogenic muscle hyperactivity or by a direct action on the muscle. This increased uptake of Ca++ may lead to a Ca++ overload and energy depletion ultimately leading to the necrosis. The alternatives are that increased Ca++ may activate proteolytic enzymes causing the muscle necrosis or that ACh itself stimulates proteolytic enzymes in the muscles. Chronic inhibition of AChE in muscles denervated by nerve crush delayed significantly the appearance of mechanical and physiological signs of reinnervation when compared with untreated control muscles.

AChE at the neuromuscular junction may in part have a protective function through its control of free ACh. By limiting the accumulation of ACh and the extent of its interaction with pre and postsynaptic membranes it may preserve and regulate morphological integrity of nerve terminals and muscle fibers.

This work was supported by NIH - NINCDS #12438, EHS #02028 and Air Force Grant #AFOSR-82-0310.

PLASMA AND RED BLOOD CELL CHOLINE IN AGING: RATS, MONKEYS AND MAN: E.F. Domino, Benjamin Mathews and Sandi Tait, University of Michigan, Ann Arbor 48109 and Lafayette Clinic, Detroit 48207.

Plasma and red blood cell choline concentrations were measured in Holtzman albino rats, Macaca mulatta monkeys, and humans (normal, demented and dyskinetic) of various ages. Choline was determined by chemical demethylation and GC-NP detection. Compared to young adults, a marked increase in plasma choline was found in geriatric monkeys and humans, but not in geriatric rats. Although red blood cell choline levels are increased above plasma choline levels in monkeys and most humans, this is not true of the adult rat. Plasma or red blood cell choline levels did not differ among patients with senile dementia or tardive dyskinesia when compared to normal volunteers of similar age. No sex differences in blood choline levels were noted.

The plasma clearance of $^{2}H_{4}$-choline, given in a dose of 10 mg/70 kg i.v., was reduced in both the plasma and red blood cells of geriatric monkeys. Oral phosphatidylcholine (15 gm/70 kg) elevated plasma and red blood cell choline four fold 3-4 hours after ingestion in normal young adults, geriatric volunteers, and in patients with senile dementia. In contrast to young adults, the clearance of plasma and red blood cell choline after oral phosphatidylcholine was slower in the geriatric normal and senile dementia patients. Although plasma and red blood cell choline levels do not correlate with brain dysfunction in either humans or monkeys, it is clear that with increasing age, plasma choline levels increase and plasma choline clearance decreases. The following conclusions can be made as a result of this study:

1. Red blood cells from adult rats do not concentrate choline in contrast to red blood cells from adult monkeys and humans. However, red blood cells from young (30 day old) rats do concentrate choline.

2. Plasma choline concentrations in adult rats, monkeys, and humans are similar. However, with increasing age plasma choline levels significantly increase in monkeys and humans but not in rats.

3. Adult rat blood is a poor model for blood choline changes in adult monkeys and humans.

4. In contrast to plasma, the choline concentrations in red blood cells from geriatric monkeys and humans do not increase with age.

5. Pharmacokinetic analyses of oral phosphatidylcholine loading in man and deuterated choline administration i.v. in monkeys show reduced plasma turnover in both species of geriatri subjects.

6. The brain cholinergic deficit in senile dementia patients or its possible imbalance in tardive dyskinesia patients is not reflected in either their plasma or red blood cell choline levels.

PHOSPHOLIPASE NEUROTOXINS AS PROBES FOR THE PRESYNAPTIC CHOLINERGIC MEMBRANE IN TORPEDO: Mike J. Dowdall, Pamela Fretten and P. Gail Culliford, Department of Biochemistry, University Hospital and Medical School, Nottingham NG7 2UH, U.K.

A number of snake neurotoxins with intrinsic phospholipase A_2 activity are potent neuromuscular blocking agents which target on the presynaptic membrane and inhibit transmitter release. This toxin group includes taipoxin (Tpx), β-bungarotoxin (βBtx), crotoxin (Crtx), notexin (Ntx) and Notechis II-5(Ntx II-5). Electrophysiological measurements of transmitter release at poisoned junctions have shown a complex sequence of events - typically triphasic: (i) Rapid decrease, (ii) Slow increase and (iii) Gradual and progressive decrease to zero output. Phospholipase A_2 activity is essential for overall neurotoxicity and phases ii and iii but not for phase i. A working hypothesis is that a phospholipase-independent binding of toxin to specific sites on the preterminal membrane is the key step in the neurotoxic response ('Corpora non agunt nisi fixata'). Biochemical and biophysical analyses of toxin-induced changes in purely cholinergic synaptosomes from Torpedo may help to identify these sites.

The effect of a number of these toxins on synaptosomal phospholipid composition, membrane potential, uptake systems (choline, acetate and adenosine) and release of cytoplasmic enzymes (LDH and ChAT) have been studied (e.g. see results for Tpx below).

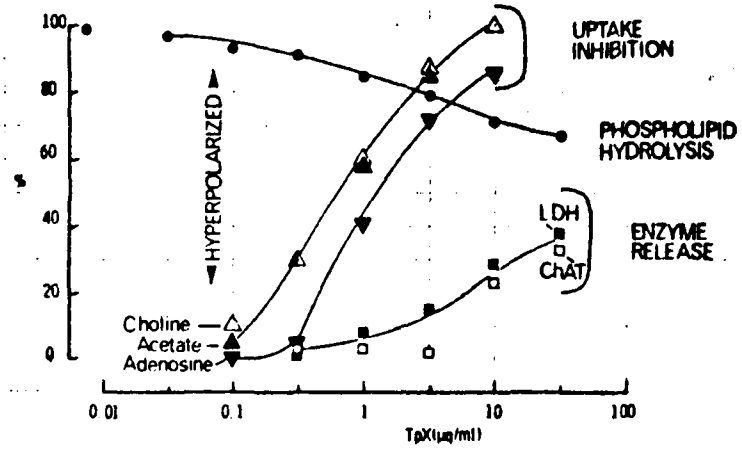


Fig. Concentration-dependent effects of TpX on Torpedo synaptosomes
Synaptosomes were incubated in Torpedo Ringer at 20-24°C for 15 min or 30 min (phospholipid hydrolysis). Enzyme release assayed after centrifugation; uptake inhibition by rapid filtration; membrane potential changes assayed using carbocyanine dye and lipophilic cation methods; hydrolysis of phospholipids (given as % survival) by phosphate analysis after 2D separation by t.l.c.

All toxins studied produced significant hyperpolarization of the synaptosomal membrane at concentrations where other changes were minimal or undetectable. Since toxins with essentially zero phospholipase activity towards synaptosomes (βBtx and p-bromophenyl-acyl bromide inactivated Ntx II-5 and α chain TpX) were also effective hyperpolarization agents, independence from phospholipase activity is indicated. This effect may correspond to phase i of the electrophysiological response and possibly reflects binding to an ion channel. The relationship between TpX binding sites and those for other toxins in this group has been tested using a radiometric phospholipase assay. Protection against TpX-induced synaptosomal hydrolysis was not seen with βBtx or PBP-NtxII-5. This suggests that toxins with similar pharmacological actions may have their own distinct target sites. (Supported by the Wellcome Trust and the MRC.)

TENTATIVE IDENTIFICATION OF THE CHOLINE TRANSPORTER IN CHOLINERGIC PRESYNAPTIC PLASMA MEMBRANE PREPARATIONS FROM TORPEDO ELECTRIC ORGAN: Ilze Ducis, Abteilung Neurochemie, Max-Planck-Institut für biophysikalische Chemie, Postfach 2841, D-3400 Göttingen, F R Germany

Vesiculated synaptic plasma membranes have been isolated from the purely cholinergic electromotor system of Torpedo marmorata. Synaptosomes, isolated on a discontinuous Ficoll gradient, were exposed to osmotic shock at 2°C, pH 8.5, in the presence of 0.1 mM Mg^{2+}. These conditions for lysis produced optimal transport of choline. Electron microscopic investigation of lysed synaptosomes showed vesiculated membranes with diameters smaller than synaptosomes and, occasionally, synaptic vesicles were observed attached to membranes. Intact mitochondria, synaptosomes, and basement membranes were not observed.

High affinity (K_m=1.7 uM) uptake of choline into these vesiculated membrane fragments showed: 1) a dependence of the Na^+ gradient (out> in); 2) dependence on the presence of Cl^-; 3) a transient Na^+ gradient-dependent accumulation of choline over the equilibrium concentration (overshoot); 4) rheogenicity, since it was further stimulated in the presence of a Na^+ gradient by the addition of valinomycin, and 5) high affinity (K_i=25 nM) hemicholinium-3 inhibition.

When the synaptosome lysate was fractionated on a 4-12% Ficoll-300 mM KCl-20 mM imidazole-0.1 mM $MgCl_2$ (pH 8.0 at 2°C) gradient, six membrane fractions were separated. The estimated concentration of choline carriers increased down the gradient. SDS-PAGE analysis of the proteins of the membrane fractions showed 5-6 components that increased pari-passu with carrier concentration; these had M_r's of 200, 140, 68 (doublet), 57 and 28 KDa. Four of these (200, 140, 68 doublet) were tentatively identified as myosin, 5'-nucleotidase, acetylcholinesterase and adsorbed choline acetyltransferase, leaving the 57 and 28 KDa components as candidates for the choline carrier.

SEROTONIN AND ITS ROLE IN MODULATION OF CHOLINERGIC TRANSMISSION IN PREVERTEBRAL GANGLIA. Nae J. Dun, Ru-chun Ma, Maria Kiraly, Lionel Barnes and Alexander G. Karczmar. Dept. of Pharmacol., Loyola Univ. Med. Ctr., Maywood, IL 60153

Synaptic transmission in neurons of the isolated guinea-pig left celiac ganglia was investigated by means of intracellular recording techniques. Stimulation of the left splanchnic nerves evoked a fast excitatory postsynaptic potential (f-epsp) that was reversibly suppressed by curare, indicating that it is mediated by a nicotinic action of ACh. Repetitive nerve stimulation (10-20 Hz, 1-2 sec) elicited, in addition to the initial f-epsp's, a slow depolarization which lasted for min. The slow depolarization was not affected by cholinergic antagonists but was reversibly abolished in a low Ca/high Mg solution indicating that the response was postsynaptic and was probably mediated by a transmitter(s) that is non-cholinergic in nature. The slow depolarization was henceforth termed non-cholinergic epsp. The mean amplitude and duration of the non-cholinergic epsp was 5.1 $\pm$ 2 mV and 158 $\pm$ 123 sec (n=305), respectively. The non-cholinergic epsp was accompanied by an increase of membrane resistance in the large majority of celiac neurons tested. As a result, the sub-threshhold f-epsp induced by heterosynaptic nerve stimulation was markedly augmented during the course of non-cholinergic epsp, leading to spike discharges. The facilitation could still be observed when the membrane potential was clamped at the resting level. This finding suggests that the facilitation of cholinergic f-epsp's could be attributed both to an increase of membrane resistance and to membrane depolarization brought about by the non-cholinergic epsp. Experiments were then carried out to identify the transmitter(s) mediating the non-cholinergic epsp. Among the several putative transmitters tested, serotonin (5-HT, 1-10 μM) caused a slow depolarization with electrophysiological characteristics similar to that evoked by nerve stimulation in a portion of the celiac neurons. Furthermore, the non-cholinergic epsp was completely and reversibly desensitized by prolonged application of 5-HT. Additional pharmacological studies showed that the slow depolarization induced by either nerve stimulation or 5-HT was markedly enhanced by fluoxetine (30-50 μM), a 5-HT reuptake blocker, and suppressed by cyproheptadine (50 μM), a 5-HT receptor blocker. Superfusing the ganglion with the 5-HT precursor, L-tryptophan (50 μM) augmented the non-cholinergic epsp while the membrane depolarization induced by 5-HT was not significantly increased. Taken together the results suggest that 5-HT is the promising candidate for the role of transmitter in generating the non-cholinergic epsp in a population of celiac neurons. As a result, the presence of 5-HT in the celiac ganglia was determined by means of biochemical and immunohistochemical methods. First, using a HPLC-ECD system it could be shown that the amount of detectable 5-HT in the control ganglia was generally small, it could be markedly increased by superfusing the ganglia in vitro with the precursor, tryptophan. Under these conditions, the average amount of 5-HT present in the ganglia was found to be 0.167 $\pm$ 0.12 ng/mg wet weight of tissues. Furthermore, 5-HT could be detected in the perfusate after incubation the ganglia in a high KCl (80 mM) solution; this release was Ca dependent. Second, immunohistochemical procedures were employed to localize the 5-HT containing structures in the ganglia. Dense and unevenly distributed nerve fibers exhibiting 5-HT immunoreactivity could be demonstrated in celiac ganglia pretreated with tryptophan. The varicose 5-HT-like immunoreactive nerve fibers were observed to run parallel and come into close proximity with many ganglionic neurons. Collectively, our data support the notion that 5-HT is the transmitter released from nerve fibers projecting into the ganglia; it appears to be responsible for the generation of a slow depolarization in a portion of celiac neurons, its primary function being to increase in a sustained manner the responsiveness of the target neuron to the incoming cholinergic-f-epsp.

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PRESYNAPTIC CHANGES ACCOMPANYING TRANSMISSION OF A SINGLE NERVE IMPULSE. AN INTERDISCIPLINARY APPROACH USING RAPID-FREEZING : Yves Dunant, Miguel Garcia-Segura*, Gregor J. Jones, Françoise Loctin, Dominique Müller and Arpad Parducz, Départements de Pharmacologie et de Morphologie", C.M.U., 1211 Genève 4, Switzerland.

We have studied the morphological and biochemical changes underlying transmission of a single impulse at the *Torpedo* nerve-electroplaque junction by freezing small pieces of tissue at very precise time, confirmed by the electrophysiological records. In a first series of experiments, we investigated the effects of 4-aminopyridine (4-AP), a drug which enhances in a dose-dependent manner the amount of transmitter released in an impulse, resulting in the generation of a long-lasting, characteristic, "giant electroplaque potential". Both the size and time-course of this giant e.p.p. were also altered by modifications of temperature and of the calcium to magnesium ratio in the bathing solution.

The results showed that extravesicular ACh -most probably cytoplasmic ACh- was rapidly used and renewed, during and after the giant impulse. In contrast, vesicular ACh remained stable and no transfer occurred from cytoplasm to vesicles for at least 1.5 s. Freeze-fracture techniques revealed changes occurring at the presynaptic membrane at the same time as transmission of the single impulse : a dramatic increase in the number of large intramembrane particles.

In recent experiments we improved to 1 ms the time intervals of our freezing system to "catch" the events occurring during a normal impulse, in the absence of 4-AP. We also found an increase in the number of large intramembrane particles.

These results support the "operator" hypothesis previously proposed to explain quantal and subquantal release of cytoplasmic ACh.

THE USE OF POSITRON EMISSION TOMOGRAPHY (PET) FOR THE EVALUATION OF REGIONAL CHOLINE METABOLISM IN THE BRAIN: Sven-Åke Eckernäs, Sten-Magnus Aquilonius, Kjell Bergström, Per Hartvig, Anders Lilja, Bo Lindberg, Hans Lundqvist, Bengt Långström, Petter Malmborg, Ulf Moström and Kjell Någren, Departments of Neurology, Radiology, Gynecology, Pharmacy, University Hospital and Department of Organic Chemistry, Gustaf Werner's Institute, Uppsala University, Uppsala, SWEDEN

Up to now studies on brain choline (Ch) metabolism have mainly been performed in small animals using ^{3}H- or ^{14}C-isotopes and for human studies only indirect methods have been available. The recent introduction of PET offers an opportunity to study regional tissue metabolism non-invasively in higher animals and in man.

10-15 mCi of a tracer dose of ^{11}C-Ch was injected i.v. into ketamine anaesthetized rhesus monkeys and the radioactivity within different brain regions was measured at different time intervals by PET. After compensation for radioactivity within the blood a reproducible biphasic uptake of Ch in the brain was seen.

An initial rapid uptake during about 2 min was followed by a slower linear increase in radioactivity which lasted as long as the radioactivity could be followed (about 1.5 hours).

By assuming that the radioactivity after 40 min represents other metabolites (mainly phosphorylcholine) than Ch and acetylcholine (ACh) an initial curve mainly representing these two compounds can be calculated by extrapolation. The mean residence time (= turnover time) can be calculated by dividing the area under the first movement curve (AUCM = uptake * time versus time) with the area under the curve (AUC = uptake versus time). The mean residence time for the presumed Ch + ACh was found to be about 20 min and was similar in all brain regions studied. The mean residence time was not conclusively affected by the injection of 1 mg atropine or by barbiturate anaesthesia.

It is probably not possible to measure changes in brain ACh metabolism by PET but the regional kinetics of radioactivity after ^{11}C-Ch-injection may represent a general Ch-metabolizing capacity. Studies in patients with different types of dementias and extrapyramidal diseases seem to be of interest in order to further evaluate the usefulness of the method in clinical research.

IMMUNOLOGICAL APPROACH OF THE CHOLINERGIC TRANSMISSION : PRODUCTION OF MONOCLONAL ANTIBODIES AGAINST PRESYNAPTIC MEMBRANES ISOLATED FROM THE *TORPEDO* ELECTRIC ORGAN : Lorenza Eder-Colli and Silvana Amato, Department of Pharmacology, Centre Médical Universitaire, 1211 Geneva 4, Switzerland.

The major goal of the present investigation is to use antibodies as a tool to analyse which components of the membrane of the cholinergic nerve endings are involved in the release mechanism of the neurotransmitter acetylcholine (ACh).

Isolation of the synaptosomal plasma membrane (SPM). The innervation of the electric organ of the fish *Torpedo* is known to be purely cholinergic. The membranes of the cholinergic nerve endings were purified from synaptosomal preparations from the electric organ as described by Morel *et al.* (J. Cell Biol., 1982, 93, 349), but with modifications (treatment of the crude SPM with high salt solutions). Two enzymatic activities were used as "markers" for the SPM : acetylcholinesterase (AChE) and choline acetyltransferase (ChAT) which remained bound to the membranes after high salt treatment (non-ionically bound ChAT). In the purified SPM preparation : (1) the specific activity (SA) of AChE was increased 4 to 6 times when compared with the SA of this marker in the initial preparation of synaptosomes; (2) the SA of the non-ionically bound ChAT was increased 3 to 4 times when compared with the SA in a crude SPM preparation and (3) a protein migrating at about 66 kdaltons on SDS-polyacrylamide gel became highly concentrated. Other polypeptides appeared to become slightly concentrated in the SPM, one at 133 kdaltons and a doublet around 35 kdaltons of molecular weight. These polypeptides were absent in other fractions isolated from the electric organ (synaptic vesicles, electric nerve membranes and a fraction highly enriched in nicotinic receptor to ACh, the marker for post-synaptic membranes).

Antisera and monoclonal antibodies against SPM. Antisera to the purified SPM were raised in Balb/c mice and specific antibodies of high titer were obtained.

Ten stable monoclonal antibodies were obtained from two hybridisation experiments (Galfré *et al.*, Nature, 1977, 266, 550). The criterion used for selecting these antibodies was : a faint or no immune reaction against electric nerve membranes but a very strong immune reaction against the purified SPM. Immunoblot analysis of these monoclonal antibodies indicated clearly that six of them (8/12, 8/13, 8/27, 8/37, 8/38, 8/43) labelled weakly two bands at 93 and 133 kdaltons and heavily one band at 66.5 kdaltons from SDS-polyacrylamide gels. Therefore these antibodies recognised antigenic sites on two polypeptides that might be considered as specific markers for SPM (133 and 66.5 kdaltons). Are these proteins related ? This is now being investigated.

In indirect immunofluorescence studies on the electric organ, the ten monoclonal antibodies obtained produced a bright fluorescence along the ventral side of the layered electrocytes corresponding to the nerve endings innervating each electroplax. This fluorescence disappeared after adsorption of the antibodies with the SPM. Some preliminary studies on species cross-reactivity have been done. The monoclonal antibody 8/43, but not the others, seemed to stain the central white matter in human but not in the other species analysed (rat, rabbit, sheep).

We are now investigating if any of the monoclonal antibodies obtained will interfere with the release mechanism of ACh.

THE INTERACTION OF ALKYLATING DERIVATIVES OF OXOTREMORINE WITH THE MUSCARINIC RECEPTOR: Frederick J. Ehlert and Donald J. Jenden, Department of Pharmacology, UCLA School of Medicine, Los Angeles, California 90024, USA.

The interaction of two β-chloroalkylamine derivatives of oxotremorine with muscarinic receptors was investigated in muscarinic receptor [^{3}H]ligand binding assays. The two compounds N-[4-(2-chloroethylmethylamino)-2-butynyl]-2-pyrrolidone (BM 123) and N-[4-(2-chloromethylpyrrolidine)-2-butynyl]-2-pyrrolidone (BM 130) form aziridinium ions in aqueous solution at neutral pH which are responsible for their pharmacological effects. BM 123 and BM 130 stimulate contractions of the guinea pig ileum, and when injected into rats these compounds cause typical muscarinic effects including chromodacryorrhea, diarrhea, hypothermia, lacrimation, salivation and tremor.

BM 123 and BM 130 caused an irreversible inhibition of muscarinic receptor binding in the rat cerebral cortex and guinea pig ileum in vitro. When tissue homogenates were incubated with BM 123 or BM 130, washed extensively, and assayed subsequently in muscarinic receptor binding assays, a loss in binding capacity for [^{3}H]N-methylscopolamine ([^{3}H]NMS) was noted without an accompanying change in affinity. The loss in receptor capacity was prevented by inclusion of atropine (1.0 μM) into the incubation with BM 123 or BM 130 and tissue homogenate.

The kinetics of receptor alkylation by BM 123 were investigated by measuring the binding of [^{3}H](-)3-quinuclidinyl benzilate ([^{3}H](-)QNB) to homogenates of the rat cerebral cortex following incubation of the homogenates with BM 123 for various times. The half-time for the loss of [^{3}H](-)QNB binding increased from 10 to 45 min as the mustard concentration decreased from 10 μM to 1.0 μM. The reduction in [^{3}H](-)QNB binding that occurred following incubation of cortical homogenates with 1.0 μM BM 123 for 1 hr was completely prevented by thiosulfate (1.0 mM) illustrating that the aziridinium ion is responsible for the persistent blockade.

The competitive inhibition of [^{3}H]oxotremorine-M ([^{3}H]oxo-M) and [^{3}H]NMS binding by the aziridinium ion of BM 123 was measured at 0°C. At this low temperature, little or no receptor alkylation occurs so that it was possible to estimate the affinity of the reversible aziridinium ion-receptor complex. The IC_{50} values of the aziridinium ion for competitive inhibition of [^{3}H]oxo-M and [^{3}H]NMS binding to the rat cerebral cortex (4 nM and 7 μM, respectively) were practically the same as those of its close structural analogue, oxotremorine-M. In contrast to the aziridinium ion, the parent 2-chloroethylamine and its alcoholic hydrolysis product were much less potent inhibitors of [^{3}H]ligand binding.

Collectively, our results establish that the aziridinium ions of BM 123 and BM 130 are potent muscarinic agonists which bind irreversibly to the muscarinic receptor.

PATTERN OF CELL LOSS IN BASAL FOREBRAIN STRUCTURES OF EARLY AND LATE ONSET ALZHEIMER CASES: Pierre Etienne, Yvon Robitaille, Douglas Hospital Research Center, Verdun, Canada and Andre Parent, Laval University, Quebec, Canada.

Price's group first reported a 90% cell loss of large neurons of the nucleus basalis of Meynert (NBM) in a patient with a familial form of Alzheimer's disease (AD)(1) and later a cell loss of over 75% in the NBM of 4 AD patients and one AD or SDAT (senile dementia, Alzheimer type) patient(2). Perry's group reported only a 33% cell loss in the NBM of 6 SDAT patients compared to 5 controls(3). McGeer's group, using a new immunocytochemical technique reported a 66% loss of ChAT positive cells in NBM of 3 SDAT cases compared to 3 controls(4). These 3 research groups did not mention the severity of Alzheimer histopathological change nor did they correlate it with cell loss in NBM.

For our morphometric studies, a brain slab containing the septal nuclei, the nucleus of the diagonal band of Broca, the NBM and a large portion of the basal ganglia was prepared according to Rossor et al(5). Two to three 20μ thick sections were taken at 400μ intervals; one served for the demonstration of AchE pattern according to the histochemical procedure of Karnovsky and Roots, whereas the other 2 were stained with cresyl violet and a silver stain respectively. We examined all sections obtained but only counted large neurons in these sections posterior to the caudal face of the anterior commissure in the mid line. The area counted is limited medially by the optic tract and laterally by the posterior limb of the anterior commissure. All neurons with a diameter larger than 30μ, a visible nucleolus and abundant "Nissl" substance were counted according to the method of Price et al. The pattern of AchE staining was examined in 5 brains (2 brains from patients aged 75 and 90 who had suffered from Alzheimer's disease, 1 case of early and another of late onset and 3 control brains without Alzheimer changes at neuropathological examination aged 66, 78 and 84). Cell loss and staining loss was intense in the NBM particularly in the early onset case of AD where the number of AchE neurons in the SI amounted to 10 - 15% of that found in normal brain. This striking cell loss also involved the large AchE neurons (maximum diameter ranging from 40 - 60μ) scattered within the external and internal medullary laminae separating the putamen from the pallidum and the external from the internal pallidal segments respectively. In contrast, the large intrinsic cholinergic neurons which exist in the neostriatum were found to be unaffected (size, number, morphological characteristics) by the illness. NBM cell counts on cresyl violet sections were done on 9 controls (aged 61 - 88) and 9 Alzheimer cases (4 early onset aged 68, 60, 75, 69 and 5 late onset cases aged 81, 90, 82, 82). Alzheimer changes were severe in all cases except 1 (moderate). Three control cases had mild Alzheimer changes. Two cases of late onset AD also had marked cell loss in substantia nigra. NBM cell loss was striking in Alzheimer cases and ranged between 60 - 90%. There was no overlap between Alzheimer cases and controls. There does not seem to be any difference in cell loss severity in NBM between early and late onset cases when Alzheimer type changes are moderate to severe. The cell loss was striking in both left and right hemispheres (3 cases). We also counted cells within supraoptic nucleus and found cell density in that structure comparable in Alzheimer and control cases.

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EFFECTS OF THE COMBINED TREATMENT OF RATS WITH FLUPHENAZINE AND CHOLINE OR LECITHIN ON THE STRIATAL CHOLINERGIC AND DOPAMINERGIC SYSTEM: Frans Flentge (1), Denise Arst (4), Ben H. Westerink (3), Michael J. Zigmond (4), and Israel Hanin (2), Departments of Biological Psychiatry (1) and Pharmaceutical and Analytical Chemistry (3), University of Groningen, Groningen, The Netherlands and Departments of Psychiatry (2) and Biological Sciences (4), University of Pittsburgh, Pittsburgh, Pennsylvania 15213, U.S.A.

While some investigators have reported that acetylcholine (ACh) synthesis in the brain can be enhanced by exogenous choline (Ch) under physiological conditions, other groups have failed to confirm this observation. However, several groups have shown that ACh levels in the striatum which have been reduced by treatment with drugs which stimulate ACh release (e.g., fluphenazine, atropine), can be partly restored by treatment with high doses of exogenous choline or lecithin. From these results it has been concluded that under stimulated conditions cholinergic nerve endings can take up exogenously offered Ch to support the elevated synthesis rate of ACh. An alternative explanation might be that choline inhibits the stimulating action of the used drugs, thereby restoring normal ACh release and ACh levels.

To investigate this hypothesis we measured not only the effects of lecithin and Ch administration on the decrease in ACh levels caused by fluphenazine (Flu), but also dihydroxyphenylacetic acid (DOPAC) levels as an index of dopamine receptor blockade. This receptor blockade is thought to be responsible for the increased ACh release by preventing the normal inhibition of the cholinergic system in the striatum by dopaminergic cells.

Lecithin (soybean phosphatidylcholine from Americal Lecithin Company U.S.A., 95% pure; or from Unilever Research Co., The Netherlands, 89% pure) was administered intragastrically (10 mmol/kg) to rats 6 hr before sacrifice and fluphenazine hydrochloride (1 mg/kg i.p.) 1 hr before sacrifice by microwave irradiation. We failed to demonstrate any effect of both batches of lecithin on either basal or fluphenazine-decreased ACh levels. Also the increase in DOPAC levels by Flu was not changed by the administration of lecithin. Choline (1 mmol/kg), administered 15 min. before Flu, was able to reverse the fluphenazine-induced decline in ACh levels, provided the animals were sacrificed at a time (45 min. after Flu) when the fluphenazine-induced increase in DOPAC levels was submaximal. At the same time, this increase in DOPAC levels was significantly lower in the choline-treated animals. In this interval of submaximal effect of Flu, ACh levels were strongly correlated with DOPAC levels ($r = -0.9058$, $p < 0.001$).

