Brief Presentation of the Story and Present Status of Studies of the Vertebrate Cholinergic System

Alexander G. Karczmar, Ph.D.

This year marks the seventieth anniversary of Otto Loewi's demonstration of chemical transmission generally and autonomic cholinergic transmission specifically and the fortieth anniversary of John Eccles's proof of the existence of central cholinergic transmission. Following these epochal findings, the subsequent studies of the cholinergic system led to discoveries of similarly important phenomena. This review concerns these phenomena, including chemical structure and molecular biology of cholinergic receptors; electrophysiologic and ionic aspects of pre- and postsynaptic cholinergic events; the quantal expression of cholinergic postsynaptic events and activities of their subunits, the elementary events; second messengers and G proteins; synthesis, storage and release of acetylcholine; cholinesterases, anticholinesterases, and war gases; central cholinergic pathways; central cholinergic functions, behaviors, cholinergic EEG and REM sleep; cholinergic ontogeny and teratology; trophic phenomena; and the clinical aspects of the cholinergic system. This review refers to the history as well as the present status of each of these phenomena; furthermore, it describes briefly the nineteenth-century work with calabar bean, pilocarpine, muscarine, and nicotine, that is, the work performed before the promulgation of the cholinergic era.
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This year marks the seventieth anniversary of Otto Loewi's (1923) definitive paper on the transmitter nature of acetylcholine (ACh) at the amphibian heart and the fortieth anniversary of a similar demonstration for the mammalian central nervous system (CNS) by Eccles et al. (1953). These anniversaries remind us of the unique historic and scientific significance of the studies of the cholinergic system: Besides providing the first demonstration of chemical transmission, these studies included the first descriptions of the trophic phenomena, the quantal nature of the biological events, the receptors and their chemical structure, the role of a transmittive system in arousal and memory, and so on. So, it is appropriate to sketch at this time the history and the present status of the vertebrate cholinergic studies.

International Cholinergic Symposia (ICS) hold an important place in this history. These symposia were initiated in 1970 by Edith Heilbronn, Anders Winter, and Bo Holmstedt in Skokloster, Sweden (Heilbronn and Winter 1970). Holmstedt and Heilbronn participated in most of the subsequent meetings, as did the late Frank McIntosh, Sir William Feldberg, Giancarlo Pepeu, George Koelle, Jean Massoulie, Peter Waser, Herbert Ladinsky, Claudio Cuello, Don Jenden, Israel Hanin, Stanislav Tucek, Victor Whittaker, Maurice Israel, the late Brian Ansell, Don Jenden, Chris Krnjevic, Konrad Loffelholz, Sir Arnold Burgen, Edson Albuquerque, Larry Butcher, the McGeers, Steve Thesleff, Hermona Soreq, John Szerb, David Colquhoun and this

From Research Services, Hines VA Hospital, Hines, and the Department of Pharmacology, Loyola University Medical Center, Maywood, Illinois.

Address reprint requests to: Alexander G. Karczmar, Ph.D., Research Services, Hines VA Hospital, Hines, Illinois 60144.

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author (Karczmar 1986, 1990; Pepeu 1993). The last, the VIIIth ICS took place in 1992 in Sainte Adele, Quebec (Cuello 1993). The participants in the ICSs pioneered much of the modern cholinergic research, and their contributions at the VIIIth ICS will be referred to.

PRECHOLINERGIC ERA

The cholinergic lore began long before the arrival of the concept of cholinergic transmission. Indeed, the calabar bean, *Physostigma venenosum*, was used for centuries in tribal rites of Western Africa and as an antidote of curare in South America (Karczmar 1970; Holmstedt 1972; Holmstedt et al. 1984). Subsequently, as the bean was brought to Scotland by Scotch missionaries active in Calabar, its effects were studied by Edinburgh botanists and pharmacologists, such as Fraser, Robertson and Christison, both on animals and themselves (Christison 1885)!

