Alexander G. Karczmar

Exploring the Vertebrate Central Cholinergic Nervous System

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Cover illustration. Several items of cholinergic interes are shown. From left to right, clockwise: Steve Kuffler, John Eccles and Paul Katz, in Canberra, Australia, in the early 1950s (from Karczmar, 2006a, with permission); brain machinery against a shadowy human profile (adopted from Time, March, 25); a common abbreviation for the bonding between a naturally occurring anticholinesterase, huperzine A and acetylcholinesterase of the electric fish, Torpedo; the diagram of circuitry of the Renshaw cell (from Karczmar, 2006a, with permission); a mouse model, used in cholinergic studies of aggression (from Karczmar, 2006b, with permission); leaves of Physostigma venenosum (from Karczmar, 2006b, with permission); a common symbol for a tridimensional structure of a protein.

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Foreword

Even if the "weapons of mass destruction" (WMD) and, among them, stocks of organophosphorus (OP) agents (also referred to as war gases and nerve gases) were not found in Iraq following the US-Iraq war, the relative ease with which these substances can be made from harmless precursors and the low cost of their manufacture will continue to fascinate power-hungry, ruthless dictators, as well as multinational and international terrorists, particularly as the close relationship between the OP agents and useful insecticides makes it easy to disguise the importation and purchase of small amounts of the precursors. Indeed, the use by Saddam Hussein of a nerve gas against the Kurds and his possible employment of the OP agents during his war with Iran, and the Sarin attack in the Tokyo underground by an extremist religious set magnetized the world with respect to the OP drugs. As these drugs exert their toxicity via their cholinergic action on the nervous, particularly central nervous, system, it is no wonder that the research in the cholinergic field attracts, and merits, our intense attention. These considerations underlie the significance of this book, as Alex Karczmar devotes an entire chapter of *Exploring the Vertebrate Central Cholinergic Nervous System* to anticholinesterases (antiChEs), and as he is an acknowledged expert in the field of cholinergic toxicity as well as a consultant to the Surgeon General of the U.S. Army.

Another and equally cogent reason for our interest in the cholinergic field is the involvement of the cholinergic system in autonomic functions, neuromuscular transmission, control of behavior and higher brain activities that are concerned with cognition, learning, memory and awareness. Furthermore, many neurological and psychiatric disorders such as presenile dementia, Huntington's chorea, schizophrenia, Alzheimer's disease, Parkinsonism, motoneuron disease, as well as a number of peripheral disorders, such as myasthenia gravis, involve cholinergic dysfunction. Accordingly, for several decades now there is no slackening in the production of books and monographs charting the progress of research into cholinergic function and its accessible components such as cholinesterases, acetylcholine and cholinergic receptors. An early, and still very important, volume is the monumental monograph edited by George Koelle, *Cholinesterases and Anticholinesterase Agents*, which was published in 1963 as supplementary volume 15 of the great Heffter-Heubner *Handbuch der Experimentelle Pharmakologie (Handbook of Experimental Pharmacology).* Then, to cite just a few of the early to recent compendia, there is *Le System Cholinergique*, edited by G.-G. Nahas, J.-C. Salamagne Paul Viars, and G. Vourch in 1962; Ann Silver's 1974 *Biology of Cholinesterases*; Alan Goldberg and Israel Hanin's 1976 *Biology of Cholinergic Function*; my own *The Cholinergic Synapse*, published in 1988 as volume 86 of the successor to the *Handbook of Experimental Pharmacology* and my 1992 *The Cholinergic Neuron and Its Target*; and Ezio Giacobini's *Cholinesterases and Cholinesterase Inhibitors* and *Butyrylcholinesterase, Its Function and Inhibitors*, published in 2000 and 2003, respectively. Parenthetically, Alex Karczmar contributed review chapters to all, except the last, of these books and monographs.