The present results suggest that the primary action of choline might be the prevention of dopaminergic receptor blockade by Flu; as a consequence normal ACh release and ACh levels may be restored. Supported by NIMH Grant

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INCREASED DENSITY OF MUSCARINIC RECEPTORS ON FIBROBLASTS ASSOCIATED WITH VULNERABILITY TO AFFECTIVE DISORDERS: Elliot S. Gershon and N. Suzan Nadi, Section on Psychogenetics, Biological Psychiatry Branch, National Institute of Mental Health, Bldg. 10, Rm. 3N218, 9000 Rockville Pike, Bethesda, Maryland 20205.

Muscarinic receptors on fibroblasts have been identified by Nadi et al[1], meeting criteria for receptor demonstration of: specific binding, saturability, pharmacologic specificity, inhibition of norepinephrine stimulated adenylate cyclase, and increased binding after incubation with antagonists. The density of the receptors on fibroblasts is about 1/10 that of brain receptors, and the K_D is 10^{-10} M, which is 1/10 the affinity observed in brain.

Sitaram et al[2] have reported that patients with major affective disorder have increased sensitivity to REM induction by muscarinic agonists, in comparison with normal volunteers. They hypothesized that this finding, which is independent of clinical state of the patient, represents a genetic alteration at the receptor level.

Fibroblast receptors are suitable for clinical investigation because of their characteristics similar to neurons (MAO, choline uptake) and because individual differences in receptor density are stable over several cellular generations (passages 5-7). We investigated fibroblasts cultured from skin biopsies of 12 volunteers with no psychiatric disorders, and 18 unrelated patients with primary affective disorder [12 Bipolar I, 5 Bipolar II with major depression, 1 Unipolar (with family history of Bipolar illness)].

B_{max} in volunteers was 227.8 $\pm$ 47.3 fmol/mg protein (mean $\pm$ S.D.), versus 335.1 $\pm$ 58.3 in patients with affective disorder ($p < .0001$). There was no age effect, but females had greater density than males, after controlling for the effect of diagnosis ($p < .05$). There were no differences in K_D associated with sex or clinical diagnosis. Increased density was observed in patients on medication as well as in patients off medications six months or longer. These findings imply that muscarinic receptor density is chronically increased in some cells of patients with affective disorders, and that this increased density is not dependent on neural or hormonal input to these cells.

A more detailed genetic investigation was pursued because of the stability of inter-individual differences. Thirteen relatives with affective disorder, of the patients, were studied (2 Schizoaffectives, 5 Bipolar I's, 5 Unipolars, 1 Anorexia). B_{max} was 307.3 $\pm$ 50.1, similar to the ill patients. Five well relatives were studied, with B_{max} 189.7 $\pm$ 16.7, even less than controls. This suggests that B_{max} is segregating with affective disorders in these pedigrees. However, in one Amish pedigree separately collected and diagnosed by Dr. J. Egeland, there was no mean difference in B_{max} between ill and well individuals. These results suggest that there is heterogeneity in affective disorders, with a substantial proportion of cases associated with increased muscarinic receptor density as a genetic vulnerability factor.

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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.

AD-A148 914 CONFERENCE ON DYNAMICS OF CHOLINERGIC FUNCTION: ACETYLCHOLINE IN HEALTH D..(U) PITTSBURGH UNIV PA I HANIN APR 84 DAMD17-83-G-9534 2/2

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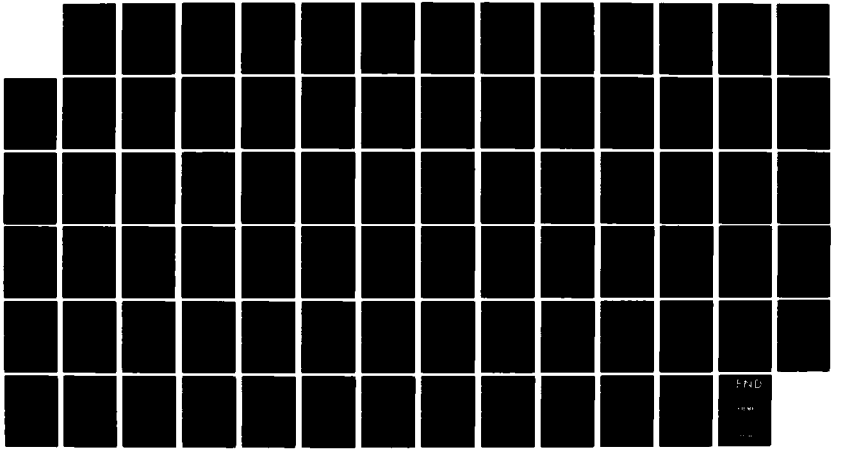

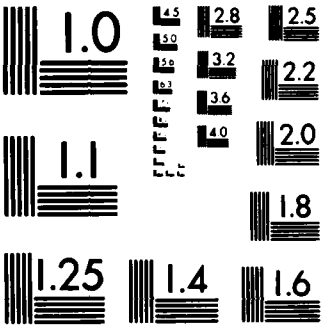

INTERACTIONS OF CALCIUM HOMEOSTASIS, ACETYLCHOLINE METABOLISM, BEHAVIOR AND 3,4-DIAMINOPYRIDINE DURING AGING. Gary E. Gibson and Christine Peterson, Department of Neurology, Cornell Univ. Medical College, Burke Rehabilitation Center, White Plains, New York, 10605, USA.

Age-related deficits in learning and memory may be due to reduced neurotransmitter metabolism that is related to altered calcium homeostasis. In brain slices from aged mice, the calcium-dependent release of acetylcholine decreased from 100 to 46 or 22% in 3, to 10 or 30 month old mice, respectively, but non-calcium-dependent release was unaltered. Therefore, calcium homeostasis was examined in brain synaptosomes from aged rats. In low potassium media (5 mM-KCl), calcium uptake decreased from 104.2% (3 months) to 100% (6 months), 76.7% (15 months) or 58.6% (27 months). In high potassium media (31 mM-KCl), calcium uptake decreased with age from 106.6% (3 months) to 100% (6 months), 67.8% (15 months) or 45.8% (27 months). This age-related reduction in calcium uptake paralleled the decrease in both the calcium-dependent release of acetylcholine and in performance on a simple behavioral measure (the tight rope test). Aging may also alter synaptosomal membranes, since the superficially bound calcium increased at 3 months (105.4%) to 6 months (100%), 15 months (116.1%) or 27 months (141.4%).

3,4-Diaminopyridine, which enhances calcium influx by nerve terminals, diminished the age-related deficits in acetylcholine release, behavior and calcium uptake. The calcium-dependent release of acetylcholine increased 15% (3 months), 89% (10 months) and 260% (30 months) in the presence of 3,4-diaminopyridine. A similar dosage stimulated synaptosomal calcium uptake by 2% (3 months), 10% (6 months), 32% (15 months) and 79% (27 months). These drug-induced alterations appeared to be behaviorally important, since 3,4-diaminopyridine improved tight rope test performance and 8-arm maze performance in aged animals.

Thus, altered calcium metabolism during aging may be the underlying mechanism that leads to behavioral deficits through diminished acetylcholine metabolism. Furthermore, these deficits can be partially reversed by drugs such as 3,4-diaminopyridine that interact with calcium transport.

(Supported in part by grants AG04171 and NS03346.)

ACETYLCHOLINE RELEASE FROM RAT DIAPHRAGM; A SEARCH FOR RELEASE REGULATORY MECHANISMS: Johan Häggblad and Edith Heilbronn, Unit of Neurochemistry and Neurotoxicology, University of Stockholm, Enköpingsvägen 126, S-172 46 Sundbyberg, SWEDEN.

In the course of a study of acetylcholine release from rat diaphragm the possible involvement of a muscarinic autoreceptor at the motor endplate was investigated. First, release of acetylcholine from rat hemidiaphragms was studied in the presence of oxotremorine (10^{-5}-10^{-4} M) and QNB (10^{-8}-10^{-6} M). Release was also studied from hemidiaphragms of supersensitized, long term atropine treated rats (20 mg/kg i.p., 20 days). In the release experiments acetylcholinesterase was inhibited by sarin and backfiring prevented by use of TTX. Evoked release was achieved by increasing the potassium concentration. Acetylcholine was determined by a chemiluminescence technique. No effects of added muscarinic drugs *in vitro* or of atropine treatment were recorded.

In order to mimic autoreceptor action, permeable cyclic nucleotide derivatives were used in subsequent release experiments. 8-Br-cGMP ($2x10^{-4}$ M) did not affect either basal or evoked release. 8-Br-cAMP ($2x10^{-4}$ M) somewhat enhanced basal release, evoked release remained unaltered.

A binding study of ^{3}H-QNB to diaphragm homogenates of the supersensitized atropine treated rats revealed no specific binding of the ligand. In contrast, the expected increase in ^{3}H-QNB binding to cerebral cortex homogenates was observed.

In parallell with binding and release experiments autoradiography on skeletal muscle was performed. No staining was obseved in endplate regions while blood-vessels were readily stained.

The data presented suggest the absence of a muscarinic autoreceptor at the skeletal neuromuscular junction of the rat.

In contrast, preliminary data suggesting a presynaptic mechanism with nicotinic properties, have been obtained. Preincubation of diaphragms with alpha-bungarotoxin was found to augment potassium evoked release, as also previously reported by Miledi and coworkers. Further, in collaboration with Dahlström immunostaining with human myasthenic IgG and rabbit-anti-Torpedo nicotinic acetylcholine receptor IgG was found in crushed rat sciatic nerve. Faint staining was also found to accumulate if rhodamine labeled alpha-bungarotoxin was used.

MANIPULATION OF DIETARY CHOLINE (Ch) AND SLEEP ELECTROENCEPHALOGRAM (EEG) PROFILE IN RATS. Israel Hanin, Shirley Y. Hill, Renato B. Reyes and Anita B. Russell, Department of Psychiatry, Western Psychiatric Institute and Clinic, University of Pittsburgh Medical School, Pittsburgh, PA. 15213, U.S.A.

These studies were designed to follow up on the reported shortened rapid eye movement (REM) latency in depression, and on the implicated involvement of cholinergic mechanisms in the modulation of REM latency both in humans and in rats. The goal of this series of studies was to establish whether one could alter the normal sleep-EEG profile of experimental animals through modification of the supply of Ch in the diet of these animals.

We explored, in rats, the effect of three different diets (in terms of their Ch content) on food consumption, body weight, plasma and RBC Ch, levels of Ch and ACh in selected brain areas, and sleep EEG profiles. Charles River Holtzman (CDBR strain; male) rats were used in these studies. Ch-containing diets: 0% (Ch deficient); 0.2% (Ch normal); and 4% (Ch-enriched) were purchased from the Ralston-Purina Company. Rats were fed for 15 or 21 days with one of these diets, then analyzed for sleep EEG changes. Parallel groups of animals were fed the same diets for the same periods of time, then killed by microwave irradiation of the head, for subsequent analysis of Ch and ACh content in cortex, hippocampus and striatum.

Results obtained were as follows:

a. The Ch-deficient diet, when administered to rats for the time period involved, did not alter any of the parameters initially tested in these rats. Animals fed with the 0% Ch diet gained weight, consumed food, and behaved in an identical manner to the "normal Ch" (0.2% Ch) group. We presume that up to 21 days of feeding with this diet does not cause major deficits in rats. Consequently, subsequent comparison studies in this line of investigation were conducted between "normal Ch" (0.2%) and "Ch-enriched" (4%) fed animals only.

b. Major differences did occur in the Ch-enriched animals by 21 days of feeding. Specifically, body weight gain as a function of time was lower in the Ch-enriched group. Furthermore, by 14 days of feeding the Ch-enriched group was already noticeably more hyperactive and stressed than the normal Ch group. Last, but not least, a significant trend towards increasing Ch and ACh levels was noticed in cortex after 21 days of feeding with the Ch-enriched diet, although levels of Ch and ACh were not affected in hippocampus or striatum of the same animals. As expected, plasma and RBC Ch levels were significantly increased in the animals fed with the Ch-enriched diet.

In spite of these noted chemical changes no differences were observed in a large number of sleep EEG parameters, tested, between rats fed with the Ch-enriched, versus the normal Ch diet. Thus, elevation of Ch levels in vivo by the dietary approach would not appear to have any effect on central sleep-EEG parameters. If these parameters are indeed indicative of an individual's affective state (as has been implied in humans), then our findings would imply that oral administration of high doses of Ch per se is not an efficient approach to altering behavioral function. Conceivably lecithin, or a combination of Ch and some other agents (e.g. piracetam ?) might have a more definitive effect on the neurochemical and sleep parameters studied.

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POLYPHOSPHOINOSITIDE AND PHOSPHOPROTEIN RESPONSES TO MUSCARCINIC RECEPTOR ACTIVATION: John N. Hawthorne and Abdel-Mohsen F. Swilem, Department of Biochemistry, Medical School, Nottingham NG7 2UH, U.K.

Bovine adrenal medulla responds to nicotinic stimulation by secreting catecholamines but simultaneous activation of muscarinic receptors depresses secretion. The calcium influx required for secretion is caused by nicotinic activation but the phosphoinositide effect is produced by muscarinic drugs only. This effect was previously thought to be initiated by hydrolysis of phosphatidylinositol and Michell suggested that it was concerned with calcium gating. Since the calcium flux in the bovine medulla is associated with nicotinic receptors, this theory does not hold.

We have prepared chromaffin cells from bovine tissue and cultured them for two days (Fisher et al., J. Neurochem. 37 (1981) 491-497). Phospholipids were then labelled by incubation for 60 min with inorganic ^{32}P. After two washes to remove excess ^{32}P the cells were incubated with carbachol (3×10^{-4}M) for 30 sec. Phospholipids were then extracted and separated by thin-layer chromatography. Carbachol produced an increase in the radioactivity associated with phosphatidate, but loss of ^{32}P from phosphatidylinositol 4-phosphate (Ptd Ins 4P) and from phosphatidylinositol 4,5 bisphosphate (Ptd Ins(4,5)P_2).

Effect of carbachol on Ptd Ins(4,5)P_2

	Ca^{++} (mM)	cpm in Ptd Ins(4,5)P_2
Control	2.2	592 ± 190
Carbachol	2.2	477 ± 123*
Carbachol	0	580 ± 144
Carbachol (0.5 mM EGTA)	0	584 ± 146

* Student t-test, differs from control, $P < 0.05$.

There was no rapid change in the labelling of phosphatidylinositol itself and we conclude that the initial muscarinic response is hydrolysis of polyphosphoinositides. This is dependent on external Ca^{++} as the Table shows and seems unlikely to be concerned with influx of this ion. Recent evidence in other tissues suggests that the diacylglycerol released when phosphoinositides are hydrolysed may activate protein C kinase, but the significance of this is unknown.

VASOACTIVE INTESTINAL POLYPEPTIDE (VIP)-MUSCARINIC CHOLINERGIC INTERACTIONS:
Britta Hedlund[1,2], Janis Abens[1], Anita Westlind[1] and Tamas Bartfai[1], Department of Biochemistry, Arrhenius Laboratory, University of Stockholm, S-106 91 Stockholm, Sweden[1], and Section of Neuroanatomy, Yale University, School of Medicine, New Haven, Connecticut 06510, USA

In the postganglionic nerves innervating the cat and rat submandibular salivary gland acetylcholine coexists with VIP:

In membranes from the rat salivary gland a number of mutual interactions take place between occupied VIP receptors and muscarinic receptors. A) VIP in nanomolar concentrations enhances muscarinic agonist binding under nonequilibrium conditions (2 min incubation, 37°C). B) Occupancy of muscarinic receptor by either agonists (or antagonists!!) enhances the VIP-mediated stimulation of adenylate cyclase in a GTP-dependent manner. C) VIP attenuates the muscarinic receptor mediated increases in cyclic GMP levels. Atropine and oxotremorine inhibit ^{125}I-VIP binding (which is easiest studied in membranes from the cerebral cortex.

Chronic treatment of rats with the muscarinic antagonist atropine (20 mg/kg/day, 14 days, s.c.) results in a muscarinic supersensitivity, as expected, in all tissues tested. Furthermore the tissue levels of VIP in salivary gland are virtually depleted and the number of ^{125}I-VIP binding sites has increased by almost 100%.

The same treatment has also induced an increase (75%) in the number of ^{125}I-VIP receptors in the cerebral cortex where coexistence of VIP and acetylcholine has not yet been reported.

The results indicate widespread interactions between VIP and muscarinic receptors.

STUDIES ON A cAMP-DEPENDENT PROTEIN KINASE OBTAINED FROM NICOTINIC RECEPTOR-BEARING MICROSACS: Edith Heilbronn, Håkan Eriksson and Robert Salmonsson, University of Stockholm, Unit of Neurochemistry and Neurotoxicology, Enköpingsvägen 126,S-172 46 Sundbyberg, Sweden.

As a part of a study concerning mode and functional consequences of nicotinic receptor phosphorylation, a cAMP-dependent protein kinase was separated from the receptor and its properties and action were studied. Receptor phosphorylation was shown to be clearly cAMP-dependent both when the receptor containing membranes were used as substrate and when isolated receptor was used,though some phosphorylating activity was seen with ^{32}P-ATP only. An inhibitor of cAMP-dependent protein kinases blocked most of the receptor phosphorylation. Phosphorylation was not found to be K^+-activated as previously reported. Phosphorylated receptor parts were identified as the γ and δ-subunits. The sedimentation coefficient of the kinase was found to be 6S which suggests a molecular weight around 170 000, for the holoenzyme.

STRIATAL DOPAMINE-γ-AMINOBUTYRIC ACID-ACETYLCHOLINE INTERACTION IN ORGANOPHOSPHATE-INDUCED NEUROTOXICITY. I. K. Ho, S. P. Sivam, John C. R. Fernando, D. K. Lim and Beth Hoskins, Dept. Pharmacology and Toxicology, Univ. Mississippi Med. Ctr., Jackson, MS 39216, USA.

Diisopropylfluorophosphate (DFP) toxicity and tolerance in rats was studied. Acute injection of DFP showed dose-dependent depressions in body weights and in food and water consumption. The animals recovered within 72 hours. Daily injections of DFP caused significant depressions in all three parameters. However, tolerance to DFP in terms of growth rates, food and water consumption occurred. Other behaviors, e.g., tremors, chewing-movements, hind-limb abduction and hypothermia induced by DFP increased in a steeply dose-dependent manner. All, except chewing, subsided after 7 hours. Chronic treatment with DFP for up to one month produced biphasic patterns of change for all the behavioral parameters. Tremor appeared in a complex spectrum of slow to intense fast types. Except for chewing, tolerance developed for all these behaviors, but at different rates. The data show dose-dependency of general toxicity during acute and subacute exposure to DFP and of tolerance during subacute exposure.

The effects of acute and subacute administration of DFP to rats on acetylcholinesterase (AChE) activity (in striatum, medulla, diencephalon, cortex and cerebellum) and muscarinic, dopamine (DA), and γ-aminobutyric acid (GABA) receptor characteristics (in striatum) were investigated. After a single injection of (acute exposure to) DFP, the striatal region was found to have the highest degree of AChE inhibition. After daily DFP injections (subacute treatment), all brain regions had the same degree of AChE inhibition, which remained at a steady level despite the regression of the DFP-induced cholinergic overactivity. Acute administration of DFP increased the number of DA and GABA receptors without affecting the muscarinic receptor characteristics. Whereas subacute administration of DFP for either 4 or 14 days reduced the number of muscarinic sites without affecting their affinity, the DFP treatment caused an increase in the number of DA and GABA receptors only after 14 days of treatment. This increase, however, was considerably lower than that observed after the acute treatment. The *in vitro* addition of DFP to striatal membranes did not affect DA, GABA or muscarinic receptors.

The effects of acute and subacute administration of DFP to rats on choline acetyltransferase (CAT) activity and on GABA synaptic function were also investigated in the striatal region of brain. Acute and subacute administration of DFP did not affect CAT activity. Acute as well as subacute treatments increased levels of GABA and its precursor (glutamate) and decreased GABA uptake and release. However, none of the treatments affected activities of the GABA related enzymes: glutamic acid decarboxylase and GABA-transaminase. After acute treatment, striatal DA and 3,4-dihydroxyphenylacetic acid (DOPAC) levels were altered with a trend such that the DOPAC/DA ratios were consistently increased within about the first two hours, suggesting an increased turnover of DA. After subacute treatment for 4 or 14 days, DA and DOPAC levels were both decreased without a change in their ratios. Daily treatment for 28 days, however, had no effect on levels of DA and DOPAC.

The results indicate an involvement of the GABAergic and dopaminergic systems in the action of DFP. It is suggested that the GABAergic and dopaminergic involvement may be a part of a compensatory inhibitory process to counteract the excessive cholinergic activity produced by DFP. (These studies were supported by a contract, DAMD 17-81-C-1238, from the U. S. Army Medical Research and Development Command.)

THE EFFLUX OF CHOLINE FROM NEURONS AND GLIA CELLS IN CULTURE : Dominique HOFFMANN, Serge MYKITA, Raphael MASSARELLI, Centre de Neurochimie de Strasbourg, 5, rue Blaise Pascal, 67084 STRASBOURG CEDEX, FRANCE.

The outward mouvement of choline has been observed in primary cultures of neurons and glia cells from chick embryo cerebral hemispheres, preloaded with radioactive choline in the cell growth medium (Dulbecco's modified Eagle's medium supplemented with 10 or 20 % foetal calf serum). Aliquots of the incubation medium (Krebs Ringer phosphate solution pH 7.4) were taken every 1 - 2 min. and the exiting radioactive material was measured in counting aliquots (100 or 200 µl) taking care to maintain the final volume constant by adding the same aliquots of incubation medium. The radioactive material exiting the cells was identified as choline and it was shown that its efflux was strongly dependent upon the ionic composition of the medium : the reduction of Ca^{++} ions increased the efflux of choline from neurons and from glia cells indicating a release mechanism Ca^{++} independant. The substitution of Na^{+} ions with the corresponding isoosmotic $Li^{+}Cl^{-}$ or with sucrose slightly enhances the efflux from neurons while very markedly increased it from glia cells. This difference between the two cell types was also observed in adding K^{+} in the incubation medium. KCl(100 mM) increased the efflux of choline from neurons while a smaller concentration (25 mM) was sufficient to increase the efflux of choline from glia to a similar extent. The presence of $BaCl_2$ in the medium (0.5 µM) reduced the effect of excess K^{+} in neurons but not in glia cells and it had no effect (at least in the concentration range between 0.1 and 1 µM) on the increased efflux produced by the reduced Ca^{++}. The efflux of choline was also temperature dependent and inhibited by cyanide.

The results would indicate that choline which is known to enter nerve cells and synaptosomes following the Na^{+} ionic gradient may leave the cells following the K^{+} ionic gradient, mediated by a channel inhibited by Ba^{++}. This hypothesis would explain the greater sensitivity of glia cells compared to neurons with respect to the effects of excess K^{+}. The balance between the ionic gradients changing during nerve activity might supply choline at the right moment for the synthesis of acetylcholine from glia cells to cholinergic neurons.

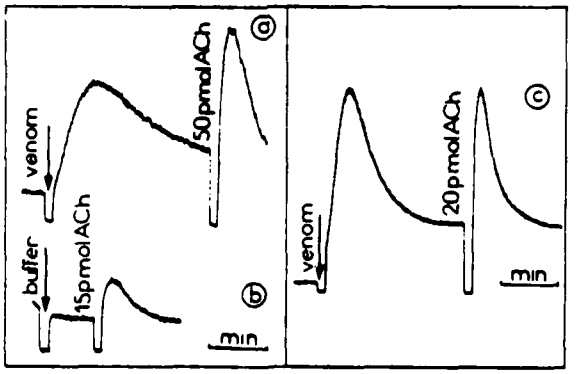
ACETYLCHOLINE RELEASE FROM PC12, A CLONAL CELL LINE: Bruce D. Howard and William P. Melega, Department of Biological Chemistry, School of Medicine, University of California, Los Angeles, California 90024, USA.

We have examined the effect of various drugs on the metabolism and secretion of acetylcholine and dopamine by PC12 cells. PC12 is a clonal line of rat pheochromocytoma cells that respond to nerve growth factor by extending neurites and acquiring the appearance of neurons. Both the undifferential cells and nerve growth factor treated cells store and secrete catacholamines (primarily dopamine) and acetylcholine. The release of acetylcholine and dopamine from PC12 can be evoked by 56 mM K^+, 1 mM carbachol or 2 mM Ba^{2+}. Evoked neurotransmitter release is inhibited by phenothiazines, dephenylhydantoin and phenobarbitol. However, release is not affected by oxotremorine, hexamethonium, β-bungarotoxin or botulinum toxin. The drug AH5183 inhibits the loading of acetylcholine into storage vesicles but it does not block the evoked release of previously loaded acetylcholine. AH5183 also inhibits dopamine transport into the cells but does not affect loading of dopamine into storage vesicles. Unlike other secretory systems evoked neurotransmitter release from PC12 does not require intact stores of ATP. PC12 appears to circumvent the ATP dependent step of secretion, perhaps by a modification of protein Ib. When mammalian brain nerve terminals are stimulated to secrete neurotransmitter, M_r 86,000 and M_r 80,000 proteins (called proteins Ia and Ib, respectively) show an increased phosphorylation. PC12 cells contain proteins equivalent to mammalian brain proteins Ia and Ib; antibody raised against bovine brain proteins Ia and Ib cross reacts with PC12 M_r 86,000 and M_r 80,000 proteins. However, the PC12 M_r 80,000 protein does not exhibit detectable phosphorylation.

A RECONSTITUTED PRESYNAPTIC MEMBRANE EQUIPPED WITH PROTEIN STRUCTURES WHICH PERMIT THE CALCIUM DEPENDENT RELEASE OF ACETYLCHOLINE : Maurice Israël, Nicolas Morel, Robert Manaranche and Bernard Lesbats, Département de Neurochimie, Laboratoire de Neurobiologie Cellulaire, C.N.R.S., Gif sur Yvette, 91190 Gif sur Yvette.

It was previously found that intact electric organ slices, synaptosomes or synaptosomal sacs filled with acetylcholine, release their cytoplasmic or free acetylcholine upon stimulation. It is possible to dissociate the release of cytoplasmic acetylcholine from late changes taking place in the bound vesicular pool, suggesting that endo-exocytosis was probably not directly ensuring the release of the transmitter. The structure which ensured the calcium dependent release of acetylcholine was searched within the membrane itself. The only common ultrastructural change found in a variety of conditions triggering the release of acetylcholine is the appearance of a category of large intramembrane particles while smaller ones disappear. It seemed possible that these particles resulting from calcium action, ensured the passage of the transmitter.

The reconstitution of a functional presynaptic membrane was recently achieved using lyophilized synaptosomal membranes for starting material. The proteoliposomeswhich were obtained contain intramembrane particles similar to the ones found on the native membrane. When filled with acetylcholine the proteoliposomes released the transmitter upon Glycera neurotoxin action or after generating an influx of calcium with the ionophore A 23187. At the peak of acetylcholine release induced by the calcium ionophore, intramembrane particles appear in one of the leaflet of the proteoliposomal membrane. The reconstituted membrane gains its calcium dependent release capabilities from a protein constituent of high molecular weight which was extracted. Several protein subunits appear as possible constituent of this large element.



Legend : a) Release of ACh from the reconstituted presynaptic membrane proteoliposome stimulated with the neurotoxin present in the venom of the annelid Glycera convoluta.

b) Control buffer is injected instead of venom

c) Shows for comparison the release of ACh from synaptosome stimulated with the venom.

ACh was measured using the chemiluminescent assay of Israël and Lesbats, 1980. C.R. Acad. Sci. Paris, 291, 713-716.

CENTRAL ACETYLCHOLINE AND STRESS INDUCED CARDIOVASCULAR, NEUROENDOCRINE AND BEHAVIORAL CHANGES:

David S. Janowsky, M.D., S. Craig Risch, M.D. and J. Chris Gillin, M.D., Departments of Psychiatry, San Diego VA Medical Center and the University of California, San Diego, La Jolla, 92093, U.S.A.

A number of central neurotransmitters have been considered important in the mediation of stressful phenomena. Such neurotransmitters include norepinephrine, vasopressin, GABA, and the opioid polypeptides. Although not widely recognized, there is considerable information from preclinical and clinical studies that acetylcholine may be an important modulator of stress.

Work of Gilad and Associates (1982) suggests that stressful stimuli can cause an increase in the uptake of choline, in the release of acetylcholine, and a downregulation of muscarinic receptors; and that these effects occur to a greater extent in stress sensitive animals.

We have reviewed a number of preclinical studies suggesting that, like aversive stress, increasing central acetylcholine activity with cholinesterase inhibitors and cholingergic agonists activates stress sensitive neurohormones such as beta-endorphin, cortisol, prolactin, ACTH, growth hormone, epinephrine, and norepinphrine.