These studies coincided with those of German, English, French, Russian, and U.S. medicinal scientists, such as Fuhner, Pal, Dixon, Winterberg, Harnack, and Bartholow, with the bean extract or its purified alkaloid, eserine (physostigmine), nicotine, muscarine, and pilocarpine; the peripheral autonomic and central effects of these substances became known toward the end of the nineteenth century (Karczmar 1970). Hunt and Taveau demonstrated in 1906 the potency of choline esters, particularly ACh, as mimics of the effect of "brief faradisation of the vagus nerve" (Karczmar 1970), physostigmine and muscarine. Henry Dale (1914) forged another link in the development of the concept of cholinergicity, as he surmised the existence of cholinesterases (ChEs); their role in the action of ACh and of the anti-ChE physostigmine was established by Fuhner (1917) and Loewi with Navratil (1926; see Karczmar 1970; Holmstedt 1972).

This work ushered in the demonstration in the 1920s by Otto Loewi of the action of ACh as a transmitter at the vagus nerve and, indeed, of the existence of chemical transmission. The subsequent work of Feldberg, Vogt, Brown, Dale, Gaddum, Kibjakov, and others established the existence of cholinergic transmission at additional parasympathetic sites, at the autonomic ganglia, and at the neuromyal junction (Karczmar 1970, 1986, 1990; Feldberg 1987). Although Henry Dale (1935) and William Feldberg (1945) conceptualized early the existence of the central cholinergic transmission, its proof was provided only in 1953 by John Eccles, Bernard Katz, and Kyozo Koketsu for the spinal synapse between the motor nerve collateral and Renshaw interneuron; ultimately, this synaptic relay generates the inhibitory response of the motoneuron. Earlier, Eccles was reluctant in accepting the notion of chemical transmission, as he could not envisage a mechanism for the termination of action of a chemical transmitter that would be sufficiently rapid to prevent clogging; however, the rapidity of action of acetyl ChE (AChE) became known in the 1950s helping Eccles's conversion (Karczmar 1991). An important technical aspect of this research was the development of microinjection cannula, and this technique served the Canberra scientists, including David Curtis, John Hubbard, John Crawford, and Kris Krnjevic at Babraham Institute of Animal Physiology to establish the presence of cholinergic transmission elsewhere in the CNS.

Objections were raised to Eccles and Renshaw's cell story. The synapse in question was identified by Renshaw and Eccles and his collaborators via electrophysiological and pharmacological analysis, not morphologically. Accordingly, Forrest Weight (1968) ascribed the inhibitory response at the motoneuron to a direct generation of current at the motor nerve terminal. Eccles (1969) referred to Weight's proposal as a "most audacious attack" and criticized it effectively on electrophysiological and pharmacological grounds. Actually, in 1966 Szentagothai (see Szetagothai 1983) and subsequently Jankowska and Lindstrom (1971) identified morphologically the Renshaw interneuron (Eccles 1987).

CHOLINERGIC ERA

The next decades provided a rich expansion of this basic concept of cholinergic transmission at peripheral and central sites. I will discuss pertinent areas in terms of both their history and their modern status.

Muscarinic and Nicotinic Receptors

Dale's and Langley's concept of two types of "receptor substances," nicotinic and muscarinic, and Claude Bernard's even older concept of the receptor for curare are the first recorded notions of the receptors as actual substances. Actually, structure-activity studies of cholinergic receptors, such as those of Collumbine, Ariens (1960), Furchgott, and Goldstein (see Ariens 1960) conveyed a somewhat abstract sense of the notion of receptors and their subtypes, and I remember how astonished were the cholinergikers when at the Rio de Janeiro Meeting of 1959 (Chagas and Paes De Carvalho 1961) Carlos Chagas, Peter Waser, David Nachmansonn, and Sy Ehrenpreis presented the first strictly chemical studies of the nicotinic receptor. The subsequent development of many types of agonists and antagonists, including neurotoxins, by Sir Arnold Burgen, Edward Hulme, Michael Schimerlik, Herbert Ladinsky, Michael Birdsall, and Bernard Witkop, and the application of appropriate molecular biology and chemical methods