Karczmar's *magnum opus* is a very welcome culmination of these efforts, and it covers more territory than most of the monographs concerned with the cholinergic field. It is essentially a one-person effort, as Karczmar wrote eight of the eleven chapters of the book and

was the coauthor of another chapter. As a result, the book has a homogeneity of approach—historical and conceptual—as well as a personal bias which adds color and interest to his monograph. Jon Lindstrom, Arthur Christopoulos, and Goerge Siegel with Neelima Chauchan and Alex Karczmar contribute important and up-to-date chapters on nicotinic and muscarinic receptors and on Alzheimer's disease.

The longest of the eleven chapters deals specifically with the physiology and pharmacology of the central cholinergic nervous system. With more than 1500 references, it is a thorough historical and conceptual review as it discusses, *inter alia*, the electrophysiology and pharmacology of cholinergic synapses, cholinoception and loci of release of acetylcholine, and central functions and behaviors exhibiting cholinergic correlates, including the involvement of the cholinergic system in cognition. The chapter even summarizes recent speculations on the metaphysics (in the literal sense of that word!) of the mind-body problem.

The chapter reminds us that the status of acetylcholine as a central neurotransmitter was for many years doubtful. Gradually, however, the electrophysiological evidence provided by John Eccles and the discovery by this author and his associates of nerve terminal localization of acetylcholine established the neurotransmitter—central as well as peripheral—role for acetylcholine. Indeed, me and my associates' work showed that that acetylcholine along with other neurotransmitters is specifically localized in subcellular fraction enriched with detached nerve terminals (synaptosomes) and, within the terminals, in the synaptic vesicles. In addition, our discovery of a cholinergic-specific surface antigen enabled us to isolate synaptosomes derived from cholinergic nerve endings in their pure form by immunoaffinity chromatography as well to enrich the synaptosomal content of acetylcholine; notably, vasoactive intestinal peptide (VIP) was enriched 20-fold in parallel with acetylcholine, serving as acetylcholine's co-transmitter. This co-transmitter role of VIP—and other polypeptides—in central cholinergic function, if thoroughly understood, might well throw a new light on the pharmacology and clinical significance of central cholinergic transmission. Karczmar discusses these matters, as well as recent controversies concerning the role of synaptic vesicles in acetylcholine release, in several chapters (particularly in a section of Chapter 2 entitled "Classical and Unorthodox Hypotheses of ACh Release").

Other chapters survey, with the same historical perspective and comprehensive treatment as the central nerve chapter, cholinergic cells and pathways; metabolism of acetylcholine and choline and cholinesterases; antiChEs, war gases and insecticides; muscarinic and nicotinic receptors (with contributions of Arthur Christopoulos and Jon Lindstrom); cholinergic ontology; and etiology of Alzheimer's disease (prepared by George Siegel, Neelima Chauhan, and Alex Karczmar). Molecular aspects of these subjects are considered as well.

Other chapters with the same historical perspective and comprehensive treatment as Chapter II and IX survey metabolic aspects of cholinergic function, muscarinic and nicotinic receptors, the organophosphorus anticholinesterases, cholinergic ontology and the etiology of Alzheimer's disease. A complete understanding of cholinergic function indeed requires a full integration of its pharmacology, physiology, and molecular biology. As an input to this partnership, this weighty monograph from a distinguished expert with a life-long perspective on the subject, aided by well-known co-authors, is a monumental contribution of enduring value.

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Preface and Acknowledgments

When they heard, some five years ago, of my intent to write, solo, a book on the central cholinergic system and its correlates, my cholinergiker friends told me that I was crazy to even try. Five years later, I agree with their assessment, as without the 3 crucial chapters by Neelima Chauhan, Arthur Christopoulos, Jon Lindstrom, and George Siegel, this book would have never been finished. My friends intimated also, rather sheepishly, that after all, in view of certain aspects of the matter and with some help, perhaps I would be able to complete the book: again, they were right; today the task, for better or worse, is finished.