Furthermore, central cholinergic agonists also increase blood pressure levels in animals, cause exaggerated blood pressure increases in the spontaneous hypertensive rat, and cause behaviors analogous to anxiety and irritability and analgesia. Atropine, a centrally acting anticholinergic agent, effectively reverses the above effects.

To explore the possibility that stressful phenomena are mediated by acetylcholine, we administered the centrally active cholinesterase inhibitor, physostigmine, (.022 mg/kg), crossed over with placebo to 64 psychiatric patients of mixed diagnosis, pretreated with 1.0 mg methscopolamine, Ten additional psychiatric patients received neostigmine (.011 mg/kg) in addition to physostigmine. We then evaluated these patients for changes in mood, behavior, serum neuroendocrines, blood pressure levels and pulse rates.

Physostigmine induced consistent increases in anxiety, depression, hostility, and fatigue and decreases in vigor, friendliness, euphoria, and behavioral activation, a behavioral profile not unlike stress. Neither placebo or neostigmine caused behavioral effects. Physostigmine also increased diastolic and systolic blood pressures and pulse rate significantly more than did placebo. Significant correlations were found between baseline blood pressures and the response to physostigine, a phenomena analogous to that which occured in the spontaneous hypertensive rat.

In addition, physostigmine, in contrast to placebo and neostigmine, caused dramatic increases in serum epinephrine, ACTH, cortisol, prolactin, and beta-endorphin, all stress sensitive neurohormones. None of the above cardiovascular, behavioral, or neuroendocrine phenomena occurred when placebo was given, or in a ten subject series, when neostigmine was given.

Finally, studies by others have shown that physostigmine can cause analgesia, a stress sensitive phenomena, and that it can reverse the soporific, (i.e. antistress) effects of diazepam.

All the above observations, coupled with an extensive animal literature on the subject, suggest that acetycholine may be a major moderator of stress, secondarily increasing sympathetic outflow and opioid polypeptide release.

BIOCHEMICAL EFFECTS OF LECITHIN ADMINISTRATION: Richard S. Jope, University of Alabama in Birmingham, Birmingham, AL 35294.

The effects of altered choline concentration on cholinergic activity are of interest both for increasing our understanding of regulatory mechanisms involved in cholinergic metabolism and clinically as a possible pharmacological means of influencing cholinergic activity. Clinically, the most widely tested design involveds the administration of lecithin to patients with Alzheimer's Disease with the hope that choline derived from lecithin will enhance the synthesis and release of acetylcholine. This report compares the effects of lecithin treatment on choline concentrations in rat and human blood and reports several effects of chronic lecithin treatment to rats.

Lecithin was administered orally to rats (250 gm body weight; 1.9 gm/rat; 10 nmole/kg) and human subjects (15 gm/subject; 0.29 mmole/kg). The time courses of the rise in plasma and erythrocyte choline concentrations in rats and humans were similar. Peak levels of 3 to 4 fold control levels were reached between 4 and 6 hours after treatment. The main difference was that the choline levels decreased at a slower rate in rat blood, perhaps due to the higher relative dose. The choline concentration in rat brain increased with a similar time course to that seen in blood but the maximum increase was only approximately 1.5 times the control level.

Chronic administration of lecithin (10 days) to rats did not cause any greater increase than did acute treatment of the choline levels in plasma, erythocytes, cortex or hippocampus but did result in a greater increase in striatal choline levels.

The lipid and fatty acid compositions of the plasma were also altered by chronic lecithin treatment: free and total cholesterol levels increased, triglycerides increased, the monoene fatty acids generally decreased and the diene and tetraene fatty acids generally increased. There was no effect of this treatment of the hepatic microsomal cytochrome P_{450} activity or on the N-demethylation of benzphetamine or methamphetamine.

In the brain, 10 days of lecithin treatment increased the concentration of choline but had no effect on the concentration of acetylcholine, the activity of choline acetyltransferase, cholinesterase activity, the apparent K_D or B_{max} of muscarinic receptors or the fatty acid composition of rat brain lipids. Further studies of the effects of chronic lecithin treatment are required to clarify its biochemical effects on the brain of normal and diseased subjects.

INFLUENCE OF CHOLINESTERASE INHIBITORS ON ACETYLCHOLINE TURNOVER IN MOUSE BRAIN

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The effect of the irreversible cholinesterase inhibitors soman, sarin and FX on the turnover of acetylcholine (ACh) has been studied in whole brain and striatum of mice. 20 µmol/kg deuterated choline (d_6-Ch) was injected i.v. as precursor and the animals were killed 15 and 45 sec later with microwave irradiation. ACh, Ch and their deuterated analogues were analysed by mass fragmentography. Soman, sarin and FX were injected in doses of 150 µg/kg sc, 330 µg/kg ip and 17.5 µg/kg sc 15 min before d_6-Ch. These doses correspond to about 70 percent of their LD 50 doses and induced strong effects typical of anticholinesterase intoxication.

In whole brain ACh was increased after soman and FX but was unchanged after sarin. Turnover of ACh was decreased after all inhibitors and was correlated to the increase of ACh. In striatum ACh was increased after sarin and FX but not after soman. Turnover was somewhat decreased after sarin and to a great extent after soman and FX. The decrease did not correlate to the changes of ACh.

The results show that the irreversible inhibitors affect the ACh levels to different degrees in the same tissue as well as in different tissues. The level of ACh is not correlated with the rate of ACh synthesis in striatum. Thus direct product inhibition seems to be ruled out as the only cause for a reduced synthesis rate. It is plausible that a direct effect on the synthesis mechanism is of importance, especially in the case of soman.

THE ROLE OF GLIAL CELLS IN NEURONAL ACETYLCHOLINE SYNTHESIS:

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Earlier reports have suggested that choline supply for ACh synthesis may originate from the blood[1], or be released[2] or synthesized de novo by different enzymes in the brain[3]. There are no data, however, on the role of glial cells and glial-neuron contacts in ACh synthesis. To shed more light on this problem we have investigated: (a) the presence of the ACh-system (ACh, CAT, AChE, Ch-uptake, AChR-s) in neurons cultured with or without glial cells, (b) the role of glial cells in choline production, and (c) the effect of contacts between glial cells and neurons on the ACh synthesis.

The procedure used to study the transport of choline from the glial cells to the neurons and its utilisation for ACh synthesis was as follows:

Glial cells from 14-day-old chick brain were cultured in vitro for 14 days and they were then treated with 10 μM ^{14}C-choline solution. Neurons were then plated from 7-day-old embryonic chick brain onto the glial cell layer to give combined cultures. After 2, 5 and 7 days the medium in which the cells were growing was changed and the ^{14}C-choline efflux into it was determined. The ^{14}C-choline incorporated into lipids, phospocholine, betaine and ACh, as well as the free ^{14}C-choline, were determined in the pure glial cell cultures in combined cultures and in pure neuron cultures.

The results obtained in these experiments suggest: (a) The steady-state level of ACh is higher in neurons cultured without glial cells, then in the mixed cultures; (b) When glial cells were cultured without neurons and were treated with 10 uM ^{14}C-Ch, and neurons were next plated onto this preformed glial cell layer, the ^{14}C-Ch was released from the glial cells and utilised in the synthesis of ACh in the neurons; (c) If the pure neuron cultures were placed above the pure glial cells without cell to cell contacts, no ^{14}C-ACh has been formed in the neurones.

From the results presented it is suggested that, apart from other choline sources the glial cells and their contacts with neurons may play a role in the neuronal ACh synthesis.

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MUSCARINIC BINDING WITH QNB IN FRONTAL CORTEX, HYPOTHALAMUS, AND PONS IS SIMILAR IN SUICIDES AND CONTROLS

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Janowsky and colleagues originally proposed the cholinergic adrenergic balance hypothesis of affective illness in 1972, suggesting that depression is associated with a relative increase in central cholinergic activity. More recently, Sitaram and Gillin (1980), Sitaram et al (1980,1982), and Nurnberger et al (1983) have proposed that muscarinic cholinergic supersensitivity is a genetically influenced, trait marker for major affective illness. This hypothesis has, perhaps, been best supported in remitted, unmedicated bipolar patients, who entered REM Sleep more rapidly than age and sex matched normal controls following an intravenous infusion of arecoline (a muscarinic agonist) between the first and second REM periods. In addition, Nadi, Nurnberger, and Gershon (unpublished observations)have reported that muscarinic binding with QNB is greater on fibroblasts obtained by skin biopsy from bipolar patients and their first degree relatives compared with controls.

To test this hypothesis, we determined muscarinic binding with QNB in frontal cortex (n=10), hypothalamus (n=10), and pons (n=8) from pairs of suicides and controls matched for age, sex, postmortem interval (from death to autopsy), and freezer time (from autopsy to assay). Bmax did not correlate with age, postmortem interval, or freezer time for the combined suicide and control groups in any brain region. Furthermore, it did not differ between suicides and controls in any brain area: frontal cortex (185 versus 170 fmol/mg protein), pons (77 versus 78), or hypothalamus (122 versus 187, $p>.05$). In addition, Kd did not differ between suicides and controls in any brain region.

These results do not support the hypothesis that muscarinic receptors are increased in major affective illness, but the hypothesis can not be rejected on the basis of this study. First, we have little data on the premorbid diagnosis of the suicide subjects in this sample; only about 50% of suicides meet the diagnostic criteria for major affective illness and only about 11% meet the criteria for bipolar illness. Secondly, the methodology of the study could not fully control for other variables which might have been important, such as use of psychoactive medications affecting the cholinergic nervous system. Third, binding studies, such as this one, have important limitations, such anatomical specificity, functional activity, and subtypes of receptors involved. Future studies with direct agonists, or measures of cGMP synthesis or phosthatidylinositol turnover, might be informative.

ON THE SENSITIVITY OF DIFFERENT GROUPS OF SKELETAL MUSCLES TO NEUROMUSCULAR BLOCKING AGENTS: Dmitry A. Kharkevich, Vladimir P. Pisenko, Department of Pharmacology, First Medical Institute, B.Pirogovskaya 2/6, Moscow 119435, USSR

The relationship between the sequence of muscle relaxation, chemical structure and mode of action of neuromuscular blocking agents was investigated. Action potentials of six groups of skeletal muscles were recorded simultaneously. The role of the mode of action was elucidated using two pairs of neuromuscular blocking drugs: decadonium-decamethonium and diadonium-succinylcholine. Decadonium and diadonium are nondepolarizing N-adamantyl analogs of decamethonium and succinylcholine, respectively. The data obtained indicate that different groups of muscles are equally sensitive to structurally similar neuromuscular blocking drugs, but having different modes of neuromuscular blocking action. However, chemically different neuromuscular blocking agents cause unequal order of relaxation in various groups of muscles. Depolarizing agents with identical cationic groups and different interonium structure (decamethonium, succinylcholine) cause relaxation of skeletal muscles in dissimilar sequences. The latter concerns also nondepolarizing drugs with different interonium structure (decadonium-diadonium, pancuronium-dacuronium). Nondepolarizing agents - -truxillic acid derivatives (anatruxonium, cyclobutonium) or steroid derivatives (pancuronium, pipecuronium), having different cationic groups but similar interonium structure are characterized by the same sequence of muscle relaxation. Thus, the structure of the interonium part of the molecule of neuromuscular blocking agents is more significant for the succession of their neuromuscular blocking action than the structure of cationic groups. The dissimilarities in the sequences of muscle relaxation, depending on the chemical structure of neuromuscular blocking agents, are maintained under unequal content of "fast" and "slow" fibers, varying temperatures, acid-base equilibrium and circulation in the skeletal muscles.

FACILITATION AND INHIBITION BY MUSCARINIC AGONISTS OF ACETYLCHOLINE RELEASE FROM PERIPHERAL NERVES: Heinz Kilbinger,

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The effects of muscarinic agonists on acetylcholine (ACh) release and smooth muscle contraction were studied in the myenteric plexus-longitudinal muscle preparation of the guinea-pig ileum. The tissue was preloaded with ^{3}H-choline and subsequently superfused with Tyrode solution.

Pilocarpine and muscarine (both 0.1 - 100 µM) caused a transient increase in spontaneous outflow of ^{3}H-ACh that was prevented by tetrodotoxin (300 nM) and scopolamine (100 nM), but not by hexamethonium (300 µM). These results suggest that the myenteric nerves are endowed with muscarine receptors whose activation increases the spontaneous release of ACh.

When the strips were stimulated electrically (1 Hz, 120 pulses) muscarinic agonists (oxotremorine, methylfurmethide, muscarine, carbachol, arecoline, ACh, pilocarpine, choline) inhibited in a concentration-dependent manner the evoked release of ^{3}H-ACh. The pD_2 values obtained for presynaptic effects (inhibition of release) and postsynaptic effects (smooth muscle contraction) were similar.

In other experiments the affinity constants (pA_2 values) for pre- and postsynaptic effects of muscarinic antagonists were determined (scopolamine, methylatropine, trihexyphenidyl, clozapine, pirenzepine). The antagonistic potency of the drugs was determined by constructing concentration-response curves for pre- and postsynaptic effects of oxotremorine in the absence and presence of the respective antagonist. It was found that pre- and postsynaptic pA_2 values for each of the antagonists did not differ significantly.

The results suggest that the myenteric plexus is equipped with three types of muscarine receptors: 1. The "classical" postsynaptic receptor that causes contraction of the smooth muscle. 2. The presynaptic receptor which causes inhibition of the evoked release of ACh. Both receptors have similar pharmacological properties. 3. A receptor that causes facilitation of ACh release. This receptor may be located on the soma-dendritic region of the neurone.

AN ENDOGENOUS NEUROTROPHIC FACTOR FOR THE MAINTENANCE OF AChE AND BuChE IN THE PREGANGLIONICALLY DENERVATED SUPERIOR CERVICAL GANGLION OF THE CAT: George B. Koelle, Gerard A. Ruch, and Eiji Uchida, Department of Pharmacology, Medical School/G3, University of Pennsylvania, Philadelphia, PA 19104, USA.

Nearly forty years ago Sawyer and Hollinshead (J. Neurophysiol., 8:137, 1945) showed that preganglionic denervation of the cat superior cervical ganglion (SCG) results within 3 days in a loss of >80% of its AChE and >30% of its BuChE content. In the present study we have found that these effects can be largely prevented by the prolonged intraarterial infusion of a crude extract of cat brain, spinal cord, and sciatic nerves.

Under sodium pentobarbital anesthesia, the SCG of cats were preganglionically denervated bilaterally; 24 hr later, they were reanesthetized, the external carotid and lingual arteries ligated bilaterally, and the right common carotid artery was infused for 24 hr with the extract. Cats were sacrificed at 48 hr post-denervation, and the AChE and BuChE contents of the SCG were compared with those of similarly denervated, arterially ligated control ganglia. Results were as follows (numbers of SCG in parentheses):

Procedure	nMol substrate hydrolyzed/mg protein/min	
	AChE	BuChE
1. Normal SCG	449 ± 42 (8)	590 ± 61 (8)
2. Preganglionically denervated, arterially ligated at 24 hr, sacrificed at 48 hr.	167 ± 10 (8)	412 ± 29 (8)
3. Ibid. 2, plus infusion of extract 24-48 hr.	354 ± 35 (6)	602 ± 65 (6)

These findings indicate that nerve tissue contains a neurotrophic factor that maintains the contents of AChE and BuChE in the cat SCG. Whether it acts by prevention of preganglionic degeneration or by maintenance of postsynaptic AChE and BuChE is now under investigation.

It was also found that in non-arterially ligated cats continual anesthesia with pentobarbital opposes the fall in AChE following preganglionic denervation.

HISTOCHEMICAL AND BIOCHEMICAL EFFECTS OF THE INJECTION OF AF64A INTO THE NUCLEUS BASALIS OF MEYNERT: Michael R. Kozlowski, Roni E. Arbogast and Nicholas G. Bacopoulos, Pfizer Central Research, Groton, CT 06340, USA.

Ethylcholine mustard aziridinium ion (AF64A) has been shown to have selective toxic effects on cholinergic neurons when injected into the cerebral ventricles or the hippocampus (Mantione et al., Science 213, 579, 1981; Mantione et al., J. Neurochem. 41, 251, 1983). We now report the effects of this substance when injected locally into the area of the nucleus basalis of Meynert (nbM).

Male Sprague-Dawley rats anesthetized with equithesin were given stereotaxic injections of AF64A (0.01 to 1.0 nmoles in 1µl; prepared as the aziridinium ion) or its vehicle (isotonic saline pH 7.4) unilaterally at two sites along the rostrocaudal axis of the nbM. The injections were made at a rate of 0.4 µl/min., and the cannula was allowed to remain in place an additional 5 min. The rats were sacrificed 7 to 14 days postlesion and their brains were removed. Cortical choline acetyltransferase (CAT) activity was measured as an indicant of the functional level of cholinergic terminals in the cortex, and acetylcholinesterase (AChE) histochemistry was used to help identify nbM neurons, which stain darkly for AChE.

Even the lowest dose of AF64A used (0.01 nmole) caused a visible decrease in AChE staining in the region of the nbM immediately adjacent to the cannula tip, however no decrease in CAT activity in the ipsilateral cortex (vs contralateral) was seen. Higher doses (0.02 and 0.05 nmole) produced a more widespread loss of AChE staining in the region of the nbM, including a substantial loss of densely staining cell bodies, and modest localized decreases (10-20%) in cortical CAT activity ipsilaterally. Larger decreases in both AChE activity in the nbM and cortical CAT activity were seen with the highest doses of AF64A used (0.2 and 1.0 nmole), however these effects were accompanied by substantial non-specific tissue damage surrounding the injection sites. Vehicle injections did not alter AChE activity in the nbM or CAT activity in the cortex. These results suggest that injections of low doses of AF64A into the nbM, which produce minimal non-specific tissue damage, can decrease cortical CAT activity by damaging the cholinergic nbM neurons that project to the cortex.

REGULATION OF STRIATAL CHOLINERGIC ACTIVITY BY THE CORTICOSTRIATAL PATHWAY:
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The influence of the corticostriatal excitatory pathway on cholinergic neuropharmacology of the striatum was investigated. As previously shown, the interruption of this pathway by undercutting the cortex reduced glutamate uptake by about 50% and depressed ACh synthesis by about 30% after two post-lesion weeks without affecting ACh and choline contents, ChAT and AChE activities and the levels of noradrenaline, serotonin, dopamine and DOPAC. The high affinity uptake of choline by the synaptosomal fraction was reduced. The results suggest that the cholinergic neurons intrinsic to the striatum are depressed by the removal of the excitatory pathway.

It was found that the marked increase in striatal ACh content induced by several classes of drugs was prevented in the decorticated rats. These drugs included oxotremorine (1 mg/kg, i.p., 20 min), muscimol (10 mg/kg, i.p., 30 min), 2-chloroadenosine (20 ug, i.c.v., 20 min) and some dopaminergic agonists, apomorphine (1.5 mg/kg, i.p., 30 min), bromocriptine (4 mg/kg, i.p., 90 min) and lisuride (200 ug/kg, s.c., 30 min). Despite the blockade of the cholinergic action of apomorphine by decortication, behavioral responses to the drug were unaltered - rotatory behavior was not induced in unilaterally decorticated rats and stereotyped behavior was not mitigated in bilaterally decorticated animals - providing evidence that the brain lesion did not interfere with the drug's disposition in the brain.

By contrast, frontal decortication did not mitigate the decrease in ACh content induced by typical (pimozide, 1 mg/kg, i.p., 240 min) and atypical (clozapine, 20 mg/kg, i.p., 60 min and L-sulpiride, 100 mg/kg, i.p., 60 min) neuroleptics. These results therefore suggest that the cholinergic neurons inhibited by the loss of the excitatory input cannot be further inhibited by drugs that depress these neurons whereas they can respond to drugs that activate them.

Acetylcholinesterase inhibitor-induced increases in striatal ACh content by physostigmine (1 mg/kg, i.p., 20 min) and dichlorvos (20 mg/kg, p.o., 30 min) were only weakly antagonized by the lesion.

Ongoing studies are designed to determine whether the excitatory pathway influences the cholinergic neurons directly or indirectly and whether the nigrostriatal dopaminergic pathway is also involved.

CHARACTERIZATION OF ^{3}H-NICOTINE BINDING IN RODENT BRAIN AND COMPARISON WITH THE BINDING OF OTHER LABELLED NICOTINIC LIGANDS: Christer Larsson and Agneta Nordberg, Department of Pharmacology, Uppsala University, Box 573, S-751 23 Uppsala, Sweden.

Specific binding of labelled nicotinic ligands (^{3}H-alfa-bungarotoxin; ^{3}H-alfa-BTX, ^{3}H-nicotine; ^{3}H-NIC and ^{3}H-tubocurarine; ^{3}H-TC) to brain homogenate have been reported by several laboratories. The physiological relevance of the ^{3}H-alfa-BTX binding site is, however, unclear, even if the criteria for in vitro ligand-neuro-receptor interaction (e.g. saturability) are fulfilled. Recently some laboratories have also measured binding of ^{3}H-NIC (with high specific activity) to brain homogenate. The outcome of these studies has, however, been found to vary among laboratories, probably due to differences in procedure. In this in vitro binding study we have therefore tried to define the conditions which yields optimal specific binding for ^{3}H-NIC to brain homogenate.

The specific ^{3}H-NIC binding increases linearly with protein concentration, between 0.05-0.3 mg. Between 0.1 - 0.2 mg of tissue protein the specific binding is 35-40% of total binding. Incubation temperature was found to effect binding capacity in an opposite manner than is ordinary for in vitro receptor-ligand interactions. The binding of ^{3}H-NIC decreases linearly with temperature, showing highest binding at 4^{o}C and lowest at 37^{o}C. The time course for association of ^{3}H-NIC to brain homogenate is rapid with maximal binding after only 5-10 min; the binding is then reduced. In a previous study (1) we have estimated equilibrium binding data for both ^{3}H-alfa-BTX and ^{3}H-TC binding to hippocampus (mouse). In the table we now compare Bmax values and affinity constants for ^{3}H-BTX and ^{3}H-TC with the values for ^{3}H-NIC binding to hippocampus (mouse). As one can see in the table, data indicate two binding sites for ^{3}H-NIC and ^{3}H-TC while ^{3}H-BTX has only one binding site. Further, it is interesting to note the striking similarities between the constants for the high affinity site of ^{3}H-NIC and ^{3}H-TC and the binding site for ^{3}H-BTX. ^{3}H-NIC binding to different brain areas indicates a differential regional distribution of ^{3}H-NIC binding sites in mouse brain.

TABLE

Ligand	Bmax (pmol/g protein)	Kd (nM)
^{3}H-BTX	50	4
^{3}H-TC	75	2
	275	14
^{3}H-NIC	60	6
	230	125

(1) Studies of Nicotine-Like Binding Sites in Brain. Christer Larsson and Agneta Nordberg. In: Neurotransmitters and their Receptors, Eds: U.Z. Littauer, Y. Dudai, I. Silman, V.I. Teichberg and Z. Vogel. 1980 John Wiley & Sons Ltd.

CORTICAL CHOLINERGIC HYPOFUNCTION AND BEHAVIORAL IMPAIRMENT PRODUCED BY BASAL FOREBRAIN LESIONS IN THE RAT: Barbara Lerer, Elkan Gamzu and Eitan Friedman, New York University Medical Center, New York, NY 10016 and Pharmacology I, Hoffmann-LaRoche Inc., Nutley, NJ 07110

The magnocellular nuclei of the basal forebrain (MNBF) provide extensive cholinergic innervation to fronto-parietal cortex and are analogous to the nucleus basalis, implicated in the cognitive dysfunction in Alzheimer's disease. Bilateral MNBF neurotoxic lesions depleted cortical choline acetyltransferase (CAT) in frontal and parietal cortex but not in striatum or hippocampus. Cortical dopamine was unaltered, while serotonin concentrations in the frontal cortex were slightly diminished.

Behavioral testing began 3 wk post-operative, after MNBF-lesioned rats recovered from the aphagia, adipsia and locomotor abnormalities resulting from the lesion. Rats were tested only if they weighed 20 g more than pre-operative baseline weight. The MNBF rats were compared to unoperated controls, sham-operated controls and control rats injected with kainic acid in the cortical area directly above the MNBF. Cortically-lesioned controls displayed the hyperactivity characteristic of MNBF rats for 7-10 hr after surgery, but were not aphagic, adipsic or behaviorally impaired.

MNBF-lesioned rats were impaired in 24 hr retention of a passive avoidance task with escapable footshock; however, their acquisition performance during the initial training trial was not different from that of the three control groups. There were no differences between the four groups in mean number of daily avoidances on a bar-press active avoidance task; however, the data suggested a slower rate of avoidance learning in the MNBF rats. Mean rates of motor activity were not different between groups and, therefore, do not account for the results obtained in these two tasks. Finally, all rats were tested in a task involving an exploratory response to one of two recessed feeding devices. The procedure required serial reversals of a spatial discrimination. MNBF-lesioned rats showed evidence of acquiring the task, as indicated by a decrease in the mean number of daily errors; however, their performance was significantly poorer than that of the three control groups.

A cortical cholinergic deficit may underlie the memory impairment and reduced cognitive functioning of Alzheimer's disease. We believe our rodent model is useful for studying the role of the cholinergic system in memory dysfunction and for developing treatment strategies to alleviate the deficits of Alzheimer's disease.

TENDENCY FOR REPETITIVE VAGAL ACTIVITY TO SYNCHRONIZE CARDIAC PACEMAKER CELLS: Matthew N. Levy and Tianen Yang, Department of Investigative Medicine, The Mt. Sinai Medical Center, Cleveland, Ohio 44106, USA

One burst of stimuli was delivered to the left or right vagus nerve each cardiac cycle in anesthetized, open-chest dogs. The timing of the stimulus bursts was varied within the cardiac cycle by a constant, small amount each heart beat, until the entire cardiac cycle was scanned. The level of vagal activity was changed by varying the number of stimulus pulses in each burst. For a given number of pulses per burst, the mean cardiac cycle length and the amplitude of the phase-response curve were significantly greater during right than during left vagal stimulation. These response characteristics increased as the number of pulses per burst was augmented. The minimum-to-maximum phase-differences of the phase-response curve were less during right than during left vagal stimulation and they decreased when the number of pulses per burst was increased. The minimum-to-maximum phase-difference is an index of the extent to which the individual automatic cells in the S-A node are synchronized with one another during phase-coupled vagal stimulation. The disparities between the minimum-to-maximum phase-differences for the right and left vagi are probably ascribable to the associated differences in the overall magnitudes of the chronotropic responses, rather than to any fundamental difference in the innervation of the effector cells by nerve fibers originating from the right and left sides.

MUSCARINIC RECEPTOR ACTIVATION INCREASES EFFLUX OF CHOLINE FROM ISOLATED HEART AND RAT CORTEX IN VIVO: Konrad Löffelholz, Renato Corradetti, Rudolf Brehm and Ruth Lindmar, University of Mainz, Dept. of Pharmacology, 6500 Mainz (FRG)

Efflux of choline (Ch) from the isolated perfused chicken heart into the perfusate and from the rat cortex in vivo into the "cup solution" was taken as a measure for cellular Ch mobilization. Ch was determined by the chemiluminescent and/or a radioenzymatic method. Mepacrine, a phospholipase A 2 inhibitor, reduced the basal cardiac efflux by about 50 % (EC 50 was 7 µM). - Corradetti, Lindmar & Löffelholz (Eur. J. Pharmacol. 85: 123, 1982) found that the basal cardiac Ch efflux was maintained constant at about 1 nmol/g min for 2 to 3 h due to constant hydrolysis of phospholipids and was facilitated by physostigmine. The effect was blocked by atropine. - The conclusion that muscarinic receptor activation increases cellular mobilization of Ch was now confirmed by showing that ACh (0.3 and 1 µM)about doubled cardiac Ch efflux, an effect that was blocked by atropine (3 µM). Cooling the perfusion medium from 37°C to 17°C reduced both basal and ACh-evoked Ch efflux to about 20 %. At 37°C, mepacrine (10 µM) caused inhibition by about 40 % of both basal and evoked efflux. - To study the involvement of cAMP on cardiac Ch efflux, we used forskolin (30 nM to 10 µM), a adenylate cyclase activator, and IBMX (0.3 mM), a cAMP phosphodiesterase inhibitor. Both substances increased cardiac Ch efflux up to 200 and 50 %, respectively, and markedly augmented force of contraction as determined on isolated chicken atria. Atropine (0.1 and 3 µM) potentiated the effect of forskolin on Ch efflux, whereas DFP (ChE inhibitor) had an opposite effect. - Similar to the heart, Ch efflux from the rat cortex in vivo was highly temperature-sensitive, was increased by physostigmine (1 µM) and by bethanechol (0,5 mM)and was reduced by atropine. - It is concluded that muscarinic receptor activation has two opposing effects on Ch efflux. First, it facilitated Ch efflux in heart and brain, an effect which, at least in the heart, appeared to have the same temperature-sensitivity and dependency on phospholipase A 2 activity as the basal Ch efflux. Second, muscarinic receptor activation possibly inhibits cAMP-mediated increase in Ch efflux. We speculate that hydrolysis of Ch-containing phospholipids such as phosphatidylcholine is influenced by ACh and possibly by other neurotransmitters and hormones.