by Kubo, Peralta, and Haga (Schimerlik 1990), led to the definition of at least five subtypes of muscarinic and nicotinic receptors, these subtypes being represented by several variants, as in the case of alpha and beta variants of the M2 receptor subtype (Birdsall and Hulme 1983; Schimerlik 1990; Brown 1989; Dahlbom et al. 1986; Kubo et al. 1986; Brown and Masters 1984; Karczmar 1986; Dorje et al. 1991; Sargent 1993; Hulme et al. 1990). However, the pharmacologic definition of the receptor subtypes was based on in vitro studies of relatively few types of isolated organs, and "none of the tested antagonists . . . and agonists . . . showed a marked selectivity for one subtype over all other subtypes" (Dorje et al. 1991).

Of the five muscarinic receptor subtypes, M1 and M2 receptors are present in the heart and ganglia, respectively (Mukaiyama et al. 1991); these two, as well as M3 and M4 receptors, are present in the CNS, the M1 and the M2 receptors being located at the CNS nerve terminals, as they regulate ACh release (see the section Release of ACh). M3 receptors may be present particularly in the hippocampus, as proposed at the VIIIth ICS by Quirion, Pepeu, and their associates (Cuello 1993).

Some 15 genes control the generation of the muscarinic receptor and its channel (see, for example, Rettig et al. 1992). According to a model of the M receptor proposed by Hulme et al. (1990) and Lai, Yamamura, and their associates (Cuello 1993), there are seven transmembrane helices that are hydrophobic, three extra, and three intracellular domains. This model has to account for the functions of the muscarinic receptor and its subtypes, namely, the binding of agonists and antagonists, the binding and activation of the second messengers and G proteins, and the generation of postsynaptic currents (see the section Electrophysiological Postsynaptic Aspects of Cholinergic Responses). The tyrosine and aspartic acid residues at the 3rd and 7th transmembrane helix may be particularly involved in ligand binding, while the sequence located at the amino terminal generates the glycosylation needed for the coupling with specific G proteins (Schimerlik 1990). It must be stressed that several muscarinic receptor subtypes may have a common second messenger signal, as they all activate the phosphatidyl inositol cascade with the interaction of appropriate G proteins, depress cAMP generation, and activate phosphokinase C (Schimerlik 1990; see also the section Second Messengers herein); similarly, they all activate several K^+ currents (see the section Electrophysiological Aspects). The subtle differences among the M receptor subtypes with respect to these various responses were not clearly defined as yet.

Similar work on nicotinic receptors was conducted by Bernard, Lindstrom, Patrick, Salvaterra, Karlin, Noda, Changeux and their associates (Sargent 1993; Barnard et al. 1987). The cholinergic nicotinic receptors consist of four subunits, alpha, beta, delta, and gamma; the alpha subunit appears twice, and the subunits are organized in a rosette. Furthermore, as shown at the VIIIth ICS by Jim Patrick, Michel Paquet, and others (Cuello 1993), several variants of these subunits exist. Genetically controlled formation and the organization of the subunits and their variants leads to the diversity and specificity of the nicotinic receptor subtypes. Thus, Changeux (1993) posited a complex, multigene "compartmentalized gene expression" mechanism that controls, via changes in the promoter regions of the genes for the four subunits and in cooperation with trophic factors and kinase activities, the structure, distribution, and ligand specificity of the nicotinic receptor subtypes; this mechanism predicates the anisotropic distribution of the nicotinic receptor in the embryonic or denervated muscle, versus its localized distribution in the mature or innervated muscle. The subunits were cloned, and their transmembrane and promoter domains are known (Ratnam et al. 1986; Paquet and Cooper in Cuello 1993; Changeux 1993; Karczmar 1990). The second transmembrane domain may constitute the channel pore (Paquet and Cooper in Cuello 1993). However, the molecular, functional, and structural differences between the nicotinic receptor subtypes were not defined as yet; for example, it is not understood why the classic alpha-bungarotoxin binding is irreversible at the neuromyal junction, reversible in the case of the ganglia and nonexistent at certain central neurons (Chiapinelli and his associates in Cuello 1993).