I dedicate the book to my late, excellent, and old friends George Koelle, Bo Holmstedt, Jack Eccles, and Stanislav Tucek; for many decades we swam together, with joy and enthusiasm, down the mighty cholinergic river.

I wish to thank, for their help and comments, Marsel Mesulam, Palmer Taylor, Peter Waser, Claudio Cuello, Jochen Klein, Giancarlo Pepeu, Chris Gillin, Edson Albuquerque, Larry Butcher, Ezio Giacobini, Pat and Edith McGeer, Israel Hanin, Mona Soreq, Abe Fisher, Victor Whittaker, Mimo Costa, Nigel Birdsall, Ed Hulme, Roger Nitsch, Brian Collier, Maurice Israel, Jean-Pierre Changeux, Agneta Nordberg, Andrzej Szutowicz, Chris Krnjevic, and Ferdinand Hucho; I owe special gratitude to Konrad Loeffelholz, Jean Massoulié, and Yves Dunant for their help with Chapters 2 and 3.

I am particularly thankful to Kyo Koketsu and Syogoro Nishi and their past student Nae Dun for giving me a special insight into the cholinergic neuron and for guiding me for many fruitful years in the laboratory. And I am grateful to Enzo Logo for introducing me to the EEG expressions of the cholinergic system.

Invaluable help was afforded to me by Dr. Logan Ludwig, the director of Media Development and Design, Health Science Library, Loyola University Medical School, and his staff, particularly the reference librarians Janet Mixter and Mary Klatt, M.A.L.S.

Enormous assistance, moral support and warm friendship were afforded to me in the course of the work on this book by Kathleen Lyons, the Scientific Editor, Springer. Painstaking effort was experted in the immense task of preparing the proofs for the book by Barbara Chernord, Chernow Editorial Services.

Jenifer Stelmack, my editorial associate, is a computer whiz; without her computer expertise the difficult job of writing, formatting, and organizing this book could not have been done. Also, she was assiduous in clarifying and editing my text. In addition, Mr. Joseph Tomaszek, computer specialist, Hines VA Hospital, assisted us on many occasions with computer vagaries.

I am also very indebted to Joseph Messer, M.D., who kept me in good health throughout the arduous years of my work on this book.

A special encomium is due to my wife, Marion. She could not have tolerated my preoccupation with the book if she were not very busy on many fronts (and if she were not a swami); even so, she needed much endurance during the years of my writing this opus, and

I am thankful to her for her patience and support. The interest of my sons Chris and Greg in my doings was most supportive, and their urging me on is much appreciated.

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Introduction: History and Scope of This Book

A. Why a Book on the Central Cholinergic System?

Unlike the Roman Empire, the central cholinergic research rises but does not fall. The history of cholinergic research can be traced back to a time when shamans, hunters, medicine men and mystics collected plants, fungi and animal materials endowed with cholinergic ingredients in order to cure mental disease, provide food to the tribe, and identify the culprits. From these exotic beginnings there arose the research on the central cholinergic system that has exploded over the last 150 years. In this book, my associates and I propose to describe these beginnings of the cholinergic lore and to shed light on the recent central nervous system cholinergic research—research that continuously points the way toward distant peaks of new discovery.

No single review of the whole system seems to be available today, which warrants the publication of this book. In addition, this publication is called for, as the discipline of the central cholinergic system occupies today a preeminent and brilliant status among the biological sciences.

Some 85 years ago, the Nobel Prize winner Otto Loewi demonstrated the chemical, cholinergic nature of communication between peripheral nervous system and effector organs at the level of the cardiovagal junction. Twenty years later, Sir John Eccles, another Nobel Prize winner (with Paul Fatt and Kyozo Koketsu), proved that Santiago Ramon y Cajal's neuronal hypothesis translates into chemical, cholinergic communication between central neurons. Eccles also established that this communication, initially excitatory, could change signals (via an interneuron) and become inhibitory in mode (Figure 1-1). These discoveries led to explaining how signals travel along defined central circuitries and culminated with elucidating the generation of functions such as respiration and behaviors such as aggression; beyond that, we are today in the process of defining the cholinergic correlates of learning and cognition and, most excitingly, consciousness or self-awareness. In sum, the cholinergic wisdom has outlined a physical image of animal and human performance, whether corporeal or mental. From neuron to behavior, cognition, and consciousness—what a beautiful road (Table 1-1)!