RELATIONS BETWEEN CHOLINE ACETYLTRANSFERASE AND MUSCARINIC BINDING IN AGING AND ALZHEIMER'S DISEASE: Edythe D. London and Steven B. Waller, Addiction Research Center, National Institute on Drug Abuse, and Gerontology Research Center, c/o Baltimore City Hospitals, Baltimore, Maryland, 21224, USA.

Studies in rodents and in tissue culture systems have demonstrated that muscarinic receptor binding, as measured with the ligand [^{3}H]quinuclidinyl benzilate (QNB), is modulated by cholinergic input (R. G. Simon, W. L. Klein, 1979, Proc. Natl. Acad. Sci. U.S.A. 76, 4141; Y. Ben-Barak, H. Gazit, Y. Dudai, 1980, Brain Res. 194, 249; M. McKinney, J. T. Coyle, 1982, J. Neurosci. 2, 97). Additional evidence indicates that aging and Alzheimer's disease (AD) can influence central cholinergic markers. Thus, it seems possible that the relations between presynaptic cholinergic markers and muscarinic binding could change with aging or AD. We therefore studied choline acetyltransferase (ChAT) and muscarinic binding in brain regions from rats and mice of different ages. Assays also were performed on autopsy samples from people who died with AD. The objective was to determine if the relations between the cholinergic markers changed with aging or AD. ChAT and muscarinic binding were assayed in brain regions from male Fischer-344 rats, Wistar rats, and C57BL/6J mice. Animals of different ages (4 to 24 mo), were used to obtain samples of cortex, striatum, and hippocampus. In the Wistar rat, only the cortex and hippocampus were sampled. ChAT was assayed radiometrically, and muscarinic binding was assessed with QNB as the ligand.

Data obtained in rats were consistent with a general decline in pre- and postsynaptic cholinergic markers. Values of Vmax for ChAT were lower in the cortex of aged Wistar rats and in the striatum and hippocampus of aged Fischer-344 rats. In aged Wistar rats, K_D and Bmax were reduced in the hippocampus, but K_D was higher in the cortex.

Age differences in mice were markedly different from those observed in rats. In the cortex, striatum and hippocampus, Vmax for ChAT was elevated in older mice. Although there also was an elevation in Bmax for QNB in the hippocampus, values of Bmax were reduced in the cortex and striatum. These findings suggest that in the cortex and striatum, increased ChAT may reflect a presynaptic compensation to maintain a critical level of cholinergic neurotransmission despite receptor losses.

In neocortical and hippocampal samples from people who died with AD, ChAT was markedly reduced as compared with corresponding controls. Muscarinic binding was not reduced in the AD samples, but was increased in several regions. These findings suggest that a presynaptic cholinergic defect in AD is associated with muscarinic receptor upregulation.

The results of this study indicate that effects on the relations between ChAT and QNB binding vary with the species and brain region assayed. Although evidence for postsynaptic receptor upregulation was obtained in samples of AD brain, no evidence for this phenomenon was seen in rodent brains. (We thank Dr. Melvyn J. Ball, University of Western Ontario for supplying postmortem tissue from AD brains.)

EFFECTS OF EXOGENOUS CHOLINE ON ACETYLCHOLINE RELEASE FROM SLICES OF RAT STRIATUM: Jean-Claude E. Maire, Jan K. Blusztajn and Richard J. Wurtman, Laboratory of Neuroendocrine Regulation, Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge, MA, 02139, USA.

The synthesis of acetylcholine (ACh) requires choline as the immediate precursor. Free choline may be supplied to cholinergic neurons by blood circulation and it is also present in all cells as a constituent of membrane choline-phospholipids. The objective of this study was to investigate the effect of the exogenous choline on ACh release and to determine whether the choline-containing compounds of brain tissues can constitute a source of choline for ACh biosynthesis.

Slices from rat striatum were superfused with a physiological solution (PhS). After a period of equilibration, the effluent was collected and assayed for choline and ACh. The tissue content of choline and ACh was also determined in samples removed from the perfusion apparatus at the beginning ("initial content") or at the end ("final content") of the collection period.

Spontaneous release of ACh into the effluent was 7.5 pmol x mg of protein^{-1} x min^{-1}. In the presence of 5 or 20 μM choline the spontaneous release of ACh was increased by a factor of 1.8 or 2.6, respectively. The addition of an inhibitor of the high affinity choline uptake, hemicholinium-3 (HC-3) (5 μM) to the choline-free PhS caused a decrease in the ACh release by a factor of 0.5. When slices were superfused with PhS, choline appeared in the effluent at a rate of 55 pmol x mg of protein^{-1} x min^{-1}. In the presence of HC-3 there was a 1.75 fold increase in the rate of choline efflux.

Evoked release. Electrical stimulation of the slices (15 Hz, 30 min.) led to an increase in the rate of ACh release. This rate was constant throughout the stimulation period. The amount of evoked ACh released during the stimulation period was 767, 773 or 1039 pmol/mg of protein, when the slices were superfused with PhS containing 0, 5 or 20 μM of choline, respectively. Electrical stimulation of the slices superfused with choline-free PhS containing HC-3 caused the release of ACh at a rate that was initially similar to that occuring in the absence of HC-3. This rate quickly declined to that characteristic to the spontaneous release. Electrical stimulation had no effect on the release of choline.

In choline-free PhS, the differences between the "initial" and "final" tissue content of choline and ACh accounted for only a fraction of these compounds found in the effluent. Thus we postulate that the major fraction of the released choline originated from the breakdown of the membrane choline-phospholipids.

The observations that ACh content of the slices and ACh release (but not choline efflux) were lowered by HC-3 suggest that the choline originating from choline-phospholipids is liberated into the extracellular space and can be used for ACh synthesis only if the high affinity uptake of choline is functional. The results of this study further support the hypothesis that the release of ACh can be stimulated by supplementation with exogenous choline.

(J-C. Maire holds a fellowship from the Swiss Foundation for Fellowships in the Field of Experimental Medicine and Biology; these studies were supported in part by NIMH grant MH-28783).

MEMBRANE TRANSPORT OF CHOLINE BY HUMAN ERYTHROCYTES: RELATIONSHIP TO INTRACELLULAR CHOLINE CONTENT: Alan G. Mallinger, Ursula Kopp and Israel Hanin, University of Pittsburgh School of Medicine, Western Psychiatric Institute and Clinic, Pittsburgh, Pennsylvania, 15213, USA.

The choline (Ch) content of erythrocytes (RBCs) has been reported to be elevated in several psychiatric and neurologic disorders, including major depression, acute mania, Gilles de la Tourette syndrome, and dementia. Although the mechanisms that regulate intracellular Ch content are not completely understood, previous investigations have demonstrated that lithium treatment results in both significant elevations of RBC Ch content and a concomitant inhibition of Ch transport across the RBC membrane. The present investigation was conducted in order to examine the relationship between in vivo RBC Ch content and Ch flux across the RBC membrane (measured in vitro) in lithium-treated and drug-free subjects.

To perform the in vitro studies, we developed a method for measuring the outward transport of endogenous Ch (D_0Ch efflux), and the concomitant inward transport of deuterated Ch (D_4Ch influx), using RBCs that were incubated in physiologic media containing 10μM D_4Ch. Incubations were conducted for 24 minutes at 17°C. Transport of the Ch isotopes, measured using gas chromatography/mass spectrometry, was linear during the incubations, so that unidirectional net flux values for D_0Ch and D_4Ch transport could be calculated directly from the slopes of the concentration-versus-time curves.

In drug-free, psychiatrically normal control subjects, D_0Ch efflux was inversely correlated with the natural logarithm of in vivo RBC Ch content ($r=-0.97$, $p<0.001$), and there was a nearly significant correlation between D_4Ch influx and the natural logarithm of in vivo RBC Ch content ($r=-0.81$, $p<0.06$). In patients with bipolar affective disorder, lithium treatment both inhibited D_0Ch and D_4Ch transport, and substantially elevated the in vivo RBC Ch content as compared to pretreatment values. However, varying degrees of transport inhibition (ranging from 46.6 to 99.0% of the pretreatment values) were produced by this drug in different subjects. Despite this variability of transport inhibition, the small group of lithium-treated bipolar patients had higher levels of RBC Ch than untreated patients or drug-free control subjects. We observed a close correlation between D_0Ch efflux and D_4Ch influx ($r=0.91$, $p<0.02$) in RBCs from drug-free control subjects; in addition, the degree of Ch transport inhibition produced by lithium treatment was similar for D_0Ch efflux and D_4Ch influx. Preliminary studies using cord blood, which is representative of the fetus at term, were also performed. RBCs from cord blood of a lithium-treated bipolar patient had substantially elevated Ch content, as compared to cord RBCs from drug-free control subjects. Moreover, cord plasma and RBCs from drug-free subjects had higher Ch content values than maternal blood. RBC Ch transport, measured in one specimen of cord blood, was elevated in comparsion with adult values.

This work demonstrates a potentially useful in vitro method for the quantitative study of Ch transport across RBC membranes, using physiologically relevant amounts of Ch. Our findings suggest that membrane Ch transport may have a role in the regulation of endogenous RBC Ch content, and that lithium treatment can produce varying degrees of transport inhibition (potentially related to specific clinical variables) in different subjects. Our findings also support the idea that a common mechanism is involved in both outward and inward transport of Ch by RBC membranes. Finally, preliminary evidence suggests that maternal lithium treatment affects Ch transport by fetal RBCs, and that, in drug-free subjects, fetal handling of Ch may differ from that of adults. Future investigations of membrane Ch transport by RBCs or other types of cells could potentially help to increase our understanding of cholinergic function in psychiatric or neurologic disorders.(Supported by NIMH Grant #MH26320)

BIOCHEMICAL AND ULTRASTRUCTURAL STUDIES OF ACETYLCHOLINE RELEASE INDUCED BY GLYCERA CONVOLUTA NEUROTOXIN : Robert Manaranche, Maurice Israel and Nicolas Morel, Laboratoire de Neurobiologie Cellulaire, Département de Neurochimie, Centre National de la Recherche Scientifique, Gif sur Yvette, 91190, France.

Several previous works in which we studied the release of acetylcholine(ACh) from Torpedo electric organ synaptosomes have shown that the cytoplasmic ACh pool was depleted in parallel to modifications of intramembrane particles.

The only ultrastructural change which was found was the occurrence of large intramembrane particles while small ones disappear. These large particles became more numerous for the strongest release conditions.

The Glycera convoluta venom (GCV), which was extracted from the venom glands of this polychaete annelid, is able to induce a high frequency of miniature end-plate potentials at neuromuscular junction and electric organ synapses. It was found that the calcium dependent ACh release from synaptosomes is considerably increased in the presence of GCV. Since this venom alters in no way the organelles in the nerve terminals, we tried to find out if the release of ACh triggered by this neurotoxin would be accompanied by substantial intramembrane particles rearrangements.

The synaptosomes were therefore quick frozen and freeze- fractured to analyse their membranes (Gulik-Krzywicki and Costello, 1978). In this technique, a thin layer (10 µm) of synaptosomes is immersed, between two copper plates, in cold freon at the peak of the ACh release as measured with chemiluminescent method (Israël and Lesbats, 1980). Intramembrane particles of 8-18 nm became very numerous in the E face some of them being also pinched-off with the P face. The small particles (5-11 nm) became less numerous in the P face. This experimental finding permits to put forward the hypothesis that the release mechanism has for morphological counterpart the rearrangement of intermembrane particles.

The presynaptic action of GCV requires the specific binding of the neurotoxin (a glycoprotein of 300 000 daltons M.W.) to an ectocellularly exposed protein of the presynaptic plasma membrane. This binding is not calcium dependent suggesting that GCV acts via the Ca^{2+} entry in the nerve terminal, hence triggering ACh release. (Morel et al 1983)

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Israël M. and Lesbats B. (1980) C.R. Acad. Sc. Paris 291, 713-716.
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POSSIBLE MECHANISMS INVOLVED IN THE PRESYNAPTIC CHOLINOTOXICITY DUE TO AF64A IN VIVO: Charles R. Mantione[1], Henriette Bech, Abraham Fisher*, and Israel Hanin, Department of Psychiatry, Western Psychiatric Institute and Clinic, University of Pittsburgh Medical School, Pittsburgh, Pennsylvania, USA, and Israel Institute of Biological Research, Ness-Ziona, Israel*.

AF64A (ethylcholine aziridinium) is an alkylating analog of choline (Ch) that, at low doses, persistently depletes acetylcholine (ACh) levels in mouse and rat brain, and abolishes cholinergic transmission in the superior cervical ganglion of the intact cat. This disruption of neurotransmitter function in vivo is specific to the cholinergic system. The mechanism of neurotoxicity appears to involve an effect on ACh metabolism by inhibiting sodium dependent Ch uptake and/or choline acetyltransferase (ChAT) activity. In synaptosomal membrane preparations, in vitro, ChAT is not potently inhibited by AF64A, yet total enzyme activity is significantly reduced by 40-60% in vivo, after AF64A injection into rat brain. We studied whether this effect could be due to acetylation of AF64A to a toxic false neurotransmitter in vivo. ChAT activity was measured in rat hippocampal tissue obtained from crude synaptosomal homogenates. Tissue (0.5-1 mg) was incubated for 30 minutes at 38°C in high ionic strength phosphate buffer containing 1 mM eserine, ^{14}C -acetylcoA (46μCi/μmole), and either Ch or AF64A (1-5mM) in a total volume of 25 μl. Quaternary amines were extracted into heptanone saturated with tetraphenylboron (75 mg/ml). Radioactivity due to ACh formed from endogenous Ch was subtracted from that counted in samples containing AF64A. Under these conditions, inclusion of AF64A in the medium resulted in a concentration-dependent formation of an acetylated product, with an apparent Km of 5mM. Thin layer chromatography of a formic acid/acetone extract of the enzyme product formed from 5mM AF64A, identified the substance as acetylethylcholine aziridinium. This substance was susceptible to acetylcholinesterase hydrolysis, since omission of eserine from the medium completely prevented retrieval of any product in the heptanone extract. At higher AF64A concentrations (2 mM), ChAT activity was even partially inhibited. Addition of Ch to the medium did not result in additional ChAT activity at saturating concentrations of both substrates. These preliminary results, along with our previous work, suggest that AF64A may disrupt ACh synthesis via, possibly, two consecutive mechanisms. Initially, AF64A reduces Ch uptake into the nerve terminal. However, after a definitive time period, AF64A may itself become accumulated into the presynaptic site, where it could serve as an alternate substrate for ChAT. Beside further reducing ACh synthesis, formation of acetylethylcholine aziridinium could conceivably cause an irreversible feedback inhibition of ChAT, as well as a potential blockade of accumulation of ACh into storage vesicles. AF64A thus may be a useful tool to study the regulation of ChAT, coupled with ACh synthesis and storage, as a function of neuronal activity.

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PRESYNAPTIC MUSCARINIC RECEPTORS: CHANGES OF SENSITIVITY DURING LONG TERM DRUG TREATMENT: Mario Marchi and Maurizio Raiteri, University of Genova, Institute of Pharmacology and Pharmacognosy, Genova, Italy.

It is widely accepted that the sensitivity of neurotransmitter receptors can be modified during long term activation or blockade of these receptors by specific drugs. Receptors mediating regulation of neurotransmitter release exist on nerve terminals of the mammalian brain (presynaptic receptors). The release of a given transmitter can be regulated by the transmitter itself through the activation of "presynaptic autoreceptors" as well as by other transmitters through the activation of "presynaptic heteroreceptors". Presynaptic receptors lend themselves to functional studies of sensitivity modulation since neurotransmitter release can be measured easily in brain tissue from animals pretreated with receptors agonists or antagonists. In the present investigation we have compared the effects of chronic drug treatments on the sensitivity of presynaptic muscarinic autoreceptors and presynaptic muscarinic heteroreceptors. The receptors selected for our experiments were: 1) the muscarinic autoreceptors mediating inhibition of acetylcholine release in the cortex; 2) the muscarinic autoreceptors present in the nerve endings of the hippocampus; 3) the muscarinic presynaptic receptor mediating potentiation of striatal dopamine release (heteroreceptors). The experiments were performed by using rat brain synaptosomes in superfusion. Male Sprague-Dawley rats were treated subcutaneously once daily for 11 days with scopolamine (10 mg/Kg) or paraoxon (0.15 mg/Kg on day 1 and 2 followed by 0.07 mg/Kg on day 3 through 11). Control animals were injected with saline. The animals were killed 48 hours after the last injection. We compared the effects of the long term treatment with those of a single administration. The results of experiments on muscarinic autoreceptors show that the inhibitory potency of exogenous ACh (5 μM) on ^{3}H-ACh release differed depending on the drug treatment received by the animals. In synaptosomes from rat chronically treated with paraoxon the release of ^{3}H-ACh was significantly less inhibited than in synaptosomes from saline treated animals (autoreceptor subsensitivity). The opposite was true in the case of rats chronically treated with scopolamine (autoreceptor supersensitivity). On the other hand extracellular ACh was equally effective in reducing ^{3}H-ACh release in nerve endings prepared from control animals and from rats pretreated with a single dose of paraoxon or scopolamine. Neither the long term nor the single treatment affected the K^{+} evoked release of ^{3}H-ACh. In contrast, the sensitivity of the muscarinic presynaptic heteroreceptors mediating potentiation of ^{3}H-DA release in striatal synaptosomes remained unchanged during chronic administration of paraoxon or scopolamine. The results presented indicate that muscarinic presynaptic autoreceptors may be more susceptible than muscarinic presynaptic heteroreceptors to changes of sensitivity elicited by long term in vivo administration of antagonist or agonist drugs. This difference in susceptibility between auto- and heteroreceptors may represent a further criterion to discriminate between muscarinic receptor subtype.

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FACTORS THAT DETERMINE THE EFFECTS OF LITHIUM ON THE CHOLINE TRANSPORT SYSTEM IN ERYTHROCYTES: K. Martin, Department of Pharmacology, University of Cambridge, U.K.

The administration of lithium to manic depressive patients abolishes choline transport in erythrocytes almost completely but there is no indication that choline transport in other cells is similarly affected. The in vitro exposure of human erythrocytes to lithium reduces choline transport only by about one third even when high concentrations of lithium and long incubation periods are used. The inhibition of choline transport by lithium is irreversible.

When lithium is administered to a patient, both choline influx and efflux are reduced progressively and to the same extent. At the same time there is a reduction in the intracellular space available to labelled choline added to the incubation medium. It appears that, over a period of several weeks, an increasing number of cells become virtually impermeable to choline. It is suggested that at a certain stage of their development erythrocytes are particularly sensitive to the effects of lithium. The 30 to 40 per cent reduction in choline transport that can be produced in vitro develops over about 2 hours if erythrocytes are incubated with 1 mM lithium; higher concentrations result in a more rapid onset but the intracellular concentration of lithium does not appear to be the relevant factor. The transport system can be protected against inhibition by lithium both by intracellular or extracellular choline as well as by competitive inhibitors, e.g. hemicholinium HC-3. When the transport system is inactivated by cystamine, exposure of the cells to lithium does not result in an inhibition of choline transport. The inactivation by cystamine can be reversed by subsequent exposure of the cells to DTE. The inhibition by lithium is not affected by DTE.

Erythrocytes from rat, rabbit, guinea pig, dog and cat show no or very little carrier mediated transport of choline. Erythrocytes from HK sheep, but not those from LK sheep, have a choline transport system that is in many ways similar to that found in human cells. However, the choline transport system in sheep erythrocytes is not irreversibly inhibited by exposing the cells to lithium in vitro. It is possible that lithium does not interact with the transport system directly but with another component present in the membrane of human erythrocytes. Such a hypothesis is consistent with another observation. The lithium induced irreversible inhibition of choline transport is still observed when ghosts are prepared from lithium treated erythrocytes. However, lithium has no effect on choline transport when the ghosts rather than the intact cells are exposed to lithium. It seems that preparing ghosts, which has little effect on the choline transport system, either removes or significantly changes the target for lithium.

PHASIC DEPENDENT EFFECTS OF DISCONTINUOUS VAGAL STIMULI ON AV CONDUCTION AND ATRIAL CONTRACTILE FORCE: Paul Martin, The Mt. Sinai Medical Center, Division of Investigative Medicine, Cleveland, Ohio 44106, USA.

The dynamic effects of discontinuous vagal stimulation on atrial-ventricular conduction and atrial contractile force were studied with paced- and unpaced canine heart preparations that had been sympathectomized. Brief vagal bursts, separated by at least a minute, were delivered to the isolated cervical vagosympathetic trunks. The time in the cardiac cycle (phase) at which the stimulus was given was varied in steps to encompass an entire cardiac cycle. Atrial and ventricular electrograms were measured with bipolar catheters inserted into the right atrium and ventricle. Data were collected and stimulus patterns were generated with real-time computer techniques that permitted a time resolution of 1 msec. The cardiac responses to a collection of stimulus bursts were combined for analysis by constructing vagal effect curves; these curves illustrate an averaged time course of the cardiac responses.

The AV conduction response is critically dependent on the time in cardiac cycle at which the stimulus burst is given (the phase of the stimulus). A 3 to 5 msec difference in phase of the stimulus can mean the difference between a large increase and no change at all in AV conduction time on the subsequent cardiac cycle. A maximal AV response is obtained if the stimulus is given about 100-150 msec prior to that time in the cardiac at which no response is obtained. The amplitude of the response also strongly depends on the (constant) cardiac cycle duration: the response amplitude increases as a power function of the pacing rate. The atrial contractile force response also depends on the phase of the stimulus, but the resulting vagal effect curves are much broader, i.e. these curves rise and fall much more gradually. Nevertheless, the magnitude of the decrease in atrial contractile force to a single vagal stimulus burst is dependent on the phase of the stimulus; a properly timed vagal burst with but 1 to 3 stimulus pulses in the burst was shown to decrease contractile force to unmeasurable levels for several seconds in some preparations. There is also an interaction of contractile force with the paced heart rate; but in opposition to the interaction of the AV conduction response, the contractile force response decreases as a function of the pacing rate. Other cardiac variables have a pronounced interaction with brief vagal activity to produce a resultant nonlinear summated response when combined dynamic influences are simultaneously applied. Two such other variables that have been studied are concurrent dynamic changes in heart period and in sympathetic activity. Another important determinant of response amplitude to vagal activity is the time that has elapsed since any previous vagal stimuli were given, i.e. the system exhibits a form of "hysteresis."

POLYMORPHISM OF CHOLINESTERASES: BIOCHEMICAL CLASSIFICATION OF MOLECULAR FORMS ; MOLECULAR INTERACTIONS AND CELLULAR POSITIONING: Jean Massoulié , Laboratoire de Neurobiologie, Ecole Normale Supérieure, 46 rue d'Ulm, 75005 Paris, France.

Both acetylcholinesterase and butyrylcholinesterase are very polymorphic. They present a number of molecular forms which are equivalent in their catalytic activity but differ in their molecular parameters and interactions.

Homologous sets of forms exist in all vertebrates. These forms may be classified as globular (G) and asymmetric (A) forms, depending on the presence of a collagen-like tail. The collagen-tailed molecules present ionic interactions which may anchor them in extracellular structures, e.g. basal lamina at neuromuscular endplates.
The globular forms can be subdivided into detergent-insensitive and amphipathic molecules, according to their interactions with micelles of non-denaturing detergents (e.g. Triton X100). Amphipathic forms may be soluble or membrane-bound: soluble amphipathic forms occur naturally in Torpedo plasma, and may be secreted by neural cells in culture. Membrane bound forms are found at the outer surface of plasma membranes.

Every tissue possesses a characteristic spectrum of molecular forms, suggesting that they play specific roles, probably because of their diverse positions. The distribution of asymmetric forms is more restricted than that of globular forms, and their expression is controlled in a specific manner, for example at neuromuscular junctions. These forms however cannot be considered as biochemical correlates of neuromuscular interactions because there are a number of situations in which they are produced by muscles in the absence of nerve.

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.

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THE CHOLINERGIC LIGAND BINDING MATERIAL OF AXONAL MEMBRANES: Henry G. Mautner, Department of Biochemistry and Pharmacology, Tufts University School of Medicine, Boston, Mass., 02111, USA.

Evidence based on the binding characteristics of a series of cholinergic agonists and antagonists suggests the presence, in crustacean axonal membranes, of a biopolymer having some of the characteristics of an acetylcholine receptor (AchR). However, careful studies of the binding of ^{125}I-labelled α-bungarotoxin showed the binding of the α-toxin to be much more reversible and much more salt-sensitive than the corresponding interaction with membranes containing nicotinic AchR. However, histochemical visualization of binding sites on axonal membranes could be achieved by treatment with a conjugate of α-bungarotoxin and horseradish peroxidase both in axonal membrane fragments and intact axons. Labelling could be prevented by pretreatment with d-tubocurarine or with unlabelled α-toxin. Treatment of axonal membranes, following disulfide reduction, with 4-(N-maleimido) benzyl trimethylammonium, a ligand believed to be capable of labelling the α-subunit of the AchR, labelled primarily a peptide with a molecular weight of 50,000 D. Labelling could be largely blocked by pretreatment with d-tubocurarine or bromoacetylcholine. Fusion of axonal membrane fragments with planar bilayers resulted in a K^+ specific increase in conductance. The conductance increase could be blocked asymmetrically with d-tubocurarine, C_{10}, or atropine. Recently (R. Coronado, R. Latorre, H.G. Mautner, unpublished data), it proved possible to study the single-channel characteristics of K^+ channels from lobster walking-leg nerve membranes and to show the frequency of channel openings to be voltage-dependent while the channels can be blocked by TEA and nonyltrimethylammonium in a voltage dependent manner.

SPECIFIC ASPECTS OF CHOLINERGIC NEUROANATOMY: Edith G. McGeer, Patrick L. McGeer, Hiro Kimura and Jeng-Hsiung Peng, Kinsmen Laboratory of Neurological Research, University of British Columbia, Vancouver, B.C. V6T 1W5, Canada.

Monospecific polyclonal and monoclonal antibodies against human choline acetyltransferase (ChAT) were used to map cholinergic structures in well fixed feline and rodent brain and in some areas of human brain. The use of well fixed tissue is essential to distinguish cholinergic from cholinoceptive cells.

At least 5 major cholinergic systems were found. In all cases the cholinergic cells are large to giant cells. These systems are:

1) The medial basal forebrain system which serves cerebral cortex and limbic system and includes cell groups in the olfactory tubercle, medial septum, the nucleus of the diagnonal band of Broca and the substantia innominata complex.
2) The large interneurons of the caudate, putamen and nucleus accumbens which serve the extrapyramidal system.
3) The large motor neurons of the cranial nerve nuclei which send fibers to either the voluntary or parasympathetic systems.
4) The parabrachial nuclear complex.
5) The giganto and magnocellular elements of the tegmental fields of the pons and medulla.

Most of the systems served by the last two groups are unknown although recent lesion data indicate these cells provide the cholinergic innervation of the substantia nigra. Other cholinergic cell clusters are found in the red nucleus, superior olivery complex, some vestibular nuclei and a few other areas where they probably serve a restricted function. Cholinergic nerve endings and cholinoceptive cells are widespread, with the highest densities occurring in areas such as the interpeduncular nucleus, caudate/putamen, medial habenula and amygdala-hippocampus. The various cell groups mapped by us have been severally confirmed by work in other laboratories with other ChAT antibodies and are generally in accord with the literature on cholinergic pathways as indicated by lesion experiments and, at least in the forebrain, by acetylcholinesterase histochemistry. A number of areas of uncertainty still exist, with the principal questions being those regarding the existence of cholinergic cells in the cortex, medial habenula and hypothalamus. Some immunohistochemical results have suggested the existence of such cells in the first two of these regions and acetylcholinesterase staining cells have been seen in the hypothalamus after DFP treatment. Moreover, the lesion data is conflicting as to the existence of cholinergic cells in these three regions. We have not been able to see such cells in well-fixed adult tissue but the evidence pro and con their existence will be reviewed.

The number of cholinergic cells in brain is surprisingly small. They account for less than 5% of the neurons in the caudate, putamen and nucleus accumbens and, in the young human basal forebrain, there are approximately 450,000 cells to serve the millions of nerve endings in the cerebral cortex.

INFLUENCE OF DIET ON MOUSE BRAIN CHOLINERGIC PARAMETERS: EFFECTS OF STRAIN AND AGE: Ronald F. Mervis (Dept. Pathology), Donald R. Meyer (Dept. Psychology), Lane J. Wallace (Dept. Pharmacology), and Lloyd A. Horrocks (Dept. Physiological Chemistry), The Ohio State University, Columbus, Ohio 43210.