Electrophysiological Postsynaptic Aspects of Cholinergic Synapses

The existence of postsynaptic potentials that result from presynaptic excitation was demonstrated first at the motoneuron (Barron and Matthews 1936; Brooks and Eccles 1947), then at ganglionic and other peripheral synapses, and finally, in the supraspinal CNS (Eccles 1963; Karczmar 1990). That changes in ionic permeability and the resulting currents (Hodgkin et al. 1952) underlie the postsynaptic potentials became clear with the advent of the voltage clamp technique (Takeuchi and Takeuchi 1960) and the subsequent development of the patch clamp method by the Nobel Prize–awarded team of Neher, Sakmann, and their associates (Hamill et al. 1981). This then led to the demonstration of nicotinic and muscarinic excitatory postsynaptic potentials and currents (fast and slow EPSPs and EPSCs; Eccles and Libet 1961). In addition, inhibitory postsynaptic potentials and currents (IPSPs and IPSCs), whether noncholinergic (today known as aminergic; Lloyd 1947) or cholinergic in nature (Bradley and Wolstencroft 1962), were demonstrated. More recently, the Kurume-Loyola University team (Katayama and Nishi 1987) observed

at the sympathetic ganglia the noncholinergic late slow EPSP; it is generated via the presynaptic activation by a peptidergic neurotransmitter.

The original discovery of Eccles and his associates concerned a nicotinic response at the Renshaw cell; however, the Renshaw cell also exhibits a weak muscarinic response. Although muscarinic, rather than nicotinic, responses predominate in the CNS (Karczmar 1967), it is apparent today that many central cholinoceptive neurons exhibit either mixed responses, as in the Renshaw cell, or predominantly nicotinic responses, as in the case of the thalamus.

In the 1950s and 1970s Fatt, Katz, and Miledi proceeded to "miniaturize" the postsynaptic responses. First, Fatt and Katz (1952) demonstrated the existence, during the quiescent state of the neuromyal postsynaptic membrane, of spontaneously arising miniature MEPPS and MEPCs. The statistical analysis of this phenomenon showed that it reflects the spontaneous, quantal release of ACh and that the evoked release fires away a packet of 6000 to 8000 quanta; the evoked response is Ca^{++}-dependent (Katz and Miledi 1965). Morphological counterparts of the electrophysiological phenomena of the miniatures are the synaptic vesicles as established elegantly by De Robertis (1967), Whittaker (1988, 1992), and Palay and Palade (1955). Newer aspects of this matter, namely, cytoplasmic release of ACh, will be discussed subsequently (see the section Release of ACh).

The second phase of "miniaturization" concerned the neuromyal phenomenon of "noise." Many investigators noticed "noise;" however, only Katz and Miledi (1970) had the serendipity to perceive—and prove via appropriate statistical analysis—that the "noise" is a biological phenomenon, as it reflects, in the presence of ACh in the synaptic gap, responses generated by probably two molecules of ACh per ionic channel. This phenomenon was termed the elementary event, and the postsynaptic entity involved may be referred to as channel-receptor macromolecule.