Several way stations along this path are no less unique and important than the total path itself. One such way station is the system generating and controlling the release of acetylcholine (ACh) from the cholinergic nerve terminals, whether at the periphery or in the central nervous system (CNS). This system, pioneered by Victor Whittaker and Eduardo di Robertis (Figure 1-2; there is a bit of priority battle here), includes organelles; enzymes; transport, uptake, and other active proteins; and ions. These elements participate in ACh synthesis and its component, the choline nerve terminal uptake; generation of synaptic vesicles, their cycling and fusion with the plasmolemma; ACh's cytoplasmic movement; and quantal ACh release (either from the vesicles, the cytoplasm, or both;[1] see Table 1-2) into the synaptic cleft or the junction with the effector organ. To safeguard teleological release of ACh, the elements of this most complex arrangement must work in perfect synchrony and, yet, in a flexible manner; in fact, this interplay is so intricate that, perhaps, a computer program capable of defining this miraculous process and predicting its outcome—ACh release—at any particular moment cannot exist (Karczmar, 1999). Furthermore, this

Figure 1-1. From left to right: Winifred Koelle (wife of cholinergiker George Koelle), Sir John Eccles, and his wife, Helena Tabanikova-Eccles, at the Symposium in Honor of A.G. Karczmar, Loyola Medical Center, Maywood, IL, 1986.

Table 1-1. Brief History of Cholinergic Transmission Studies

Period	Provenance	Individuals	Drug and Materials
Second millennium BC – present	Arab, Egyptian, Roman, South American, African ethnographic medicines	Shamans and healers	Curare, ordeal bean or esere, bella donna, nicotine[1] (see Chapter 6 A-1)
1850–1880	Calabar missionaries, anthropologists, military men, and seamen	William Daniell, Donald Simmons, Hope Wadell	Ordeal bean (esere, eserine, physostigmine)[2] (see Chapter 6 A-1)
1870–1920	Scotch, German, and British investigators (Edinburgh, Dorpat, Halle)	Thomas Fraser, Robert Christison, John Balfour, John Langley, Oswald Schmiedeberg, William Gaskell, Walter Dixon	Nicotine and muscarine; choline derivatives[3] (see Chapter 6 A-1)
1870–1920	British, German, US investigators	Reid Hunt, Walter Dixon, Rudolf Lenz, Roberts Bartholow, Heinrich Winterberg	Choline derivatives, acetylcholine, atropine (atropia), ordeal bean (eserine, physostigmine)[4]
1870–1910	British and Russian investigators	William Gaskell, A.E. Smirnov, John Langley	Peripheral cholinergic pathways[5]
1890–1950	International investigators, including Russian and Spanish	Otto Loewi, Sir John Gaddum, Sir Henry Dale, Sir William Feldberg, Ramon y Cajal, Sir Geoffrey (Lindor) Brown, Alexander Kibjakov, Wilhelm Witanowski, Robert Volle, Kyozo Koketsu, Syogoro Nishi	Autonomic synaptic cholinergic transmission, cholinesterases; nicotinic and muscarinic receptors[6] (see Chapter 6 A-1 and Chapter 8 A-2)
1870–1925	US, French (Polish), and German investigators	Max and Michel Polonowski, Percy Julian, J. Jobst, Otto Hesse, E. and E. Stedman, G. Barger	Isolation, purification, structurization, and synthesis of physostigmine and physostigmine analogs[7] (see Chapter 7 A-1 and BI)