As part of a continuing series of investigations evaluating the effects of chronic choline and choline-containing diets on various parameters (e.g., behavior, morphology, neurochemistry) in adult and aging mice, we have used two closely related strains: C57BL/6J (or JAX mice, from Jackson Laboratories) and C57BL/6NNIA mice from Charles River.

Bartus, et al., had previously shown using the JAX strain that chronic dietary choline-enrichment (ChE) or deficiency (ChD) for 4.5 months, beginning at about 8.5 months-of-age, had resulted in improved or impaired performance, respectively, in retention of learning. This behavioral effect also indirectly correlated with a subsequent morphological finding from Golgi studies in these same mice by Mervis showing that after 11 months of dietary treatments, 19 month old ChE mice had greater dendritic spine density on layer V neocortical pyramindal cells than aged-matched dietary controls. This implied a structural-functional relationship, mediated by the chronic dietary choline. Moreover, in comparison to younger (8 month old) controls, deridritic spine loss, a normal concommitant of aging, was repressed in the 19 month-old ChE Mice. Also, in collaboration with Bertom-Fredarri, it was found that in the cerebellar glomerulus, age-related loss of synaptic surface area was also repressed by chronic choline-enrichment. Although it is feasible that choline-enrichment, by increasing brain levels of acetylcholine, could modulate post-synaptic parameters of cholinoreceptive neurons, there is another attractive alternative: dietary choline could also influence glycerophospholipid synthesis and thereby modulate the plasticity and/or integrity of the neuronal/synaptic membrane. This latter possibility,--a membrane effect--would not be limited to neurons of the cholinergic system.

The present series of investigations was designed to replicate and expand on the original Bartus study. These studies used C57BL/6J mice from 8 to 13 months-old and 8 to 19 months-old, and C57BL/6NNIA mice from 8 to 13 and 13 to 24 months-old. Each group was given 12 different diets, including increasing levels of free choline, phosphatidylcholine (PC), and oil-free lecithin, choline-deficient, and control diets.

Behavioral testing using the Bartus paradigm has been completed for the 13 month old mice of both strains following 5 months of dietary treatments. Initial findings showed that NIA mice consistently demonstrated better retention of learning than the JAX mice regardless of diet. In general, (unless retention was already quite high) for both strains, increasing levels of dietary choline, either as free choline, PC, or lecithin resulted in a modest improvement in performance levels. This is of interest since between 8 to 13 months old, the NIA mice do not show a performance decrement in passive-avoidance behavior. We have subsequently determined that data from the JAX mice has been seriously compromised: Jackson Laboratories sent us mice from different housing conditions, and with significant differences in initial levels of learning.

Binding studies for muscarinic and alpha-adrenergic receptors of all mice show that choline supplementation has negligible effects on receptor densities. However, there was some positive correlation between cortical adrenergic receptors and the behavior of the mice.

We anticipate that older mice will show significant decrements in various parameters. Under these circumstances, long-term choline supplementation should prove beneficial in repressing some age-related changes.

EVOKED RELEASE OF ACETYLCHOLINE AT THE MOTOR ENDPLATE: Peter C. Molenaar and Rob L. Polak, Department of Pharmacology of the University of Leiden, Wassenaarseweg 72, 2333 AL Leiden, The Netherlands and the Medical Biological Laboratory/TNO, Lange Kleiweg 139, 2288 GJ Rijswijk, The Netherlands.

The theory of vesicular release of ACh quanta has been challenged by results from biochemical experiments, notably those on the electric organ of Torpedo [for reviews see 1,2]. The present work is an attempt to test the theory at the neuromuscular junction of the frog sartorius muscle by a combination of biochemical and electrophysiological techniques. ACh was determined by mass fragmentography and miniature endplate currents (min.e.p.cs) by conventional electrophysiological techniques. This work was done in collaboration with R. Miledi, University College London.

It was found that a 15 min exposure to La^{3+} ions (2 mM), known to cause an irreversible loss of synaptic vesicles from motor terminals and a virtual disappearance of min.e.p.cs [3], induced a great but transient outburst of min.e.p.cs and release of chemically detectable ACh. Thereafter the ACh content of the preparation gradually recovered while the vesicles remained absent. After 4 h it was not possible to increase ACh release by a second dose of La^{3+}, by KCl (50 mM) or by the calcium ionophore A 23187 (50 μM).

After 5 h the bound ACh (i.e. the ACh protected against cholinesterase upon homogenization of the muscle in Ringer) was greatly reduced by La^{3+}, indicating that it predominantly derived from vesicles. Muscles exposed for 5 min to isotonic potassium propionate (known to induce a decrease of the number of vesicles) lost bound ACh, whereas free ACh (total minus bound) was not affected.

The size of the vesicular pool and the amount of ACh in a vesicle were estimated by two methods: 1. Loss of total ACh under the influence of La^{3+} (overestimate because of the possible recycling of vesicles); 2. loss of bound ACh caused by denervation (underestimate because of the possible breakdown of vesicles). The amount of ACh in a quantum was estimated by correlating the ACh released by KCl with the number of min.e.p.cs detected at the endplates. Assuming 10^3 endplates per muscle and 10^6 vesicles per endplate we estimated that a vesicle contains between 11000 and 15000 ACh molecules, and a quantum 13000 molecules.

In conclusion, our results with frog muscle are in favour of the theory and suggest that the min.e.p.c is caused by the discharge of the whole content of a vesicle.

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CHARACTERIZATION OF PLASMA MEMBRANE PROTEINS OF CHOLINERGIC SYNAPTOSOMES.

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Pure cholinergic synaptosomes prepared from Torpedo electric organ (1) were subfractionated and their plasma membrane purified on discontinuous sucrose gradients (2). We looked for proteins in the presynaptic plasma membrane which would be specific for this membrane and directly involved in the ACh release process.

After polyacrylamide gel electrophoresis in the presence of SDS, only one major protein (MW 67 000 dalt.) was found to be enriched when the presynaptic plasma membrane is purified. We raised a monoclonal antibody (C1-8) to this protein. Using this monoclonal antibody it was possible to show that the 67 000 dalt. protein is specific or highly enriched in the presynaptic plasma membrane since it was not detected in other membrane fractions prepared from Torpedo electric organ, electric nerves or electric lobes. In addition, a hydrophobic form of Acetylcholinesterase (AChE) was found to be associated to the presyn--aptic membrane (3). It amounts to up to 25 % of the total AChE activity in Torpedo electric organ. This presynaptic AChE activity is ectocellularly oriented.

By selective proteolysis of the presynaptic plasma membrane from the outside (by incubating intact synaptosomes in the presence of Pronase), it was possible to remove from the presynaptic membrane about 35 % of the membrane proteins, including the totality of AChE activity and of the 67 000 dalt. C1-8 binding protein. In these conditions, ACh release induced either by high KCl concentra--tions or by Gramicidin was not affected. Therefore neither the 67 000 dalt. C1-8 binding protein, nor the presynaptic membrane bound AChE are directly involved in the ACh release process. In spite of close biochemical similarities, several immunological evidences suggested that these two glycoproteins are different molecules.

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EFFECT OF CDP-CHOLINE ON HYPOCAPNIC NEURONS: Serge MYKITA, Henri DREYFUS, Dominique HOFFMANN, Michele DURAND, Louis FREYSZ, Francis GOLLY and Raphael MASSARELLI, Centre de Neurochimie de Strasbourg, 5, rue Blaise Pascal, 67084 STRASBOURG CEDEX, FRANCE.

It is well known that treatment with cytidine diphosphocholine (CDP-Ch) may have an important effect in the case of cerebrovascular affections, which may come from various sources, such as ischemia or cerebral oedema produced by traumatic concussions. Some authors have, thus, suggested, that CDP-Ch could be favourably used in anoxic neuropathies.

The present report reveals the effect of CDP-Ch on hypocapnic neurons and open up a new possible treatment for neuropathies originated by acidosis.

Isolated neurons in culture prepared under standard conditions and grown in a atmosphere 95%/5%,air/CO_2, were kept for 12 hours (from 2nd to 3th days of culture) under a reduced atmosphere of CO_2 (comparable to the atmospheric CO_2). Control cells shown a slight increase in the number of cells clumps and of neurites, while in hypocapnic cells, this effect was much more pronounced. Control hypocapnic cells were vacuolised and with a much reduced number of neurites. This results were obtained in adding CDP-Ch 10^{-6}M and 10^{-8}M at the beginning of the culture. Similar data have been obtained with reduced CO_2 content at the 5th day of culture and adding CDP-Ch 10^{-6}M afterwards.

It has been shown that the effect is produced by CDP-Choline and not by the isolated nucleotide(or other nucleotide), nor by choline.

The metabolism of choline in normal and hypocapnic neurons has been followed as function of incubation time, with ^{3}H choline and no difference was observed in the acido-soluble, nor in the lipids soluble fractions. The single acido-soluble and lipid compounds are presently under investigation to detect a possible difference namely at the level of phosphatidylcholine.

THE AGING OF CHOLINERGIC SYNAPSES: ONTOGENESIS OF CHOLINERGIC RECEPTORS: Agneta Nordberg, Department of Pharmacology, University of Uppsala, Box 573, S-751 23 Uppsala, Sweden.

The cholinergic synapse in brain undergo dynamic changes during development, maturation and aging. Studies on the ontogenesis of nicotine- and muscarine-like binding sites and their correlation to the development of other cholinergic parameters as well as changes after maturation might provide a better insight into the neurochemical basis for the cholinergic function in brain, its possible susceptibility for early exposure of neurotoxic agents and involvement later in life in certain pathological states and neurological diseases.

Nicotine-like binding sites have recently been measured in both rodent and human brain using different ligands. The physiological function of the binding sites is somewhat unclear and studies of their ontogenesis might provide further understanding.

When the postnatal development of the cholinergic neurotransmitter system was studied in the cortex, hippocampus, midbrain and cerebellum of 3, 7, 12, 17 and 30 day old mice (1) the concentration of muscarine-like binding sites measured by ^{3}H-quinuclidinyl benzilate (^{3}H-QNB) increased progressively with age up to adult level and was paralell but proceded the development of the activity of the enzyme choline acetyltransferase (ChAT) (presynaptic marker). The nicotine-like binding sites were studied using ^{3}H-alfa-bungarotoxin (^{3}H-Btx) and ^{3}H-tubocurarine (^{3}H-TC). The ^{3}H-Btx binding gradually increased with age in the cortex, hippocampus and midbrain with a peak between day 7 and 12 followed by a decrease towards day 30. Using ^{3}H-TC as ligand the binding was high at day 3 and gradually decreased with age. The data thus indicate that different sub-populations of nicotine-like receptors might be determined when the different ligands are used.

Recently the development of ^{3}H-Nic binding sites in different areas of brain has been investigated and a somewhat more complex pattern has been obtained (2). Primarily a general increase in number of ^{3}H-Nic binding sites up to day 30 was seen in the hippocampus, midbrain and cortex when the incubation was performed at 25^{o}C during 60 mins. These experiments have now been repeated at a lower temperature (+4^{o}C) and shorter incubation time (10 mins) since new data indicate the importance of these conditions for a optimal ^{3}H-Nic binding (see Larsson and Nordberg, this symposium). Preliminary data using these conditions shows a pattern of development more similar with the other nicotinic ligands.

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SUCCINYLCHOLINE - A METHOD OF DETERMINATION. DISTRIBUTION AND ELIMINATION: Ingrid K. Nordgren and Bo R. Holmstedt, Department of Toxicology, Karolinska Institutet, Box 60 400, S-104 01 Stockholm, Sweden.

A method has been developed for the identification and quantitation of the neuromuscular blocking agent succinylcholine (SCh) in biological material. Stability, tissue distribution and elimination from plasma has been studied in man and experimental animals. This research was originally prompted by a forensic case and the experimental results include also studies of embalmed material.

SCh is extracted from plasma or tissue homogenates into an organic phase as an ion pair with hexanitrodiphenylamine. To enable gas chromatography SCh is demethylated with sodium benzenethiolate to form the corresponding tertiary amine which is identified and quantitated by gas chromatography - mass spectrometry. In the quantitative analysis deuterated SCh is used as internal standard.

In order to study the stability of SCh, under conditions encountered in forensic cases, rats were injected with SCh in doses ranging between 10 and 200 mg/kg i.m. Muscle, liver and kidney were embalmed and stored under different temperature conditions for 6 months. We found SCh to be still present 6 months after death. The concentration of the drug in the tissues appears to be dose dependent.

To study tissue distribution and elimination from plasma dogs were injected i.v. with SCh in doses ranging between 2 and 106 mg/kg. Of the organs we analyzed, the highest amounts of SCh were found in the kidney. The distribution between the organs was the same irrespective of the dose. It was also found that in plasma the levels of SCh rapidly decreased when the dogs were kept under artificial respiration, while the levels of SCh stopped decreasing upon cessation of circulation and death. It has generally been accepted that SCh disappears rapidly from blood and tissues due to hydrolysis by cholinesterases. Our results show that SCh is extensively distributed to tissues and that the tissue distribution plays an important role in the elimination of SCh from plasma.

IN VIVO AND IN VITRO STUDIES ON A PRESYNAPTIC MUSCARINIC ANTAGONIST, and POSTSYNAPTIC AGONIST: BM-5: Ove Nordström[1], Anders Undén[1], Veronica Grimm[2], Brina Frieder[2] and Tamas Bartfai[1], Arrhenius Laboratory, University of Stockholm, 106 91 Stockholm, Sweden[1] and Isotope Department, Weizmann Institute, Rehovot, Israel[2]

N-methyl-N-(1-methylpyrrolidino-2-butynyl acetamid (compound BM-5) an oxotremorine analogue synthetized and studied by Resul et al. (1) was examined with respect to its ability to interact with muscarinic autoreceptors in the rat cerebral cortex, hippocampus and in the guinea pig ileum (2). Compound BM-5, similarly to atropine enhanced the stimulus evoked release of ACh in all three systems. BM-5 stimulated cGMP synthesis via occupancy of muscarinic receptors in the rat hippocampus and in human lymphocytes. BM-5 displaced ^{3}H-3-quinuclidinyl benzilate from receptors with a IC_{50} value of 3 μM.

Injected s.c. (at doses 1, 2 and 5 mg/kg) into rats causes tremor similar to that caused by oxotremorine. The same doses were used in experiments on its effect on memory in trials consisting of two runs in a black and white maze subsequent to eight training trials: The BM-5 injected animals used significantly longer time and made more errors than controls. BM-5 at all doses depressed rearing in the open field studies ($p<0.05$).

Thus the same doses of BM-5 which produce tremor via a muscarinic agonist like action (i.e. would be blocked by atropine (1 mg/kg) produce an impairment of maze performance resembling of the effect of muscarinic antagonists such as scopolamine and atropine.

These dual actions may depend on the partial agonist nature of the ligand in addition to some degree of regional specificity in its action.

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INHIBITION OF ACETYLCHOLINE SYNTHESIS IN VITRO: J.J. O'Neill, P.H. Doukas, F.J. Ricciardi and G.H. Sterling*, Temple University School of Medicine, 3420 N. Broad Street, Philadelphia, Pennsylvania 19140, *Hahnemann School of Medicine, Broad and Vine Streets, Philadelphia, Pennsylvania 19102, USA.

The cholinergic system is highly regulated with concentrations of acetylcholine (ACh) maintained within narrow limits; however the factor(s) controlling ACh synthesis remain controversial. To assess the role(s) of choline-acetyltransferase (CAT) activity and precursor availability, more potent or specific inhibitors of choline uptake and CAT would be highly desirable. The design and synthesis of several quinuclidine derivatives has been undertaken based upon the work of Cavallito (1970) and of Baker (1967). The π-bonding properties of Cavallito's vinyl-compounds has been incorporated into azulene compounds and their CAT-inhibitory properties measured. One of these 4-Azulyvinylpyridine is equipotent (A_{50}=10^{-6} M) with Cavallito compound, hydroxyethylvinylpyridinium (HEVP). The design and synthesis of several quinuclidine derivatives has been undertaken based upon this homology, the known affinity of quinuclidine derivatives for cholinergic ultrastructure, and, the inhibitory effect of 3-hydroxy quinuclidine methyliodide on high affinity choline (HACU) uptake. To assess the roles of CAT activity and precursor availability, we have also prepared acetylseccohemicholinium, a known inhibitor of HACU and a potent inhibitor of CAT (10^{-6} M) and derivatives of naphthyl vinylpyridinium and quinuclidine, measuring their effects on ACh synthesis in rat brain cortex slices. Tissue was incubated at 37°C in Krebs-Ringer bicarbonate buffer with 5uCi [U-^{14}C] glucose (10 umoles), choline chloride, eserine sulfate and various concentrations (10^{-7}-10^{-3} M) of our potential inhibitors. Potassium stimulation (60mM) approximately doubled ACh synthesis to 1.97 nmoles ACH/mg prot/H. Several compounds including acetylseco-HC-3 and methyl (3-hydroxyquinuclidinium) iodide significantly reduced ACh synthesis by as much as 60-70 percent at the concentrations examined. A major aim is the development of inhibitors capable of entering the CNS from systemic sites of administration. To this end we have focused on the activity of tertiary amines, varying steric, electronic and lipophilic properties. Results on brain CAT and brain slice preparations will be presented. (Supported by USAMRDC #DAMD 17-83-C-2183).

NEW PHARMACOLOGICAL TOOLS TO STUDY ACETYLCHOLINE STORAGE IN NERVE TERMINALS: Stanley M. Parsons, David C. Anderson, Ben A. Bahr, Jr. and Gary A. Rogers, Department of Chemistry, University of California, Santa Barbara, CA, 93106, USA.

The drug AH5183 (dl-2-(4-phenylpiperidino)cyclohexanol) recently was shown to be a potent inhibitor of the acetylcholine (ACh) active transport system of synaptic vesicles obtained from the electric organ of Torpedo californica. Mechanistic aspects of the drug action are being studied. Freshly isolated vesicles incubated at 25° spontaneously leak endogenous ACh when exogenous MgATP is absent but not when it is present. AH5183 had no effect on these results, demonstrating that the drug does not inhibit uptake of exogenous ACh by inducing efflux of internal ACh. An apparently different result was obtained when action of the drug on uptake of exogenous [^{3}H]ACh was monitored. If active uptake of [^{3}H]ACh by purified vesicles was allowed to proceed for 30 minutes before AH5183 was added about one fourth of the bound [^{3}H]ACh was lost rapidly. About one half of the bound [^{3}H]ACh was lost when the protonophoric uncoupler FCCP was added after 30 minutes of uptake, which is comparable to the loss of endogenous ACh. When the concentration dependence for the AH5183-mediated loss was etermined, $4*10^{-8}$ M drug (IC_{50}) was required for loss of half of the total released [^{3}H]ACh, which corresponded to an estimated $5*10^{-7}$ M. The estimated vesicle concentration was $1.5*10^{-8}$ M. Since the drug binding site concentration probably is no more than twice the IC_{50} value of the stereospecifically active isomer of the drug (see below), it appears that occupation of less than $4*10^{-8}$ M binding site by AH5183 caused release of about $5*10^{-7}$ M [^{3}H]ACh from the vesicles. The apparent IC_{50} increased at higher vesicle concentration, and in preliminary initial velocity measurements the drug appeared to be a pure noncompetitive inhibitor of [^{3}H]ACh active transport. The data suggest that the AH5183 binding site acts on the transport mechanism in some indirect amplified way.

The optical isomers of AH5183 were resolved by fractional recrystallization of the d- and l-di-p-toluoyltartrate salts. The l-isomer of AH5183 was found to be the active isomer with an IC_{50} value of about 20 nM, whereas the d-isomer has an IC_{50} of about 1100 nM. Forty one other derivatives and analogs of AH5183 have been synthesized and their IC_{50} values determined. The potency of the drug is highly sensitive to small modifications in the structure. For example, methylation of the amine or of the hydroxyl bearing carbon, a change of the cyclohexanol ring to the cyclopentanol ring, or acetylation of the hydroxyl reduces the potency of the drug by 30, 150, 37 and 50 fold, respectively. One drug changing the cyclohexanol ring to a decalinol ring system has been found to have an 8 fold lower IC_{50} value, thus confirming the apparent substoichiometric inhibitory capability of this class of drug. LD_{50} values for intraperitoneal injection of a wide variety of AH5183 analogs in mouse correlate well with the in vitro IC_{50} values. This suggests that an important mode of action of AH5183 in vivo is inhibition of ACh storage by synaptic vesicles in cholinergic terminals.

EFFECTS OF NOOTROPIC DRUGS ON BRAIN CHOLINERGIC MECHANISMS: BIOCHEMICAL AND BEHAVIORAL INVESTIGATIONS: Felicita Pedata, Flavio Moroni,* Silvano Banfi and Giancarlo Pepeu, University of Florence, Department of Pharmacology, Viale Morgagni 65, 50134 Florence and *I.S.F. Pharmacological Laboratory, 20090 Trezzano, Italy

Compounds that improve cognitive function in a variety of experimental and clinical situations, of which piracetam is the prototype, have been named nootropic drugs (Giurgea C. Actualités Pharmacol. 25 115, 1972). The observations that piracetam induces a small decrease in hippocampal ACh in aged rats (Bartus, R.T. et al., Neurobiol. Aging 2, 105, 1981) and that its derivative aniracetam antagonizes scopolamine-induced amnesia in mice (Cumin, R. et al., Psychopharmacol. 78, 104, 1982) prompted us to investigate the interactions of two nootropic drugs with central cholinergic mechanisms. The effect of oxiracetam (4-hydroxy-2-oxo-1-pirrolidineacetamide) (Banfi, S. et al. Il Farmaco ed Sc., 1983 in press) and piracetam on brain ACh and choline (Ch) levels was investigated by a gas chromatographic method in male Wistar rats (150 - 200 g body weight). Oxiracetam (100 - 300 mg/Kg i.p.) did not modify steady state ACh and Ch levels in the cerebral cortex, hippocampus or striatum.

Thirty min after the i.c.v. administration of hemicholinium (HC3, 15 µg), a drug which inhibits ACh synthesis, ACh level in the hippocampus showed a 58% decrease. The decrease was 73% when the rats were pretreated with oxiracetam 100 mg/Kg i.p., 78% with oxiracetam 300 and 71% with 300 mg/Kg i.p. of piracetam. The differences were statistically significant. Likewise, oxiracetam potentiated HC3 effect also in the cerebral cortex. This findings suggest that oxiracetam and piracetam stimulate ACh utilization.

Pretreatment with oxiracetam (100 mg/Kg i.p.) significantly antagonized the impairment of the acquisition of an active avoidance conditioned response (pole climbing) brought about by i.c.v. administration of HC3. Oxiracetam also antagonized in a dose-dependent manner the amnesic effect of scopolamine (1 mg/Kg i.p.) as measured through a passive avoidance conditioned response in mice.

In conclusion, these experiments suggest that oxiracetam and pirecetam reduce the cognitive impairment brought about by inhibition of ACh synthesis or blockade of muscarinic receptors by stimulating ACh utilization.

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NEUROTRANSMITTERS THAT ACT ON CHOLINERGIC MAGNOCELLULAR FOREBRAIN NUCLEI INFLUENCE CORTICAL ACETHYLCHOLINE OUTPUT; Giancarlo Pepeu, Fiorella Casamenti, Piero Mantovani and Maurizio Magnani, Department of Pharmacology, Viale Morgagni 65, 50134 Florence, Italy

ACh release from the cerebral cortex, evaluated by the cortical cup technique in vivo, is an indication of the activity of the cholinergic network which originates in the magnocellular forebrain nuclei. A unilateral electrolytic lesion of the nucleus basalis (Ch4, according to Mesulam, M.M. et al., J.Comp.Neurol. 214, 170, 1983) was followed by a 40% decrease in ACh output from the ipsilateral fronto-parietal cortex in unaesthetized freely moving rats. Conversely, direct electrical stimulation of Ch4 elicited in mid-pontine transected rats a 50% ipsilateral increase in cortical ACh output.

Amphetamine, an indirect dopaminergic agonist, in freely moving rats stimulated in a dose-dependent manner ACh release from the cerebral cortex: a 100% increase was observed after 1.0 mg/kg i.p. The effect of amphetamine on ACh output was strongly reduced in rats with a Ch4 lesion and abolished by a lesion of the nigra. These results suggest that dopaminergic fibres can influence the cholinergic neurons which project to the cerebral cortex. Apomorphine (10 mg/kg i.p.), a direct dopaminergic agonist, was less effective than amphetamine and its stimulatory effect on ACh output was abolished by a Ch4 lesion but also by a nigra lesion. Therefore a direct dopaminergic influence on the cholinergic neurons seems unlikely. On the other hand, the stimulatory effect of amphetamine on ACh output was not affected by pretreatment with p-chlorophenylalanine (300 mg/kg i.p.) which depletes 5-HT stores, or picrotoxin (5 mg/kg i.p.) a GABA antagonist.

ACh output from the cerebral cortex was affected by the administration of CCK-8 and its analogue ceruletide in doses ranging from 1.5 to 20 μg/kg i.p. At doses up to 5 μg/kg both peptides brought about a dose-dependent increase in cortical ACh output with a maximum of 150%. Beginning with 10 μg/kg i.p. a decrease in ACh output was observed. Both effects were strongly reduced by Ch4 lesions. The stimulatory effect was antagonized by proglumide (100 mg/kg i.p.) and the inhibitory effect by naloxone (1 mg/kg i.p.), indicating that two different receptors are involved.

These findings demonstrate that there exists a possibility of modulating the cholinergic neurons of the nucleus basalis by acting through different neurotransmitter systems.

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TARGET TISSUE INFLUENCES ON CHOLINERGIC DEVELOPMENT OF PARASYMPATHETIC MOTOR NEURONS: Guillermo Pilar and Jeremy B. Tuttle, Physiology Section, Biological Sciences Group, The University of Connecticut, Storrs, CT 06268, USA.

The avian ciliary ganglion and iris system allows the study of several aspects of the interrelationship between neurons and target tissues during embryonic development. The present study was undertaken to examine in some detail the retrograde influence of functional target interaction upon the physiological capacities of developing ciliary ganglion neurons.

Neuromuscular transmission by the terminals of ciliary ganglion neurons in the iris prior to hatching was incapable of sustaining contracture at 20 Hz for more than a few seconds. Mature iris junctions can maintain this level of activity essentially indefinitely, and it falls within the normal range of operating frequencies of this junction. Extracellular recording methods established that transmission failure in the developing iris was due to a failure of transmitter release.

Acetylcholine synthesis in the iris terminals, studied by radio-chemical labeling 3H-choline, becomes responsive to release demand at the same developmental time that transmission becomes adult and secure. Thus, depolarization of mature iris terminals in the presence of Ca^{++} prior to incubation in labeled choline results in a several-fold increase in synthesis of ACh, while the synthetic capacity of immature terminals is unaffected by depolarization and release. We conclude that the transmission failure of the embryonic junctions is due to a failure in synthetic capacity for ACh, and that demand responsive synthesis reflects an adult functional status for the cholinergic nerve terminal.

Ciliary ganglion neurons can be removed from the embryo during the autonomous stage of development prior to dependency upon the target tissue and prior to the period of naturally occurring cell death and maintained in cell culture by including in the medium a trophic survival protein. These cultured embryonic neurons acquire high levels of cholineacetyltransferase (CAT), synthesize ACh, and release ACh in response to depolarization with Ca^{++}. However, ACh synthesis remains unresponsive to release demand at 7 da. in culture. In addition, the neurons cultured alone lose sensitivity to ACh as measured by iontophoresis from high-resistance pipets.

If the neurons are plated onto pectoral myotubes in culture, and allowed to contact and innervate the target, the neurons retain sensitivity to ACh and acquire the ability to increase ACh synthesis following a depolarization. Thus, interaction with the target tissue has a general effect in supporting the development of the neurons and acquisition of adult functional status. However, interaction with a live target tissue is not entirely necessary for this effect. Culture of the neurons on a substrate of myotube membrane remnants is sufficient for the retention of neuronal chemosensitivity, and increases the synthetic capacity of the neurons. We conclude that contact with the target membrane may be an important interaction during the development of cholinergic neurons, and the maintenance of adequate functional status in the adult nervous system.
Supported by the U.S. Army Research Office, NIH grant NS-10338, and the Univ. of Connecticut Research Foundation. JBT is the recipient of an RCDA from NIH.

RESTING RELEASE OF ACETYLCHOLINE AT THE MOTOR ENDPLATE: Rob L. Polak and Peter C. Molenaar, Medical Biological Laboratory/TNO, Lange Kleiweg 139, 2288 GJ Rijswijk, The Netherlands and the Department of Pharmacology of the University of Leiden, Wassenaarseweg 72, 2333 AL Leiden, The Netherlands.

It is well-known that ACh diffusing from resting skeletal muscle is secreted predominantly in a non-quantal form. However, the origin and the physiological significance of non-quantal release are still uncertain. In the present experiments we studied non-quantal release in rat, mouse and frog skeletal muscle with mass fragmentography.

Denervation experiments on the rat diaphragm (early: degeneration of nerve terminals; late: degeneration of motor axons) showed that 80 % of the resting ACh release originated from motor nerve terminals, and the remainder from muscle fibres. It is possible that the resting release of ACh from the nerve terminals is due to the discharge of 'giant' ACh quanta outside the normal release sites, a small proportion of which would be observed electrophysiologically as the so-called giant miniature endplate potentials (giant min.e.p.ps, with a typical slow time course). However, the drug 4-aminoquinoline, which is known to enhance the frequency of giant min.e.p.ps 10-fold [1], did not increase the resting release of ACh in the rat diaphragm. Non-quantal ACh release increased with temperature, was partially (50 %) dependent on Ca^{2+} ions, and was reduced by C. botulinum A toxin (experiments on rat and mouse diaphragm and rat extensor digitorum longus muscle).

It has been reported [2] that in a Ca-free medium no (non-quantal) endplate response can be obtained by stimulation of the motor nerve. In view of the effect of Ca^{2+} on resting release it was of interest to know whether, in the presence of Ca^{2+}, depolarization of the nerve terminals leads to an increased non-quantal release. The following results (for technical reasons obtained on frog muscle at 4°C) suggest that this is not the case. 1. When the KCl concentration of the medium was raised from 2 to 10 mM, the resting release of ACh was not altered appreciably, although the miniature endplate current (min.e.p.c.) frequency increased 10-fold (from 0.2 to 2 sec^{-1}, equivalent to 0.1 $pmol.h^{-1}$ ACh superimposed on a resting output of about 1 $pmol.h^{-1}$). 2. With KCl concentrations between 10 and 20 mM the liberation of ACh and the min.e.p.c. frequency were dependent in the same way on the KCl concentration in the medium.

It is concluded that, even if depolarization increases non-quantal ACh release in the presence of Ca^{2+} ions, such an increase is negligible in comparison with depolarization-induced quantal release. Consequently, a significant non-quantal contribution to quantal ACh release induced by the action potential is improbable.

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CHOLINERGIC DYSFUNCTION AND MEMORY: IMPLICATIONS FOR THE DEVELOPMENT OF ANIMAL MODELS OF AGING AND DEMENTIA. Michael J. Pontecorvo, Raymond T. Bartus, and Charles Flicker
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Both aged humans and other mammals show a pattern of behavioral deficits in which a decline in recent memory is superimposed on a more global cognitive deterioration that often includes failures of attention and retrieval from reference memory, as well as increased behavioral rigidity and susceptibility to interference from previous events. That this pattern of deficits can be simulated by administration of cholinolytics to young animals suggests that a dysfunction in cholinergic transmission may contribute to the cognitive decline in the aged. Recent reports of decreased cortical CHAT in Alzheimer's patients, high concentrations of ACHE in immature senile plaques, and specific degeneration of cholinergic cell bodies in the nucleus basalis further support this hypothesis, particularly with respect to cognitive decline in Alzheimer's patients.

When considered collectively, these data offer suggestions for developing more valid animal models of this problem, as well as for identifying effective pharmacological treatment. These findings suggest that destruction of the cell bodies in the nucleus basalis may produce behavioral effects analagous to the deficits suffered by Alzheimer's patients. We have induced lesions in this brain region via stereotaxic injection of the neurotoxin, ibotenic acid and compared the effects on behavior with young sham controls and naturally aged rats. These tests revealed interesting similarities and differences between the groups that will be discussed in detail along with plans for more extensive investigations.

These findings are also consistent with previous suggestions based on biochemical and electro-physiological data, that post synaptic stimulation of cholinergic receptors via muscarinic agonists or similar agents may provide the most effective means of cholinergic treatment for cognitive disorders in the aged. Results from a series of tests using aged monkeys have produced initial confirmation of this hypothesis. Implications of these and other findings for future clinical trials will be discussed.

BASAL FOREBRAIN CHOLINERGIC SYSTEMS IN PRIMATE BRAIN: ANATOMICAL ORGANIZATION AND ROLE IN THE PATHOLOGY OF AGING AND DEMENTIA.

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In the primate, the basal forebrain cholinergic (Ch) system is made up of large, chromophilic, isodendritic neurons in the medial septum (Ch1), diagonal band of Broca (Ch2 and Ch3), and nucleus basalis of Meynert (Ch4). Containing acetylcholinesterase (AChE) and choline acetyltransferase (ChAT)-like immunoreactivity, these neurons provide the major cholinergic innervation of the forebrain. Cytochemical and anterograde transport tracing studies have delineated four pathways: Ch1 and Ch2 to hippocampus primarily via the fornix; Ch4 to amygdala via a ventral pathway; Ch4 to medial cortex via the cingulum bundle; and Ch4 to dorsolateral cortex via the external capsule and corona radiata. Retrograde tracing studies have shown that neurons within Ch subdivisions project preferentially to certain cortical and subcortical targets, but there is overlap of terminal fields served by various subdivisions. For example, large injections of horseradish peroxidase into the primate occipital cortex result in a preponderance of labeled neurons in the posterior Ch4, but a number of labeled cells occur in the intermediate and anterior portions of Ch4 and in the dorsal Ch2. Receptor autoradiographic techniques have demonstrated muscarinic receptors at targets in the hippocampus, amygdala, and neocortex. Ch neurons receive a heterogeneous population of synaptic terminals, some of which derive from brainstem nuclei, including neurons in the pontomedullary reticular formation, locus coeruleus, and dorsal raphe.

Evidence from several laboratories indicates that neurons of Ch1, 2, and 4 are reduced from 25-90% in individuals with Alzheimer's disease (AD) and in demented patients with Parkinson's disease. The most severe loss generally occurs in younger AD patients. The Ch neurons appear to dysfunction before they degenerate, and this pathology is thought to underlie reductions in cholinergic markers occurring in AD. The correlation between the presence of dementia, the presence of neuritic plaques, and reductions in cortical cholinergic markers, suggests that some of the abnormal axon terminals in neuritic plaques may be derived from the Ch system. This hypothesis can be tested in aged monkeys who are known to show cognitive and neuritic plaques. In these animals, histochemical (AChE) investigations, as well as preliminary immunocytochemical (ChAT) and autoradiographic studies, suggest that neurites in plaques may form as a consequence, at least in part, of a distal axonopathy involving Ch fibers. Similar processes may contribute to the memory and cognitive deficits in AD and related disorders.

STRUCTURAL GENE MUTATIONS AFFECTING ACETYLCHOLINESTERASE AND CHOLINE ACETYL-TRANSFERASE.

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The soil nematode Caenorhabditis elegans has a very simple nervous system (302 neurons in total) and a mode of reproduction which facilitates the isolation of genetic mutations. We have been interested in how and to what extent genetically specified developmental programs direct specific synaptogenesis in this simple system. As one approach we have sought (and found) mutations affecting the metabolism of acetylcholine, which appears to be an excitatory neuromuscular transmitter in this system, as in the vertebrates. We have also used these mutations to perturb acetylcholine metabolism, in order to determine what effect such perturbations have on the formation of specific, identifiable synapses.

Mutations in two genes which we have identified, ace-1 and ace-2, affect acetylcholinesterase (AChE). These two genes are unlinked (i.e. on different chromosomes) and control two different classes of AChE, class A and class B. These two classes are kinetically distinct, and each gene is apparently a structural gene for the class it controls. Individuals with mutations in either gene lack the corresponding AChE class, but show no behavioral consequences; however, double mutants with mutations in both genes lack both classes and are severely uncoordinated. We infer that class A and class B AChE must overlap functionally, and histochemical staining suggests that they also overlap anatomically. We believe that this situation may parallel that of acetyl- and butyrylcholinesterases in the vertebrate CNS.

Mutations in a third gene, cha-1, affect choline acetyltransferase (ChAT). This gene is on yet a different chromosome, and appears to be a structural gene for the single class of ChAT observed. A mutations in this gene which reduces ChAT activity levels to 10-15% of normal has no obvious behavioral consequences, indicating a considerable "safety factor" for ChAT. Different mutations which reduce the ChAT activity level to 1-2% of normal produce marked uncoordination. Interestingly, such mutations also produce a constellation of additional secondary consequences, including slow growth, small adult size, and resistance to AChE inhibitors. These properties are not shared by equally uncoordinated mutants affected in other genes, suggesting that acetylcholine may have a developmental role more extensive than that of a simple neurotransmitter. Recent genetic and biochemical evidence suggests that cha-1 is a complex gene, encoding a ChAT with two functional domains; in this interpretation, one of these putative domains would carry out the catalytic activity, while the other would execute some essential, but as yet not understood function (perhaps correct localization). Work is in progress to test this possibility.

Synaptogenesis has so far been examined comparatively only in wild type and in severely ChAT-deficient (cha-1) mutants. Levels of cholinergic synapse formation are reduced in the mutants, implying a possible role for acetylcholine in either the formation or the maintenance of these synapses.

REGULATION OF ACETYLCHOLINE RELEASE FROM RODENT CEREBRUM BY PRESYNAPTIC RECEPTORS, METHIONINE ENKEPHALIN AND SUBSTANCE P: B. V. Rama Sastry, N. Jaiswal and O. S. Tayeb. Department of Pharmacology, Vanderbilt University School of Medicine, Nashville, Tennessee 37232, USA.

Two feedback systems have been postulated to regulate acetylcholine (ACh) release from nervous tissue, a positive feedback or ACh-amplification system and a negative feedback system. In order to evaluate components of these systems, we measured the simultaneous release of ACh, Substance P (SP) and methionine enkephalin (MEK) from mouse cerebral slices. Mouse cerebral slices were incubated in a Krebs Ringer buffer containing (methyl ^{3}H)choline (0.1 mM; 0.25 µCi/ml) for 60 min. They were filtered, washed, and transferred to a microbath set up for superfusion with the above buffer containing hemicholinium-3 (10 µM). The release of ^{3}H-ACh into the superfusate was measured. SP and MEK in the superfusate were measured by specific radioimmunoassays. This method for measuring release of ^{3}H-ACh was validated by (a) the effect of depolarizing concentrations of K^+ on ^{3}H-ACh release, (b) lack of release of ^{3}H-ACh in the absence of Ca^{++}, (c) identification of released ^{3}H-ACh by thin layer chromatography, and tissue viability as determined by tissue lactic dehydrogenase.

The above investigations gave the following results; (1) The rate of ACh release increased initially for the first 5 min, reached a peak, and then declined exponentially (half-time, 35 min). The highest rate of release for SP was observed within 2 min preceding the peak release of ACh. A broad peak for the release of MEK followed the peak release of ACh. Electrical field stimulation during the exponential phase caused significant increases in SP, ACh and MEK. (2) SP (0.6 µM) increased ACh release (35%) and Ca^{++} uptake (154%). The long acting enkephalin (D-ala-enkephalinamide, DALA, 34 nM) decreased the release of ACh (65%) as well as Ca^{++} uptake (48%). (3) 5-Hydroxymethylfurfuryltrimethylammonium (5-HMFT, 1.9 nM) inhibited the release of ACh from mouse cerebral slices. This inhibition was due to activation of muscarinic receptors (M2) which were blocked by scopolamine (10 nM) but not atropine (1µM). It did not activate the muscarinic receptors (M1) in the smooth muscle. In a medium containing Ca^{++} (2.6 mM), 5-HMFT (1.9 nM) decreased both spontaneous and electrically evoked release of ACh by 50%. Its effect was not significant in Ca^{++} free medium. It blocked K^+ (20 mM) induced ACh release. At 2.5 mM Ca^{++}, disteroylphosphatidic acid (DSPA, 43 µM) increased ACh release (40%) which was blocked by 5-HMFT. These studies indicate that 5-HMFT activates an M2 receptor and decreases Ca^{++} influx which is necessary for release of ACh. (4) 5-HMFT (1.9 nM) increased both spontaneous (14 times) and evoked (2-3 times) release of SP. It decreased spontaneous (16 times) and evoked (20 times) release of MEK. These observations indicate that the positive feedback mechanism for ACh release operates through a presynaptic muscarinic receptor (M1), SP, and activation of Ca^{++} influx. Similarly, the negative feedback control operates through a muscarinic receptor (M2), MEK, and inhibition of Ca^{++} influx.

If the rate of release of ACh were to be regulated by the above feedback mechanisms, they should be affected in conditions producing ACh deficits (e.g., aging and senile dementia). Therefore, the patterns of release of ACh, MEK and SP were measured from the cerebral cortical slices of Fischer 344 rats, ages 3-33 months. The rates of release of ACh and MEK decreased while the rate of release of SP increased as a function of age. Alterations in the release of MEK may be a regulatory consequence of the decreased rate of ACh release as a function of age. (Supported by US PHS-NIH grants AG-02077, HD-10607 and The Council for Tobacco Research, U.S.A., Inc.)

CHOLINE UPTAKE IN THE HIPPOCAMPUS: INDIRECT INHIBITION OF SEPTAL-HIPPOCAMPAL CHOLINERGIC NEURONS BY BARBITURATES: Judith A. Richter, Indiana University School of Medicine, Indianapolis, Indiana, 46223, USA.

Simon et al. (J. Neurochem. 26:909, 1976) first reported that in vivo administration of pentobarbital caused an inhibition of high-affinity, sodium-dependent choline uptake which was measured in vitro in hippocampal synaptosomes. This effect is believed to be related to a decrease in activity in the septal-hippocampal neurons. One of our goals has been to determine just where in the brain the drug acts to cause this effect.

The experiments have been done in male Wistar rats with chronic indwelling cannulae so that injections of the drug can be given in the awake animal. After injection the rats are decapitated and sodium-dependent choline uptake is measured in hippocampal synaptosomes using 0.5 uM ^{3}H-choline.

As previously reported (Richter & Gormley, J. Pharmacol. exp. Therap. 222: 778, 1982) injections of pentobarbital into the dorsal hippocampus were without effect on choline uptake even though levels of drug in the hippocampus were at least as high as those found there when the drug was given i.p. and inhibition of choline uptake was observed. Injections of the drug into the medial septum were also ineffective. Acute lesions of the medial septum (1 hr) inhibited choline uptake and pentobarbital (i.p.) then had no further effect. We observed a transient recovery of choline uptake at 3 hr after medial septal lesions, but even under these conditions, the drug had no effect. These results suggested that the inhibition of choline uptake in the hippocampus is caused by an action of the drug at another site which sends projections to the septum. A lesion separating the septum from rostral, dorsal, ventral and lateral (but not caudal) inputs was itself without effect on hippocampal choline uptake and did not alter the action of i.p. pentobarbital.

In more recent experiments, bilateral ventricular injections of phenobarbital were effective in inhibiting choline uptake. Injections of the drug into one lateral ventricle had similar effects on choline uptake in both hippocampi. These results indicated that the site of action is probably in a region near the third or fourth ventricular spaces. When injections were made into one lateral ventricle and the drug was prevented from spreading caudally by a block of Nivea cream injected into the anterior-dorsal portion of the third ventricle, the effect on choline uptake was still observed. Further experiments with injections directly into the ventral portion of the third ventricle (near the preoptic and anterior hypothalamus) also caused a significant inhibition of hippocampal choline uptake. Since barbiturates are known to enhance GABAergic inhibitory effects, it is possible that they inhibit choline uptake in the hippocampus by such a GABA-mimetic effect in the hypothalamus or nearby structure, and this effect is then transmitted by another synaptic link to the septal-hippocampal neurons.

Previous results from our laboratory provided some evidence that the inhibition of choline uptake in the hippocampus was not related to the sedative-hypnotic effects of these drugs (Richter et al., J. Neurochem. 39:1440, 1982; Miller and Richter, in preparation). In these recent experiments lateral ventricular injections of phenobarbital still caused loss of the righting reflex with or without the caudal block. However there was less frequent loss of righting reflex in rats injected with phenobarbital in the ventral portion of the third ventricle. Thus the sedative-hypnotic effects of barbiturates may be mediated elsewhere by another action of these drugs and the inhibition of cholinergic nerve funtion in the hippocampus (and perhaps the cortex also) may only have other consequences to the animal.

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AFFINITY AND EFFICACY OF OXOTREMORINE ANALOGUES AT ILEAL MUSCARINIC RECEPTORS: Björn Ringdahl, Department of Pharmacology, School of Medicine, University of California, Los Angeles, California 90024, USA.

Oxotremorine (N-(4-pyrrolidino-2-butynyl)-2-pyrrolidone) is a potent and specific muscarinic agent. Structure-activity relationships among its congeners have been extensively studied. As an extension of these studies, the muscarinic activities in the isolated guinea pig ileum of several oxotremorine analogues, including some enantiomeric pairs, were resolved into affinity and efficacy components. The method used involved analysis of dose-response data before and after fractional inactivation of receptors with propylbenzilylcholine mustard (PrBCM). When applied to partial agonists, this method gave affinity constants that were almost identical to those estimated pharmacologically by two independent methods, one of which did not make use of PrBCM. The affinity constants of some competitive antagonists were the same before and after inactivation of about 90% of the receptors with PrBCM. Furthermore, the affinity constants of oxotremorine (1.5×10^{6} M^{-1}), oxotremorine methiodide (1.5×10^{5} M^{-1}), oxotremorine-M (3.4×10^{5} M^{-1}) and carbachol (6.1×10^{4} M^{-1}) agreed with reported low affinity binding constants (K_L) of these compounds determined in receptor binding studies. Collectively, these results appear to justify the use of PrBCM for the determination of affinities and relative efficacies of muscarinic agonists.

There was no correlation between relative affinities and efficacies of the compounds studied, indicating different structural requirements for occupation and activation of muscarinic receptors in the guinea pig ileum. Although oxotremorine had higher affinity than its agonist analogues, many of the latter had substantially greater efficacy than oxotremorine. Thus replacement of the pyrrolidine ring in oxotremorine or in its acetamide analogue (N-methyl-N-(4-pyrrolidino-2-butynyl)acetamide) by azetidino, dimethylamino or trimethylammonium groups was accompanied by a dramatic increase in efficacy. The trimethylammonium derivative of oxotremorine (oxotremorine-M) and of the above-mentioned acetamide were equally or more efficacious than carbachol. They required less than 0.5% receptor occupation in the guinea pig ileum for half-maximal response. Structural modifications in parts of the oxotremorine molecule other than the cationic head were accompanied by a decrease in efficacy often yielding partial agonists or antagonists. Potency differences between enantiomers could be ascribed mainly to differences in affinity for the receptor.

The significance of the work described lies primarily in the fact that it provides an estimate of the relative efficacy of an agonist since this parameter is not readily available by other methods. Efficacy directly relates to the ability of an agonist to produce a stimulus that leads to a physiological response. Estimate of agonist efficacies therefore should be useful in investigating the mechanism of activation of the receptor-effector system (Supported by USPHS MH-17691).

MUSCARINIC SUPERSENSITIVITY OF ANTERIOR PITUITARY ACTH AND B-ENDORPHIN RELEASE IN MAJOR DEPRESSIVE ILLNESS: S. Craig Risch, David S. Janowsky and J. Christian Gillin, Department of Psychiatry, M-003, University of California, San Diego, La Jolla, CA 92093.

Studies from a number of centers have suggested that there may be an "up-regulation" or supersensitivity of muscurinic cholinergic receptor function in major depressive illness. We have previously demonstrated that patients with major affective disorders may be behaviorally supersensitive to the dysphoric-inhibitory effects of centrally active cholinesterase inhibitors. In addition, Siraham, Gillin and co-workers have reported that early rapid eye movement (REM) latency, a major biological marker for depressive illness, may reflect muscarinic supersensitivity of the REM axis in sleep. Finally, Nadi and co-workers have recently reported an increased number of muscarinic receptors on fibroblasts in patients with major depressive illness which may possibly be a genetically transferrable trait marker for depressive illness. Consequently, a number of investigations have implicated muscarinic supersensitivity in major depressive illness.

Numerous studies have suggested that central acetylcholinergic activity may stimulate hypothalamic-pituitary adrenal function. Thus, in vitro, in vivo animal and human investigations suggest that cholinergic agonists may activate the HPA axis to increase adrenal cortisol secretion. We have recently demonstrated that centrally active cholinergic agonists also increase plasma concentrations of ACTH and B-endorphin.

Since, as reviewed above, numerous physiological systems displayed muscarinic receptor supersensitivity in major depressive illness, we have hypothesized that anterior pituitary release of ACTH and B-endorphin immunoreactivity may also be muscarinically supersensitive in depression. Subjects with major depressive illness had greater increases in plasma concentrations of ACTH and B-endorphin immunoreactivity in response to central muscarinic stimulation by physostigmine, than did normal control subjects, or psychiatric control subjects without major depressive illness. These findings provide further evidence for muscarinic receptor supersensitivity in depression and may implicate an abnormality of peptide physiology in the pathogenesis of depression.

CONTRIBUTION OF THE DORSAL NORADRENERGIC BUNDLE TO THE EFFECT OF AMPHETAMINE ON ACETYLCHOLINE TURNOVER. Susan E. Robinson. Department of Pharmacology and Toxicology, Medical College of Virginia, Richmond, Virginia 23298, USA.

Amphetamine increases the turnover rate of acetylcholine (TR_{ACh}) in several brain areas. The action in the cortex and hippocampus is known to involve a noradrenergic mechanism. In order to determine the contribution of noradrenergic neurons in the locus coeruleus to the action of amphetamine in the cortex, hippocampus and hypothalamus, the dorsal noradrenergic bundle was lesioned with stereotaxically-placed injections of 6-hydroxydopamine (6-OHDA). Male Sprague-Dawley rats were injected bilaterally with 6-OHDA (23.6 nmol in 2 ul of 0.02% ascorbic acid, 0.9% saline) at the coordinates of AP + 1.0, L ± 0.8, V - 0.6, according to the atlas of Konig and Klippel. Control animals were injected with vehicle alone. Ten days later, the rats were injected with either amphetanine (27 umol/kg, i.p.) or saline 60 min before being killed by microwave radiation focussed to the skull. TR_{ACh} was monitored mass fragmentographically by measuring the incorporation of deuterium into ACh and Choline after infusion with deuterated phosphorylcholine. Brain areas were homogenized in 0.4 N perchloric acid and prepared for mass fragmentographic analysis (J. Pharmacol. Exp. Ther. 208: 476, 1979). An aliquot of the 0.4 N perchloric acid supernatant was taken from each sample for analysis of norepinephrine content by HPLC to ascertain the extent of lesion. None of the above treatments significantly affected the level of ACh or choline in the areas studied. Amphetamine significantly increased TR_{ACh} in the cortex, hippocampus and hypothalamus of unlesioned rats. Lesions of the dorsal noradrenergic bundle reduced the norepinephrine content of the cortex, hippocampus and hypothalamus, but did not significantly affect K_{ACh} or TR_{ACh}. On the other hand, 6-OHDA lesions of the dorsal noradrenergic bundle significantly reduced the amphetamine-induced increase in TR_{ACh} in the cortex (by 42%) and in the hypothalamus (by 50%). The lesions also slightly reduced (by 16%) the increase in TR_{ACh} in the hippocampus. The lack of complete blockade of the amphetamine-induced increases in TR_{ACh} may be due to a contribution of other noradrenergic neurons (as in the ventral noradrenergic bundle) to the action of amphetamine or due to supersensitivity to norepinephrine released from remaining noradrenergic terminals.

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MECHANISMS OF ACETYLCHOLINE SYNTHESIS: COUPLING WITH CHOLINE TRANSPORT:

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At least two hypotheses have been proposed for the synthesis of ACh in the mammalian nerve terminal involving the roles of cytoplasmic choline pools and high affinity transport of choline, similarly, about the intraterminal localization and functional distribution of choline acetyltransferase (ChAT). A coupling between the high-affinity choline carrier and ChAT may function as an integral component responsible for at least part of ACh synthesized, and may be of especial importance during repetitive neuronal activity. The existence or precise nature of such a coupling has not been deduced, not has its quantitative role in ACh synthesis been unequivocally established. Some of the discrepancies, relating in particular to the coupling of high-affinity choline transport to ACh synthesis, could result from the use of brain synaptosomes from two different species i.e. the rat and the guinea pig. Currently, studies on these mechanisms are being carried out in our laboratory; initially, basic comparative parameters were assessed between the two species. We found significant differences between rat and guinea-pig with respect to the efficiency of acetylation of choline transported into synaptosomes by a sodium-dependent mechanism; 72% of ^{3}H-choline if formed into ^{3}H-ACh in rat compared to 55% in guinea-pig. Potassium-induced depolarization of synaptosomes stimulated high-affinity choline transport in both species, but there was a significant increase in the efficiency of acetylation of choline in guinea-pig (55% to 71%), with no apparent change in rat. Of significance was the finding that the increased choline entering the nerve terminals following depolarization in guinea-pig was used exclusively for the manufacture of ACh. In some of the experiments a nitrogen mustard analogue of choline, choline mustard aziridinium ion (ChM Az), was used as a probe as we have shown previously this compound can act as a selective and irreversible inhibitor of sodium-dependent choline transport in brain synaptosomes. Using rat forebrain synaptosomes, we found that ChM Az (2 uM) produced a time-dependent and irreversible decrease in synaptosomal content of ChAT in parallel with alkylation of the high-affinity choline carrier. This effect was strictly sodium-dependent. When ChAT preparations, either crude brain homogenates or as the partially purified enzyme (12-fold), were incubated with ChM Az, irreversible inhibition of the enzyme could only be achieved after long incubation times with high concentrations of the ChM Az (mM; 30 min; 37^{o}C). In contrast to the results obtained with rat forebrain synaptosomes, incubation of synaptosomes prepared from guinea-pig forebrain with ChM Az did not result in the loss of intrasynaptosomal ChAT activity even though a time-dependent inhibition of high-affinity choline transport was produced. Separation of ChAT from rat and guinea-pig forebrain synaptosomes by isoelectric focussing revealed three major molecular forms for rat and one for guinea-pig (as demonstrated by Fonnum and Malthe-Sorensonn, 1973). Preliminary experiments showed that incubation of rat brain synaptosomes with ChM Az, followed by separation of synaptosomal ChAT by isoelectric focussing revealed greatest losses of enzyme activity in the basic pH range indicating loss of enzyme which displays most membrane binding capability. A model for the synthesis of ACh will be proposed.

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BIOCHEMISTRY AND IMMUNOCYTOCHEMISTRY OF CHOLINE ACETYLTRANSFERASE: Paul M. Salvaterra, Garrett D. Crawford, Carolyn R. Houser, DeeAnn Matthews, Robert P. Barber and James E. Vaughn, Beckman Research Institute of the City of Hope, Duarte, California, 91010, USA.

Monoclonal antibodies selective for choline acetyltransferase (ChAT, EC 2.3.1.6) were prepared by standard techniques to antigen derived from rat or Drosophila nervous system. Five anti-rat ChAT cell lines and several anti-Drosophila cell lines were established in culture and as ascites tumors. All antibodies produced were of the IgG_1 subclass and were purified by Protein A-Sepharose chromatography.

Nearly all the anti-Drosophila ChAT antibodies are directly inhibiting. The two most extensively studied antibodies seem to bind ChAT at or near the acetylCoA binding site. In contrast, all five anti-rat antibodies show no direct inhibition of enzyme activity. The anti-Drosophila antibodies appear species-specific, while some of the anti-rat antibodies cross-react with ChAT from species as diverse as insects and primates, and others react primarily with rodent enzyme. All five anti-rat antibodies seem to bind to a small region of the enzyme surface, which may be a main immunogenic region. The anti-Drosophila antibodies have been useful for immunoblot staining of SDS gels. The two higher molecular weight proteins observed in completely pure ChAT are stained (67 K and 54 K daltons) while no staining of the 13 K dalton polypeptide was observed. The anti-rat antibodies have been negative in immunoblot experiments. Anti-rat antibodies have been useful, however, for immunopurification of rat brain ChAT as well as for immunocytochemical studies.

The specificity of one of the anti-rat ChAT antibodies for immunocytochemical studies has been confirmed by demonstrating ChAT-positive neurons in a number of well defined "cholinergic" systems. For example, ChAT-positive reaction product was present in the cell bodies, dendrites and axonal terminations of spinal cord motorneurons. In addition, no ChAT-positive cells were found in groups of neurons, such as the substantia nigra, which are thought to use neuroactive substances other than acetylcholine. An immunocytochemical survey of rat brain indicated that ChAT had an extensive intraneuronal distribution throughout many neurons. In addition to cell body staining in "known cholinergic" nuclei such as the medial septum, we have also observed staining of intrinsic cells in hippocampus and cerebral cortex. Terminal-like punctate structures have also been observed in numerous brain regions and, in some cases, have been shown to be synaptic terminals by electron microscopy.

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EXCITATORY AMINOACID INFLUENCE ON STRIATAL CHOLINERGIC TRANSMISSION: Bernard Scatton and Dominique Fage, Synthélabo-LERS, Biochemical Pharmacology Group, 31 avenue Paul Vaillant Couturier, 92220 Bagneux, FRANCE.

Evidence has been provided that striatal cholinergic interneurons receive an excitatory input from the cerebral cortex which utilizes an excitatory aminoacid (L-glutamate or L-aspartate) as its neurotransmitter. Excitatory aminoacid receptors have been classified into three subtypes: the N-methyl-D-aspartate (NMDA), the quisqualate- and the kainate-preferring receptors. In an attempt to characterize the pharmacological nature of the receptor mediating the excitatory aminoacid influence on striatal cholinergic neurons we have investigated the effects of specific excitatory aminoacid receptor agonists and antagonists on the release of ^{3}H-acetylcholine (^{3}H-ACh) from slices of the rat corpus striatum.

In the absence of magnesium, agonists of excitatory aminoacid receptors evoked an increase in ^{3}H-ACh release. The relative order of potency was the following: NMDA > (±)ibotenate $\geq$ N-methyl-DL-aspartate > L-glutamate > L-aspartate $\geq$ cysteate > kainate = quisqualate with IC_{50}'s ranging from 15 to 800 µM. Concentration response curves for the agonists were sigmoïdal in shape suggesting the involvement of a single saturable receptor.

Low concentrations of magnesium (1.2mM) antagonized the release of ^{3}H-ACh evoked by all excitatory aminoacid receptor agonists tested, with the exception of kainate. The release of ^{3}H-ACh evoked by N-methyl-DL-aspartate (50 µM) and L-glutamate (300 µM) was antagonized in a competitive manner by the NMDA-type antagonists (-) 2-amino-7-phosphono-heptanoate (IC_{50}'s 40 and 90 µM, respectively), 2-amino-5-phosphonopentanoate (IC_{50}'s 280 and 320 µM, respectively) and D-α-aminoadipate (IC_{50} 1 mM) but not by the quisqualate-type antagonist glutamic acid diethylester (up to 1 mM). Phencyclidine, which has been shown electrophysiologically to possess NMDA-type receptor antagonist properties, also blocked the release of ^{3}H-ACh evoked by N-methyl-DL-aspartate (IC_{50} 0.2 µM). At concentrations effective in blocking N-methyl-DL-aspartate or L-glutamate responses, the NMDA-type antagonists only marginally inhibited the release of ^{3}H-ACh evoked by other depolarizing agents e.g. potassium or veratridine. Altogether, these results suggest that the excitatory aminoacid receptor mediating ^{3}H-ACh release from striatal slices is of the NMDA-type.

Chemical lesion of the nigro-striatal dopaminergic pathway failed to affect the ability of N-methyl-DL-aspartate (50 µM) or L-glutamate (300 µM) to increase ^{3}H-ACh release from striatal slices. However, addition of tetrodotoxin (0.5 µM) to the medium abolished the release of ^{3}H-ACh evoked by the excitatory aminoacid agonists in magnesium-free medium. Moreover, N-methyl-DL-aspartate failed to evoke ^{3}H-ACh release from slices of hippocampus, interpeduncular nucleus and olfactory bulb, where cholinergic afferents, rather than interneurons, are found. These results suggest that excitatory aminoacids activate NMDA-type receptors located on dendrites of striatal cholinergic interneurons giving rise to action potentials and release of ACh from cholinergic nerve terminals. These NMDA-type receptors may play a part in transducing excitatory aminoacid transmission carried by cortico-striatal afferents.

CONTROL OF THE RELEASE OF ^{3}H-ACETYLCHOLINE FROM RAT HIPPOCAMPAL SLICES BY AMINOPYRIDINES AND PHENCYCLIDINE: Roy D. Schwarz, Carolyn J. Spencer, Adele A. Bernabei, and Thomas A. Pugsley. Warner-Lambert/Parke-Davis Pharmaceutical Research, Ann Arbor, Michigan, 48105, USA.

Release of acetylcholine (Ach) by depolarization has been induced by increasing the extracellular K^+ concentration, applying Veratrum alkaloids (e.g. veratridine), or by electrical stimulation. Recent electrophysiological data has suggested that blockade of K^+ channels will also enhance transmitter output by increasing Ca^{++} entry due to the prolongation of the repolarization period. Aminopyridines (APs) (Thesleff, Neurosci 5:1413, 1980) and phencyclidine (PCP) (Albuquerque, PNAS 78:7792, 1981) have been reported to increase transmitter release by blocking specific K^+ channels. The purpose of our experiments was to examine the effect of APs and PCP on the release of ^{3}H-Ach which has been preformed by incubating rat brain slices with ^{3}H-choline. The depolarization-evoked release of prelabelled stores of Ach has been shown to physiologically reflect the release of Ach.

The rat hippocampus (dorsal and ventral portions) was removed, sliced with a McIlwain tissue chopper (0.3 x 0.3 mm), and incubated with ^{3}H-choline (0.01 uM) for 15 minutes at 37^{o} C in Krebs-Ringer Hepes buffered media, pH 7.2. After washing 3x with normal media the slices (10-15 mg tissue) were further incubated for 15 minutes in normal media or media with 20 mM K^+ in the presence or absence of test compound. At the end of incubation slices were separated from media by rapid centrifugation and radioactivity was determined in both fractions by scintillation counting. Results were expressed as % ^{3}H-Ach released into the media fraction.

Of the simple mono- and diaminopyridines tested, 3,4-, 2,3- and 4-AP significantly increased spontaneous (basal) ^{3}H-Ach release at 10^{-6} -10^{-4}M, while 2-, 3-, 2,5-, and 2,6-AP were inactive. The same APs which increased spontaneous release also markedly reduced K^+-stimulated ^{3}H-Ach release. The order of potency for affecting both types of release was: 3,4-AP > 4-AP = 2,3-AP. The increase in spontaneous release was Ca^{++} dependent. Further, while the release of ^{3}H-Ach induced by veratridine was tetrodotoxin (TTX) sensitive, the release induced by APs was not. PCP at concentrations of 10^{-9} - 10^{-4}M failed to alter either spontaneous or K^+-stimulated release.

At least four types of K^+ channels have been found in various vertebrate and invertebrate neurons. The APs have been suggested to act at voltage dependent fast K^+ channels which are linked to Ca^{++} while PCP may act at a non Ca^{++} - linked K^+ channel. Our results would suggest that the AP sensitive K^+ channel is more important in the control of ^{3}H-Ach release from rat hippocampal cholinergic neurons than the PCP sensitive K^+ channel. In addition, activity among the various APs tested would suggest that there are strict structural requirements necessary for that activity.

NICOTINIC CHOLINERGIC RECEPTORS LABELED BY ^{3}H-ACETYLCHOLINE IN BRAIN: CHARACTERIZATION, LOCALIZATION AND IN VIVO REGULATION: Rochelle D. Schwartz[1] and Kenneth J. Kellar[2], [1]NIMH, Bethesda, MD, 20205 USA, [2]Georgetown University Schools of Medicine and Dentistry, Washington, DC 20007 USA.

Nicotinic cholinergic receptors (nACHR) have been identified in brain using ^{3}H-acetylcholine (^{3}H-Ach) of high specific activity. In the presence of a cholinesterase inhibitor to prevent hydrolysis and atropine to block muscarinic cholinergic receptors, ^{3}H-Ach binds rapidly, reversibly and with high affinity to rat brain membranes associated with the synaptosomal fraction. In competition studies, nicotinic agonists are at least 100 times more potent than ganglionic and neuromuscular blocking drugs in displacing ^{3}H-Ach binding. The ^{3}H-Ach binding sites are unevely distributed throughout the brain; the highest levels of binding are found in the thalamus, cortex and striatum, while the lowest are found in the pyriform cortex and hippocampus. Additional studies of the nACHR structure have revealed that disulfide bonds (known to be important in peripheral nACHR function) are critical for ^{3}H-Ach binding but may not be located directly at the receognition site.

After characterization of the ^{3}H-Ach labeled nACHR, its anatomical (pre vs post synaptic) location and in vivo regulation was investigated. When rats were lesioned intraventricularly with 6-hydroxydopamine or 5,7 dihyroxytryptamine, ^{3}H-Ach binding sites were reduced in the striatum and hypothalamus, indicating that these sites are located presynaptically on catecholamine and serotonin nerve terminals in these areas. In contrast, these lesions did not affect binding in the cortex or thalamus. Ibotenate lesions of the nucleus basalis magnocelluaris had no effect on ^{3}H-Ach binding in the cortex. Thus it is unlikely that presynaptic ^{3}H-Ach binding sites are present on cortical cholinergic neurons arising in the nucleus basalis magnocellularis.

The in vivo regulation of nACHR was examined by repeated administration of various drugs which increase stimulation of nACHR directly or indirectly. After 10 days administration of the cholinesterase inhibitor, diisopropylfluorophosphate, the density of ^{3}H-Ach binding sites was decreased in the cortex, thalamus and striatum. However, repeated administration of nicotine (5-21 days) resulted in an increase of ^{3}H-Ach binding sites in the cortex, thalamus and striatum, and a decrease in the hypothalamus. The differential effects produced by these drugs in various brain regions may be related to the proportion of presynaptic ^{3}H-Ach binding sites associated with these regions. Thus pre and post synaptic binding sites might be regulated differently.

These data indicate that ^{3}H-Ach of high specific activity can be used to identify binding sites in brain with characteristics of nACHR. Studies of this kind will provide a basis for more extensive investigations of the molecular properties of central nACHR and of nicotinic cholinergic transmission in brain and its functional implications .

RED BLOOD CELL CHOLINE: PREDICTOR OF RESPONSE TO "ACETYLCHOLINE PRECURSOR" THERAPY? Kathleen A. Sherman and Eitan Friedman, Department of Psychiatry, New York University School of Medicine, New York, NY 10016, USA.

To investigate whether blood choline (Ch) levels are affected in patients with senile dementia of the Alzheimer's type, we compared plasma and red blood cell (RBC) Ch concentrations in elderly patients with mild to moderate cognitive impairment to age-matched control values and to those in young controls. Both plasma and RBC Ch concentrations were significantly elevated in subjects 60-80 years old in comparison to those 20-50 years of age. However, patients with cognitive impairment did not differ significantly from elderly controls with regard to plasma or BRC Ch. Further analysis of these data indicates that the incidence of high (>15 nmol/ml) Ch content of RBC is greater in the aged subjects than in young controls, but again the incidence of high RBC Ch values was not related to cognitive impairment. However, the increased incidence of high RBC Ch in the elderly may be important since in several studies we have shown a striking correlation between the baseline RBC Ch value and the rise in RBC Ch which occurs during therapy with "acetylcholine precursors", choline or lecithin, whether administered alone or in combination with piracetam. By contrast, the plasma Ch level at baseline was not predictive of the concentration achieved during precursor treatment. Lastly, the relatively small percentage of subjects who improved clinically during precursor treatment were distinguished from those who failed to respond in that they had higher RBC Ch content both at baseline and during treatment.

CHOLINERGIC EFFECTS OF HI-6 IN SOMAN POISONING. Tsung-Ming Shih 1, Christopher E. Whalley 1, James J. Valdes 2, Paul M. Lundy 3, and Peter A. Lockwood 4. 1 Basic Pharm. Br., US Army Med. Res. Inst. Chem. Def., 2 Tox. Br., Chem. R & D Ctr., Aberdeen Proving Ground, MD, USA, and 3 Biomed. and 4 Chem. Sections, Def. Res. Establishment Suffield, Ralston, AB, Canada.

The treatment of poisoning induced by organophosphorus(OP) cholinesterase (ChE) inhibitors has usually been a combination of atropine sulfate(ATS) and an oxime, such as 2-PAM. This treatment regimen is effective against most OP compounds, but not against Soman. Recently new oximes such as HI-6 have been used successfully with ATS to antagonize Soman intoxication. This observation, combined with its relatively low toxicity, presents HI-6 as a very attractive potential antidote for Soman poisoning. The mechanism of this protective effect is not completely understood; however, the primary antidotal action of these oximes is thought to be due to reactivation of the Soman-inhibited ChE. We have conducted a series of studies to evaluate the role of cholinergic mechanisms in the protective effect of HI-6 against Soman poisoning. We examined the effects of HI-6 on Soman-induced depression of ChE and elevation of acetylcholine(ACh) or choline(Ch) levels in vivo, on muscarinic receptor binding and sodium-dependent high affinity Ch uptake (SDHACU) in vitro in discrete rat brain areas. In some cases 2-PAM was also studied to compare its effects with HI-6. In the toxicity study, rats given HI-6 (125 mg/kg, i.p.) alone were protected from 2.5 LD50's of Soman while treatment with 2-PAM (43.2 mg/kg, im), ATS (16 mg/kg, im), or atropine methylnitrate (AMN; 17 mg/kg, im) alone afforded protective ratios(PR) of 1.0, 1.2 or 1.0, respectively. Combination of ATS plus HI-6 provided a PR of 5.5, while AMN plus HI-6 only 2.4. In ATS-pretreated rats, HI-6 (25 ug/rat) given via intracerebroventricular(i.c.v.) cannulae did not protect against Soman lethality. Animals given HI-6 i.p. were protected from about 5.7 LD50's of Soman while the addition of HI-6 i.c.v. had no additional beneficial effect. In the in vivo study, HI-6 administered either i.c.v. alone or plus i.p. failed to reactivate Soman-inhibited ChE in brain areas. HI-6 (i.p.) reactivated significantly the Soman-inhibited ChE in blood and some peripheral tissues. 2-PAM or HI-6 had no effect on brain ACh or Ch levels, but ATS treatment alone significantly reduced ACh in striatum, cortex and hippocampus (38, 25, and 16%, respectively) and increased Ch from 20 to 89% in all brain areas studied. In the brain regions neither ATS, 2-PAM nor HI-6 showed any effect on Soman-induced elevation of ACh except in the striatum, where ACh levels were reduced by these treatments individually. Pretreatment (15 min) with ATS plus 2-PAM or HI-6 treatment produced an even more significant, but similar, reduction in striatal ACh levels. In the in vitro study, the IC50 values of synaptosomal SDHACU for HI-6 was 2.5×10^{-3} M and the IC50 values for the binding of [3H]-methylscopolamine to brain muscarinic receptors for HI-6 or 2-PAM were 5×10^{-5} or 4×10^{-4} M, respectively. These data indicate that although HI-6 and 2-PAM had similar effects upon Soman-induced ACh elevation in the different brain areas, HI-6 produced a higher PR than 2-PAM against Soman intoxication. Although HI-6 alone could protect against Soman toxicity to a certain degree, the additional central protection provided by ATS administration was crucial. This protective effect of HI-6 may be due to its peripheral cholinergic effects, since (1) HI-6 administered either centrally or peripherally does not reactivate brain ChE, but reactivates peripheral ChE activities inhibited by Soman in vivo, and (2) HI-6 possesses some effects on SDHACU and antimuscarinic potencies in vitro in concentrations likely to be presented in the periphery during the treatment.

CHIOLINERGIC REM-INDUCTION AS A MARKER OF ENDOGENOUS DEPRESSION: Natraj Sitaram, Don Jones, Robert Pohl, and Surendra Kelwala. Wayne State University and Lafayette Clinic, Detroit, Michigan, 48207, USA

SPECIFIC OBJECTIVES: 1) To test the sensitivity and specificity of the cholinergic REM-induction response as a biological marker of specific subtypes of affective disorders such as endogenous (E) and non-endogenous (NE) depression; 2) study the interaction of coexisting anxiety and panic disorder on the marker.

METHODS: Fifty-seven patients were studied with 3-4 nights of sleep recordings. On 2 of these nights they received an infusion of arecoline (0.5 mg) and placebo, in random order, during the second non-REM sleep, 25 min after end of the first REM period. The time from infusion to onset of second REM was the index of arecoline response. Other sleep parameters such as REM latency (RL), duration of the first REM period (RT1) and density of eye movements of the first REM period (RD1) were also used both for univariate comparisons as well as factors in a discriminant analysis of 3 groups of patients: (I) Endogenously depressed patients with no anxiety (N = 22); (II) Major depressive disorder with concurrent or antecedent panic or generalized anxiety disorders (N = 19); (III) Non endogenous or nonaffective patients with no anxiety. (N=16)

RESULTS: Group I showed significantly shortened RL (mean 57 min) and supersensitive arecoline response (Inf-REM2 latency = 18.8 min) compared to both Group II (71 min, 41.7 min) and Group III (78 min, 51.2 min). Discriminant function analysis with arecoline response, RL, RT1, RD1 showed significant separation of Group I from both II and III. Groups II and III were not discriminable.

ACETYLCHOLINE CONTENT, RELEASE, AND LEAKAGE AT THE NEUROMUSCULAR JUNCTION OF MATURE ADULT AND AGED RATS.

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At the diaphragm neuromuscular junction, there is an age-related increase in the number of nerve terminals per end plate. To assess correlated changes in synapse function, transmitter content, leakage and release were measured at the neuromuscular junctions of rats aged 10 and 28 mos. The ACh content per end plate was assayed using radiochemical procedures. Values in nonstimulated tissue decreased from 29.3 fmol/end plate in 10-mos rats to 17.2 fmol/end plate in 28-mos animals; following stimulation (1000 impulses at 3 Hz) in the presence of physostigmine, the corresponding values were 27.1 fmol/end plate and 12.8 fmol/end plate, respectively. Choline uptake and choline acetyltransferase activity were measured radiochemically, and in both cases Vmax was lower in the older animals. These changes were probably too small to explain the reduced ACh content of the aged rats, however. Leakage of ACh from cytoplasmic sources was determined by measuring the hyperpolarization following application of d-tubocurarine. The hyperpolarizing responses were of similar magnitude (1 mV) in both age groups. However, the older rats were less sensitive to ACh at these agonist concentrations (10^{-9} to 10^{-8} M), and ACh flux due to cytoplasmic leakage was calculated to be 0.02 and 0.08 amol/s/terminal in the 10- and the 28-mos animals, respectively. This might explain the age-related drop in ACh content.

Miniature end-plate potentials were significantly smaller in the older rats; they also occurred more frequently in the aged animals, but this increase could be attributed to the increased terminal number. In curarized preparations, end-plate potentials were recorded intracellularly during repetitive stimulation at rates ranging from 5 to 50 Hz. Synaptic depression was observed to be more extensive in the older animals. In both age groups, depression could be described quantitatively by models based strictly on depletion of transmitter. Estimates of release probability, p, were derived from these data, and the average values were 0.17 and 0.16 in the young and the old rats, respectively. As these values were significantly greater than zero, quantal release could be described using binomial statistics. Average quantal content, m, was 75 and 116 in the 10- and the 28-mos rats; this difference is significant at the 0.05 level. However, the corresponding average values are 6 and 7 per nerve terminal, and this difference is not significant statistically. Furthermore, the binomial parameter n was estimated to be 444 and 817 in the young and the aged rats, respectively; when normalized for the number of nerve terminals per end plate, however, the values were 34 and 47, which are not significantly different (0.17 level). Biochemical assays revealed that 3.2 and 3.9 fmol of ACh were released per end plate in response to 1000 presynaptic action potentials in the young and the older rats, respectively; this difference is significant at the 0.05 level. The corresponding values per nerve terminal were 0.24 and 0.22 fmol, which are not significantly different. Furthermore, in the aged animals this represents a significantly larger fraction of the total ACh present in the nerve terminals, which may explain the enhanced synaptic depression. These results indicate that an age-related increase in transmitter release may be attributed to greater numbers of nerve terminals within the end plate.

LATERALIZATION OF CHOLINERGIC AND ENERGY RELATED ENZYMES IN HUMAN TEMPORAL CORTEX: Sandro Sorbi, Laura Bracco, Silvia Piacentini, Antonio Morandi, and Luigi Amaducci, University of Florence, Florence, Italy.

Anatomical and functional studies have shown that animal brains, including human brain, differ between the two sides (1,2). We have provided evidence of a significant lateralization of the cholinergic markér choline acetyltransferase (CAT) in the human first temporal gyrus (3). To investigate the relationship between the cholinergic lateralization in the human temporal cortex and the energy metabolism of the brain, we have extended our study analyzing in this cortical region the activity of the cholinergic marker CAT and the density and affinity of quinuclidinyl benzylate (QNB) muscarinic receptors, and the activities of some enzymes related to the energy metabolism of the brain, namely phosphofructokinase (PFK), esokinase (EK) and pyruvate dehydrogenase complex (PDHC). Cerebral cortex samples were from 7 right handed males who died suddenly with no clinical or pathological signs of neurological diseases. The mean age ($\pm$ SD) was 61.3 $\pm$ 11 yrs. (range 51-79) and the autopsy was performed after 25 $\pm$ 3 hrs. (mean $\pm$ SD) after death. Brains were handled and dissected and samples collected exactly as previously described (3). In the 7 brains of the present study the activity of the cholinergic marker CAT was always lateralized confirming our previous results. Muscarinic receptors Bmax and K_D were studied in 3 brains at the level of punch four, and they were always lateralized. However, they were right-biased in all brains ($P < 0.05$) by t-student test. Correlational analysis have also shown a significant positive correlation between the asymmetry in Bmax and K_D indicating that in the human temporal lobes there is a significant negative correlation between number and affinity of muscarinic receptors lateralization. All energy metabolism related enzymes studied had every similar pattern of lateralization. Correlational analysis showed a significant positive correlation between PDHC, PFK, AND EK values in all four punches. Correlational analysis of asymmetries in the posterior portion of the first temporal gyrus (punch 4), the portion most interesting for asymmetry studies because of anatomical asymmetry and lateralization of language function, showed a prevalence of positive correlations not only between the different energy metabolism enzymes but also between these enzymes and cholinergic activity. The results of correlational analysis of left-right asymmetries observed in this study provide further evidence of chemical lateralization in the human brain.

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CHOLINERGIC RECEPTOR BINDING IN THE FRONTAL CORTEX OF SUICIDE VICTIMS: Michael Stanley, Ph.D., Departments of Psychiatry and Pharmacology, Wayne State University School of Medicine and Division of Pharmacology, Lafayette Clinic, Detroit Michigan, 48207

A number of different studies have provided data which support a role for the cholinergic system in persons suffering from affective disorders. Patients diagnosed as having an affective disorder have been reported to display increased sensitivity to cholinergic agonists as measured by changes in REM sleep latency. Physostigmine has been reported to reduce manic symptoms in manic-depressive illness and to exacerbate the symptoms of depression in depressives. In addition to modifying symptoms in those with affective disorders, it has been reported that physostigmine induces depressive symptoms in normals. The latter observation is consistent with reports that individuals exposed to organophosphate insecticides (cholinesterase inhibitors) show symptoms of depression. Recently, increases in the density of muscarinic receptors in skin fibroblasts of manic-depressive patients has been reported. Because there is a high incidence of individuals diagnosed as having affective disorders among those who commit suicide, we thought it would be of interest to determine whether differences in central muscarinic receptors occurred in this population. At autopsy frontal cortex samples were dissected and immediately frozen on dry ice then stored at -80° C until assayed. Individuals in the suicide and control groups were carefully matched so that there were no significant differences between the two groups with respect to age, sex and post mortem delay. In our study we examined QNB binding in 22 suicides and 22 controls (18 male and 4 female in each group). Muscarinic binding was determined according to previously described methods using 10 separate concentrations of QNB per brain. Scatchard analysis of the data showed no significant differences in the number of binding sites (B max) for the suicide (492.68 f moles/mg/protein) or control groups (491.77 f moles/mg/protein). Nor were there any significant differences in affinity (Kd) between the two groups (suicide 14 pM and controls 13.68 pM). These findings do not offer support for the hypothesis that there is increased tone in the cholinergic system of individuals suffering from affective disorders. Thus, it would seem that the changes in mood induced by cholinergic agents in patients as well as controls need not be accompanied by changes in CNS cholinergic receptors.

CORTICAL CHOLINERGIC INNERVATION: DISTRIBUTION AND SOURCE IN MONKEYS.

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In Alzheimer's disease, cortical cholinergic markers, including choline acetyltransferase (ChAT), are significantly decreased. This decrement is thought to result from dysfunction and, eventually, death of neurons in the basal forebrain cholinergic (Ch) system located in the medial septum, diagonal band of Broca, and nucleus basalis of Meynert (nbM). In order to better understand this system in health and disease, we have examined the distribution of cholinergic markers in normal monkey neocortex and in monkeys with unilateral excitotoxic lesions in the basal forebrain. In these studies, ChAT activity was measured in samples from multiple sites in the neocortex. In seven normal cynomolgus monkeys, ChAT activity was highest in the motor strip and superior temporal gyrus, moderately high in prefrontal cortex, and lowest in occipital cortex. There was a 2.5-fold range from lowest to highest ChAT activities in these neocortical regions. After multiple injections of ibotenic acid (a neurotoxin which destroys neuronal perikarya and spares axons of passage) into the nbM, ChAT activity was reduced in cerebral cortex; in some instances, reductions in ChAT activity exceeded 60%. The topographical distribution of changes in ChAT activity generally correlated with the site of the lesion and were congruent with anterograde and retrograde tracing studies performed in our laboratory and with the published work of other investigators.

In conclusion, our studies of normal brain demonstrate considerable heterogeneity in cortical ChAT activity, probably reflecting the density of innervation derived from the Ch system. Moreover, biochemical studies following lesions provide additional information concerning the topographical nature of neocortical projections derived from neurons of the Ch system. Finally, the ability of microinjected neurotoxins to selectively destroy components of the Ch system offers an opportunity to carry out behavioral studies of animals with lesions in forebrain cholinergic pathways. Investigations of lesioned animals should provide new information about the functions of cholinergic innervation of hippocampus, amygdala, and neocortex, and this information should prove very helpful in delineating the roles of cholinergic abnormalities in human neurological disorders.

RELATIONSHIP BETWEEN CALCIUM ENTRY AND ACH RELEASE IN K^+-STIMULATED RAT BRAIN SYNAPTOSOMES, **Janusz B. Suszkiw, Michael E. O'Leary and Gregory Toth**
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Effects of drugs on acetylcholine (ACh) turnover and release are frequently assessed in vitro by prolonged K^+-stimulation of isolated nerve endings (synaptosomes). Interpretation of such experiments and their relevance to neurogenic ACh release is uncertain because the K^+-depolarization-ACh release coupling in synaptosomes has not been adequately characterized. In this work we examined temporal relationship between calcium entry and Ca^{++}-dependent ACh release in rat brain synaptosomes exposed to 45-52.5 mM K_o^+ from 3 sec to 30 min. Synaptosomes prepared according to Gray and Whittaker[1] were prelabelled with [^{3}H]Ch. The release of [^{3}H]ACh was studied by a rapid filtration technique and in superfused synaptosomal beds. Time course of ^{45}Ca uptake by synaptosomes was determined by rapid filtration on Millipore (0.65 μ) filters. In agreement with earlier reports (2,3) the K^+-evoked [^{3}H]ACh release exhibited a rapid temporal decay ($t_{\frac{1}{2}} \simeq 18$ sec). The amount of [^{3}H]ACh released during the first 60 seconds of K^+ stimulus did not exceed 12% of total, or 40% of the releasable intrasynaptosomal [^{3}H]ACh. Therefore, the cessation of release was not due to the exhaustion of [^{3}H]ACh stores in synaptosomes. Following the first phase release, slow efflux of [^{3}H]ACh amounting to no more than 7% of total initial, intrasynaptosomal [^{3}H]ACh could be detected over subsequent 27 min of sustained K^+ challenge. The rate of the slow phase was about 1/40th the rate of the fast phase release. The K^+-evoked ^{45}Ca uptake in synaptosomes reached a plateau within 60 sec of K^+-stimulus ($t_{\frac{1}{2}} \simeq 8$ sec). As first suggested by Nachshen and Blaustein (4), the time-course of ^{45}Ca entry in synaptosomes reflects in part the process of Ca^{++}-channels inactivation. By measuring ^{45}Ca accumulation during short (1-10 sec) pulses of ^{45}Ca injected into a suspension of synaptosomes predepolarized for various time periods in high K^+ solutions without Ca^{++}, three components of inactivation process could be qualitatively resolved: fast ($t_{\frac{1}{2}} < 5$ sec), slow ($t_{\frac{1}{2}} \simeq 40$s) and very slow or "noninactivating" ($t_{\frac{1}{2}} > 300$ sec).

Conclusions: (1) During sustained K^+-stimulus, the release of ACh from synaptosomes consists of major, rapidly terminating "phasic" and minor, sustained "tonic", components (similar relations have been reported for dopamine release (5)). (2) Although the heterogeneity of synaptosomal preparations precludes the determination of stoichiometry between Ca^{++} entry and ACh release, our results indicate that the temporal characteristics of Ca^{++}-dependent ACh release are a function of Ca^{++}-entry inactivation. (3) The rapid inactivation of the Ca^{++}-dependent ACh release from K^+-stimulated synaptosomes suggests that measurements of ACh turnover during prolonged exposure of preparations to high K^+ may not reflect the turnover of that ACh pool which contributes to the Ca^{++}-dependent release evoked by more physiological means of stimulation.

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MOLECULAR RELATIONSHIP BETWEEN ACETYLCHOLINESTERASE AND ACETYLCHOLINE RECEPTORS : Ladislav Tauc, Philippe Fossier and Gérard Baux, Laboratoire de Neurobiologie Cellulaire, C.N.R.S., 91190 Gif sur Yvette, France.

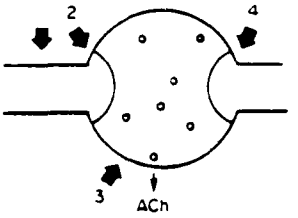
Most of the effects of actylcholinesterase (AChE) on synaptic transmission are considered to be related to its acetylcholine (ACh) hydrolysing properties. However on the Aplysia H-type synapse (Cl^- channels), the response to ionophoretically applied carbachol (which is not hydrolysed by AChE) on somatic acetylcholine receptors (AChR) increases after inhibition of AChE. This increase is observed with all kinds of AChE inhibitors (organophosphates, carbamates. No change was observed in the conductance or life time of unitary Cl^- channels opened by the carbachol activated receptor when AChE was inhibited. The increase in the total response appears thus to result from activation of an increased population of receptors. Inhibition of AChE induced an increase in evoked postsynaptic response and in the size of miniature postsynaptic currents. The response to injected carbachol on a D-type cell (cationic channels) did not increase after AChE was inhibited neither were the postsynaptic responses facilitated.

The observed results are in agreement with a hypothesis which postulates a molecular interaction between AChE and AChR : when enzymatic activity of AChE is inhibited the number of AChRs which can be activated is increased. This hypothesis was also supported by the results obtained with small concentrations of detergents. Triton-X-100 or Na-deoxycholate mimicked the facilatory action of AChE inhibitors, probably by removing the molecular action of AChE on AChR. Indeed the effect of detergents was not cumulative with the action of AChE inhibitors and was not observed for D-type cells.

PRESYNAPTIC RECEPTORS MODULATING ACETYLCHOLINE RELEASE: E.Sylvester Vizi and Georg T. Somogyi, Institute of Experimental Medicine, Hungarian Academy of Sciences, H-1450 Budapest, P.O.B. 67.

Evidence has been obtained that the cholinergic varicosities are equipped with presynaptic inhibitory receptors (cf. Vizi, 1979, see Fig.1). The release of acetylcholine (ACh) can be inhibited by stimulation of presynaptic α- (Vizi, 1968, Paton and Vizi, 1969, Beani et al. 1969, Kosterlitz et al. 1970) later turned out to be α_2- (Drew, 1977, Wikberg, 1978) adrenoceptors, adenosine- (Vizi,1975, Vizi and Knoll, 1976) opiate (μ) and muscarinic receptors (Szerb and Somogyi, 1973).

The question arises whether each cholinergic branch is equipped with these receptors or each neuron possesses only one type of inhibitory receptors. If the former is the case, the stimulation of one type of receptors will produce an inhibition of ACh release without leaving space for another presynaptic modulator to further reduce the release. If each neuron has only one recognition site the total release is reduced to the extent of the size of population. The problem has been studied on guinea-pig ileal isolated longitudinal muscle strip with intact Auerbach's plexus and on rat striatal slices measuring the release of ^{3}H-ACh. Since within an experiment the ratios between ^{3}H-ACh release evoked by two consecutive stimulation (1 Hz, 300 shocks) periods were constant, 0.80±0.05 (n=16) in the absence and 0.76±0.03 (n=14) in the presence of eserine, the effect of drugs on the ratios ($S_{n+1}:S_n$) was studied. When either α-methyl-noradrenaline (1 μM) or adenosine (10 μM) was added there was a reduction of 85 % in the ratios. However when they were added together there was no further reduction. Both teophylline (170 μM) and atropine (1 μM) enhanced the release of ACh indicating an endogenous tonic control of ACh release by adenosine and/or by ACh. In the presence of an antagonist (theophylline or atropine) the agonist of the other antagonist was able to inhibit the release. These findings indicate that there is no population of cholinergic neurons which has only one type of receptors. Similar conclusion has been drawn from the data obtained with striatal slices (Vizi, Hársing and Zsilla, Brain. Res. 212: 89-99, 1981).



Inhibitors

Noradrenaline	(α_2)	1 Axonal conduction
Adrenaline	(α_2)	2 Depolarization invasion
Dopamine	(α_2)	3 Secretion - coupling
Acetylcholine	(muscarinic)	4 Varicosity hillock
Adenosine	(P)	
Opioid peptide	(μ)	

From these data it seems very convincing that interneuronal modulation of both peripheral and central cholinergic neurons is likely to be mediated via different receptors and these recognition sites are located in each neurons. Their proprotional involvement in the modulation is time and space dependent: the type of modulatory influence depends on the regional localization of the non-synaptic and synaptic inhibitory input.

AF64A PRODUCES LONG-TERM LEARNING AND MEMORY IMPAIRMENTS IN THE RAT:

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It has recently been suggested that neurotoxic analogs of choline (Ch) might be used to develop animal models of chronic cholinergic hypofunction (Fisher and Hanin, Life Sci., 1980). AF64A is a mustard analog of Ch which has been demonstrated to exhibit selective cholinotoxic properties in vivo (Mantione et al., Science, 1981). The studies described here examined the behavioral and biochemical effects of AF64A. Measures of activity and behavioral reactivity to an aversive stimulus were included with assessments of both shock and food-motivated learning and/or memory, to better characterize the effects of AF64A on cognitive processes.

AF64A (15 or 30 nmol) was found to produce a variety of biochemical and behavioral effects following bilateral administration into the lateral ventricles. The compound had transient and dose-related effects on sensorimotor function, but produced long-term deficits in cognitive behavior. Rats dosed with either 15 or 30 nmol of AF64A reacted faster than CSF-injected controls in a hot-plate test 14 (but not 1, 7, 21 or 28) days following dosing. The group administered 15 nmol was also significantly more active than controls 28 days following dosing. The activity level of this group was comparable to controls at other times and hyperactivity was never observed in the 30 nmol group. Retention of a step-through passive avoidance task, assessed 35 days after dosing, was impaired in both AF64A-treated groups. Their retention latencies were significantly shorter than the controls, and they exhibited more partial entries during the 24 hr retention test. Radial-arm maze performance, measured 60-80 days after treatment, was markedly impaired in both the 15 and 30 nmol AF64A groups. Treated animals made more repeated feeder entries during a session, required more total selections to complete the task, and had an altered pattern of spatial responding in the maze. The neurochemical changes produced by AF64A, determined 120 days post-dosing, were specific to the cholinergic system and consisted of decreases of acetylcholine in both the hippocampus (15 and 30 nmol groups 55 % of controls) and the frontal cortex (30 nmol group 50 % of controls). The concentrations of catecholamines, indoleamines, their metabolites and Ch in various brain regions were not affected by AF64A. Furthermore, histological analysis revealed that the doses of AF64A used in the present study did not produce nonspecific damage to the hippocampus, caudate nucleus, the nucleus basalis or the septum.

The data presented here support the contention that cholinergic processes in the hippocampus and/or frontal cortex play an important role in the modulation of learning and memory processes. Furthermore, based upon the behavioral and biochemical data, it is suggested that AF64A could be a useful tool for examining the neurobiological substrates of putative cholinergic disorders such as senile dementia of the Alzheimer type.

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AUTORADIOGRAPHIC LOCALIZATION OF SUBTYPES OF MUSCARINIC AGONIST AND ANTAGONIST BINDING SITES: ALTERATIONS FOLLOWING CNS LESIONS: James K. Wamsley and Donald R. Gehlert, Departments of Psychiatry and Pharmacology, University of Utah School of Medicine, Salt Lake City, Utah 84132, U.S.A.

Autoradiographic techniques for receptor localization have been employed to identify subpopulations of receptors for both muscarinic agonists and antagonists. The agonist sites exist in both high and low affinity states. High affinity agonist sites can be localized by both direct and indirect labeling methods. The high affinity site has been demonstrated to be undergoing the process of orthograde axonal transport in the peripheral nervous system and the high affinity state can be converted to the low affinity state in the presence of guanine nucleotides. High affinity sites also accumulate adjacent to a fimbria lesion indicating they may be involved in axonal transport in the brain, as well as the periphery. However, attempts to interconvert the two affinity states in the brain have been unsuccessful and thus they could be subject to different forms of regulation. The development of chronic thiamine deficiency in laboratory rats increases the sensitivity of muscarinic receptors in many brain regions. This increase in sensitivity is manifested predominantly by the low affinity state of the agonist receptor. Chronic spinal animals also show an increase in the sensitivity of muscarinic receptors in the spinal cord below the level of the lesion. The predominant effect of spinal cord transection, however, is represented by a shift of the high affinity state of the receptor to the low affinity state in the ventral horn gray matter. The high affinity sites normally predominate the population of muscarinic receptors found in the ventral horn of the spinal cord and it is this population of sites which is lost in the human disease amyotrophic lateral sclerosis. Lesions of the medial septum also cause a shift in the agonist states represented in the hippocampus. A small population of high affinity sites is lost while the low affinity agonist sites increase. The relationship of these alterations to the subpopulation of muscarinic antagonist sites labeled with pirenzepine is unknown. However, the distribution of antagonist sites directly labeled with [^{3}H]-pirenzepine show a marked overlap with regions showing the presence of carbachol insensitive low affinity agonist sites.

These studies demonstrate alterations in muscarinic receptor populations following CNS lesions. Many of these changes are represented by shifts in the affinity states of the receptor which may be overlooked in binding studies employing radiolabeled classical antagonists which bind to muscarinic receptor subtypes with equal high affinity.

CHOLINERGIC REGULATION OF CATECHOLAMINE SYNTHESIS IN ADRENAL CHROMAFFIN CELLS THROUGH MULTIPLE SITE PHOSPHORYLATION OF TYROSINE HYDROXYLASE.

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SPLANCHNIC NERVE STIMULATION CAN RELEASE AT LEAST 50% OF THE CATECHOLAMINE (CA) STORES FROM ADRENAL CHROMAFFIN CELL, BUT THE CHROMAFFIN CELLS ARE ABLE TO MAINTAIN CA LEVELS BY INCREASING THE ACTIVITY OF TYROSINE HYDROXYLASE (TH), THE RATE-LIMITING ENZYME IN CA BIOSYNTHESIS. MECHANISMS PREVIOUSLY PROPOSED TO MEDIATE THIS EFFECT HAVE, HOWEVER, BEEN RELATIVELY INFERENTIAL. ORIGINALLY, ACTIVATION OF THE CELL WAS THOUGHT TO DIMINISH A STRATEGICALLY LOCATED POOL OF CA THAT MAINTAINED A TONIC END-PRODUCT INHIBITION OF TH. BUT, BECAUSE THIS POOL WAS POSTULATED TO BE TOO SMALL TO BE CHEMICALLY DETECTED, THIS MODEL WAS NOT DIRECTLY TESTABLE. SINCE THEN, cAMP-DEPENDENT PROTEIN KINASE (KINASE A) WAS FOUND TO PHOSPHORYLATE AND ACTIVATE TH IN VITRO. THIS LED TO THE HYPOTHESIS THAT KINASE A MEDIATED A STIMULATION-DEPENDENT ACTIVATION OF TH.

ALTHOUGH TH HAS BEEN SHOWN TO BE A PHOSPHOPROTEIN IN SITU AND TO BE PHOSPHORYLATED AND ACTIVATED IN VITRO BY KINASE A, NEITHER STIMULATION-DEPENDENT PHOSPHORYLATION OF TH IN SITU NOR THE INVOLVEMENT OF KINASE A THEREIN HAS BEEN REPORTED. TO ADDRESS THESE ISSUES, WE STUDIED THE EFFECTS OF ACETYLCHOLINE (ACH, THE NATURAL SECRETAGOGUE) ON VARIOUS ASPECTS OF CA METABOLISM IN ISOLATED, INTACT ADRENAL CHROMAFFIN CELLS.

ACH INCREASED CA SECRETION, CA BIOSYNTHESIS AND THE PHOSPHORYLATION AND ACTIVITY OF TH. ALL OF THESE EFFECTS REQUIRED EXTRACELLULAR CALCIUM. IN ADDITION, 8-BR-cAMP ALSO INCREASED CA BIOSYNTHESIS AND PHOSPHORYLATED AND ACTIVATED TH. WHILE DEMONSTRATING THE REGULATION OF TH PHOSPHORYLATION IN SITU BY TREATMENTS THAT ACCELERATE CA BIOSYNTHESIS, THESE DATA DO NOT SPECIFY THE KINASE ACTIVITY(IES) INVOLVED IN THE EFFECTS OF ACH. TO ADDRESS THIS ISSUE, WE PHARMACOLOGICALLY SCREENED CHROMAFFIN CELL SUPERNATANTS FOR ENDOGENOUS KINASE ACTIVITIES CAPABLE OF BOTH PHOSPHORYLATING AND ACTIVATING TH IN VITRO. IN CRUDE SUPERNATES, BOTH CALCIUM (IN THE PRESENCE OF KINASE A INHIBITOR AND LEUPEPTIN) AND cAMP PROMOTED THE PHOSPHORYLATION AND ACTIVATION OF TH. PHOSPHATIDYLSERINE BUT NOT CALMODULIN ENHANCED THE CALCIUM-DEPENDENT PHOSPHORYLATION OF TH. THUS, TWO CANDIDATES FOR THE MEDIATION OF ACH'S EFFECTS IN SITU WERE ESTABLISHED: KINASE A AND CALCIUM-DEPENDENT, LIPID-SENSITIVE PROTEIN KINASE (KINASE C).

IN AN ATTEMPT TO DISTINGUISH THE TWO KINASE ACTIVITIES IN SITU, WE LOOKED AT TRYPTIC DIGESTS OF TH PHOSPHORYLATED IN SITU AND IN VITRO. IN SITU, TH WAS PHOSPHORYLATED AT TWO TRYPTIC PEPTIDES. ACH INCREASED THE PHOSPHORYLATION OF BOTH PEPTIDES WHEPEAS 8-BR-cAMP INCREASED THE PHOSPHORYLATION OF ONLY ONE OF THE TWO. IN VITRO, CALCIUM INDUCED THE PHOSPHORYLATION OF BOTH PHOSPHOPEPTIDES WHEREAS cAMP INDUCED THE PHOSPHORYLATION OF ONLY ONE.

THUS, ALTHOUGH THE EFFECTS OF 8-BR-cAMP IN SITU SUGGEST THAT AT LEAST SOME KINASE A HAS ACCESS TO TH WITHIN NORMAL CELLULAR COMPARTMENTATION, IT DOES NOT APPEAR THAT KINASE A ACTIVITY IS REQUIRED FOR THE EFFECTS OF ACH. IN FACT, FROM THE PRESENT DATA, KINASE C ACTIVITY MAY BE THE SOLE MEDIATOR OF STIMULATION-DEPENDENT PHOSPHORYLATION AND ACTIVATION OF TH.

THE UTILIZATION OF SUPPLEMENTAL CHOLINE BY BRAIN: Lynn Wecker, Department of Pharmacology, Louisiana State University Medical Center, New Orleans, Louisiana, 70112, USA

The chronic administration of choline, either as the free base or in an esterified form, has been of interest as a potential therapeutic agent for the treatment of neuropsychiatric disorders postulated to involve hypocholinergic activity. To investigate the utilization of supplemental choline by brain and determine the pharmacological and biochemical consequences resulting from chronic administration, male Sprague-Dawley rats were maintained on a 12 hour light/dark cycle and fed either basal choline (0.2% choline chloride) or choline-supplemented (2.0% choline chloride) chow for 28-32 days. Maintenance of rats on the supplemented diet led to a significant 117% and 63% increase in the concentration of choline in plasma and red blood cells, respectively, as compared to basal chow animals. To determine whether these increases altered the transport of choline across the blood brain barrier, the intracarotid injection technique was utilized. Choline-supplementation did not alter the BUI for choline as compared to basal animals. Analysis of various cholinergic parameters in striata and hippocampi indicated no changes induced by chronic choline supplementation in the steady-state concentration of either acetylcholine or choline, the activity of cholineacetyl-transferase or acetylcholinesterase, or in the density of cholinergic receptors as analyzed by both agonist and antagonist binding. However, when challenged with pentylenetetrazol or atropine, choline-supplemented rats did not exhibit the decrease in the levels of acetylcholine in brain observed in basal choline animals. Furthermore, the toxicity of nicotine in supplemented rats was significantly decreased as compared to controls. To determine the possible mechanisms responsible for these effects of chronic choline administration, the concentration of phospholipids was measured in microsomal and synaptosomal membrane fractions of brain. The phospholipid: protein ratio in microsomes isolated from striata of choline-supplemented rats was 24% greater than that in basal animals. In addition, the concentration of phosphatidylcholine was also significantly increased by 15%. No effects were noted in the phospholipid profiles of the synaptosomal membrane preparations. Results indicate that although chronic choline supplementation does not alter various biochemical measurements associated with cholinergic neurons in the brain, it does affect the responsiveness of these neurons to pharmacological manipulation. Furthermore, evidence to date suggests that the effects of supplementation may be mediated by alterations in the metabolism of phospholipids in brain. (Supported by USDHHS Grant # MH-33443 and USAMRDC Contract # DAMD-17-83-C-3012.)

SUBSTANTIA INNOMINATA - CORTICAL CHOLINERGIC PATHWAY: REGULATORY AFFERENTS:
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The recent definition of the substantia innominata (SI) as the source of cholinergic innervation of the cerebral cortex has stimulated research into the role of this pathway in cognitive processes. In this report, we will present a summary of our studies of regulatory inputs into the cholinergic cell body region of the SI:

1. GABAergic Inputs. Parenteral GABAergic agents (kojic amine, THIP, AOAA, diazepam and muscimol) all potently depress cortical acetylcholine turnover (TR_{ACh}). Local injections of these agents into the SI reproduce this action in a picrotoxin-reversible manner. Biochemical and immunohistochemical studies have indicated that this GABAergic input to the SI originates in the ipsilateral N. accumbens. Electrical stimulation of the N. accumbens was therefore examined and found to decrease cortical TR_{ACh}. In summary, the SI appears to receive a potent inhibitory GABAergic input from the N. accumbens.

2. Serotonin Agents. The serotonergic agonists quipazine and m-trifluoropiperazine as well as the antagonists mianserin and metergoline do not alter cortical TR_{ACh}. These data do not support any actions of serotonergic agonists on SI neurons and indicate that a tonic serotonergic input is unlikely to be present in this nucleus.

3. Adrenergic Agents. The β agonist isoproterenol and the β_2 agonist salbutamol did not alter cortical TR_{ACh} as was also the case for propranalol. These data would therefore also argue against a β adrenergic regulatory influence on SI cholinergic neurons. Similarly, the α_2 antagonists piperoxan and CP 14304-18 and the α_2 agonist clonidine do not alter cortical TR_{ACh}. However, preliminary studies indicate that the α_1 antagonist prazosin produces a dose-dependent depression of cortical TR_{ACh}.

4. Opioid Regulation. Mu and delta opioid receptor agonists produce a dose-dependent and naloxone-reversible depression of cortical TR_{ACh}. These actions are not reproduced by local opiate injections into the SI. Current data indicate that a subcortical site caudal to the SI may be involved.

In summary, our data support potent inhibitory GABAergic and opioid regulation of the SI - cortical cholinergic pathway.

DIFFERENTIAL LIGHT MICROSCOPIC AUTORADIOGRAPHIC LOCALIZATION OF MUSCARINIC CHOLINERGIC RECEPTORS IN THE BRAINSTEM AND SPINAL CORD OF THE RAT USING RADIOLABELED PIRENZEPINE: Henry I. Yamamura, James K. Wamsley, Donald Gehlert, Thomas W. Vickroy, Mark Watson and William R. Roeske, Departments of Pharmacology, Biochemistry, Psychiatry and Internal Medicine, University of Arizona Health Sciences Center, Tucson, Arizona, 85724, and Departments of Psychiatry, Anatomy and Pharmacology, University of Utah Medical Center, Salt Lake City, Utah, 84132.

Pirenzepine (PZ), a non-classical muscarinic cholinergic antagonist is currently used in the treatment of peptic ulcer disease. This drug has shown significant differences in potencies at atrial and ganglionic muscarinic receptors suggesting the presence of different classes of cholinergic muscarinic receptors in these tissues. We have recently demonstrated that radiolabeled PZ bound to a high affinity population of muscarinic binding sites in the rat cerebral cortex, hippocampus and corpus striatum. However, in the rat cerebellum, heart and ileum a low density of high affinity binding was seen in these tissues. These data suggest that radiolabeled pirenzepine labels a subpopulation of muscarinic cholinergic receptors. Thus we examined the localization of muscarinic receptors using radiolabeled PZ and compared its localization with radiolabeled 3-quinuclidinyl benzilate (QNB). Preparation of rat brain sections for light microscopic autoradiography followed the method as described by Wamsley and Palacios (Handbook of Neurochemistry, 2, 27-52, 1983). Under the conditions of our assay specifically bound ligands accounted for about 80 to 90% of the total binding in slices and was similar to that previously observed in our rapid filtration assay (Watson, Yamamura and Roeske, Life Sciences, 32, 3011, 1983). A distinct regional distribution of specific receptor binding was observed with both muscarinic ligands. Specific binding was highest in the CA1 region of the hippocampus, the molecular layer of the gyrus dentatus, the corpus striatum and the superficial laminae (I-III) of the parietal cortex and was lowest in the corpus callosum, a known white matter region for both ligands. However, an interesting differential localization of QNB and PZ binding was seen in the brainstem and the cervical spinal cord. Although QNB labeled the grey matter of the spinal cord uniformily, specific PZ binding was observed only in the substantia gelatinosa of the dorsal horn. Radiolabeled QNB also labeled the hypoglossal nucleus (nXII) of the caudal medulla oblongata but low density of specific binding was observed with radiolabeled PZ. From our preliminary autoradiographic studies, we conclude that radiolabeled PZ can be used to label a subpopulation of muscarinic cholinergic receptors in specific regions of the rat brain.

FACTORS WHICH INFLUENCE THE AVAILABILITY OF CHOLINE TO BRAIN:

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Choline is necessary for normal brain function as it is a precursor for the biosynthesis of acetylcholine (ACh), and of phosphatidylcholine (PC), sphingomyelin (SM), and choline plasmalogen. It is obvious that large supplies of choline molecules must be used for normal growth and function of the central nervous system. Despite this requirement, more unesterified (free) choline leaves the brain, in vivo, than enters it.

Brain can synthesize choline molecules de novo by methylating phosphatidylethanolamine (PE), forming PC. Free choline can then be generated when this PC is catabolized. The amount of choline produced by this pathway (approximately 10 pmoles/g/min) is small. The brain also contains storage pools of choline-esters from which free choline might be derived, PC and SM being likely candidates. Even pools as large as these would be insufficient to maintain continuous choline efflux from brain. These observations lead to the natural conclusion that significant amounts of choline-containing compounds (such as lysoPC) must enter brain from the blood. Even though estimates of the relative contribution of exogenous and endogenous sources of the choline molecule may be still be a matter of controversy, it is clear that, whatever the derivation of free choline may be, mechanisms must exist for its liberation from choline esters.

We, and others have observed a rapid increase in choline content within rat brain which was homogenized and incubated in vitro. This was mediated by enzymatic activities, as microwave irradiation stopped the accumulation of choline, and these reactions were temperature and pH dependent. We were interested in determining which ester was the initial reservoir from which free choline was produced. We observed a significant excess capacity for the liberation of choline from phosphorylcholine (PCh) within brain. However, it is unlikely that this was the primary pool from which choline was derived, as we, and others, have observed a net increase in PCh concentrations during incubations. Brain glcerophosphorylcholine (GPC) could have been a source of free choline, as we observed a small, but significant fall in GPC concentration during incubations. However, the fall in GPC content would only account for a small fraction of the free choline formed by brain homogenates. We noted significant stimulation of choline production by magnesium, with saturating concentrations being much higher than those needed to stimulate GPC diesterase. In addition zinc ions do not inhibit this enzyme, yet we found that zinc was a potent inhibitor of choline formation. GPC is a water soluble compound; we found that all membrane-containing subcellular fractions of brain produced free choline and that there were no factors present in the soluble fraction that increased choline production. PC and SM concentrations remained relatively constant during incubations, as both pools were large. We were able to demonstrate significant amounts of GPC, PCh and free choline which were derived from radio-labeled PC and SM.

ATP was an inhibitor of choline formation. Addition of magnesium was able to reverse some of ATP's inhibitory influence. We also found that added ATP inhibited the degradation of added lysoPC. Three routes for lysoPC metabolism exist: It can be reacylated, hydrolyzed to release free choline, or hydrolyzed to form GPC. Reacylation requires ATP to form the acyl-CoA moiety, and is rapid. It is possible that, in the presence of adequate ATP concentrations, most lysoPC is reacylated, making little available for further degradation to form free choline. If this is the case, then fatty acids as well as free choline should accumulate after treatments which lower ATP availability. Several other investigators have reported that there is a large increase in brain FFA content within 30 seconds after decapitation or the ligation of the arterial supply of brain. We suggest that ATP availability may influence the liberation of choline by brain.

CHOLINERGIC AND PEPTIDERGIC REGULATION OF GANGLIONIC TYROSINE HYDROXYLASE ACTIVITY: Richard E. Zigmond and Nancy Y. Ip, Department of Pharmacology, Harvard Medical School, Boston, MA 02115 USA.

The activity of tyrosine hydroxylase, the enzyme which catalyzes the rate-limiting step in catecholamine biosynthesis, has been shown to be regulated by a variety of factors. We have examined the acute regulation of this enzyme in the rat superior cervical ganglion. Ganglia were removed from adult rats, de-sheathed, and incubated at 37°C in an atmosphere of 95% O_2/5% CO_2. Tyrosine hydroxylase activity was measured by adding a dopa decarboxylase inhibitor (brocresine) to the incubation medium and measuring the rate of accumulation of dopa. Carbachol (0.1 mM) increased the rate of dopa accumulation by 4- to 6-fold. This effect of carbachol was blocked by about 80% by the nicotinic antagonist hexamethonium (3 mM) but was not significantly affected by the muscarinic antagonist atropine (6 μM). The response to carbachol was completely blocked by addition of both hexamethonium and atropine. Electrical stimulation of the preganglionic cervical sympathetic trunk at 10 Hz for 30 min also increased the rate of dopa accumulation by 4- to 6-fold. This increase in enzyme activity was partially blocked by addition of hexamethonium (3 mM) but not affected by addition of atropine (6 μM). In the presence of both antagonists, preganglionic nerve stimulation still produced a 2- to 4-fold increase in dopa synthesis. Increasing the concentration of both antagonists by an order of magnitude did not further decrease this biochemical response to nerve stimulation. These data suggest that the increase in tyrosine hydroxylase activity produced by preganglionic nerve stimulation is mediated in part by acetylcholine and in part by a second (non-cholinergic) transmitter. As a first step in an attempt to identify this non-cholinergic neurotransmitter, the ability of 14 neuropeptides to increase tyrosine hydroxylase activity was examined. Secretin and vasoactive intestinal peptide both stimulated enzyme activity at a concentration of 10 μM, whereas angiotensin II, bombesin, bradykinin, cholecystokinin octapeptide, glucagon, insulin, luteinizing hormone-releasing hormone, [D-Ala^2]-Met -enkephalinamide, motilin, neurotensin, somatostatin, and substance P produced no effects. The EC_{50} for the effect of secretin was approximately 5 nM while the EC_{50} for the effect of VIP was approximately 0.5 μM. Though more potent than VIP, secretin consistently produced a smaller maximal increase in enzyme activity. Since tyrosine hydroxylase activity can be elevated by cholinergic stimulation, we sought to determine whether these neuropeptides produced their effects indirectly by causing the release of acetylcholine from preganglionic nerve terminals. Thus, we examined the ability of the peptides to increase enzyme activity in decentralized ganglia and in intact ganglia in the presence of the cholinergic antagonists hexamethonium and atropine. Under both conditions, the effectiveness of secretin and VIP in elevating tyrosine hydroxylase activity was unaltered. To examine the possible interaction between cholinergic and peptidergic regulation of tyrosine hydroxylase activity, the effect of various concentrations of secretin from 1 nM to 10 μM were examined in the absence and in the presence of a low concentration (3 μM) of carbachol. The presence of both carbachol and secretin caused a potentiation of the enzyme response at all concentrations of secretin examined.

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