The voltage clamp and patch clamp methods, as well as the analysis of the elementary events, provided the ionic and dynamic characteristics of the postsynaptic responses. In the case of the nicotinic channel–receptor macromolecules, the characteristic fast EPSC is generated by the increase in the Na^+ and K^+ conductance. There are several subtypes of the nicotinic channel responses; they depend on the subunit composition and organization of the channel-receptor macromolecule (see the section Receptors), and they differ in the kinetics of their response and binding properties. Another factor is Ca^{++}, as Ca^{++} may regulate the channel kinetics (Colquhoun et al. 1990; Ogden et al. 1987; Kuba and Nishi 1987). Altogether, the channels may "show multiple conductance states" (Colquhoun et al. 1990), and their response may depend on the state.

Similarly, the muscarinic responses, the slow EPSP and EPSC, whether at the ganglia or in the CNS, involve diverse currents that include several types of K^+ and Ca^{++} currents; particularly well studied is the voltage-dependent Paul Adam's K^+ current (M– current; Adams and Brown 1982; see also Schimerlik 1990). Second-messenger mechanisms mediate these muscarinic responses (see the section Second Messengers), and these mechanisms and the currents that they generate are specific for the different muscarinic channel-receptor macromolecule subtypes. For example, at the VIIIth ICS, Malcolm Caulfield, David Brown, and their associates (Cuello, 1993) related the activation of the M2 and M4 receptors to inhibition of adenylyl cyclase and the generation of Ca^{++} currents and Ca^{++}-dependent K^+ currents, whereas the activation of the M1 and the M3 receptors generates the Ca^{++}-dependent K^+ current via the stimulation of phosphatidyl inositol cascade and the release of IP3 and inhibits the M-type, voltage-dependent K^+ current. These muscarinically evoked currents may not be transmittive in nature; rather, they modulate the excitability of the neurons, as proposed early by Chris Krnjevic (1969; see the section Interactions, Modulations, and Gene Expression).

Second Messengers

In the 1960s Sutherland, Michell, and the Hokins, and subsequently Paul Greengard, demonstrated that the generation of the current requires intermediaries, termed second messengers (Greengard 1987; Lambert and Nahorski 1990; Berridge 1987); to promote permeability changes, the second messengers cause structural channel-receptor modifications via activations of phosphokinases and resulting protein phosphorylation. A number of second messengers are distinguished today, including Lowell Hokin's phosphatidyl inositol cascade, Paul Greengard's several cyclic nucleotides, and Ca^{++}-calmodulin system (Greengard 1987). The phosphatidyl inositol cascade and diacylglycerol and inositol triphosphate (Berridge 1987) and the cyclic guanosine monophosphate (GMP) respond to muscarinic activation, whereas cyclic adenosine monophosphate (AMP) is sensitive to other agonists or transmitters than cholinomimetics and ACh, such as dopamine. The specific second messenger status of the nicotinic response is not clearly established; the evidence presented at the VIIIth ICS by Francesca Grassi, Chuan-qui Liu, and their associates (Cuello, 1993) suggests that both at the periphery and in the CNS the nicotinic agonists activate, similarly to the muscarinic agents, the phosphatidyl inositol cascade and related Ca^{++} fluxes (Cuello 1993). Finally, several guanosine triphosphate–binding proteins (G proteins), whether of heterotrimeric or monomeric (small) type (Yamane and Fung 1993) "function as . . . additional . . . intermediates in transmembrane signal-

ling pathways" (Gilman 1987), preparing the channel-receptor macromolecule for the action of second messengers or otherwise interacting with the latter (Dunlap et al. 1987). Covalent modifications of G proteins induced by transmitters and resulting phosphorylations, acylations, and so on are "critical in controlling the proper interaction of the G protein with other proteins, as well with the appropriate membrane compartments" (Yamane and Fung 1993). It is important in the present context that the G proteins respond to muscarinic stimulation and specific G proteins may recognize different, specific receptor subtypes. Although the phenomena described so far concern the postsynaptic actions, second-messenger mechanisms obtain as well with respect to presynaptic events (see the section) and ACh metabolism (see the section Release of ACh), as well as underlie certain nonsynaptic events, such as postsynaptic desensitization (see the section Interactions, Modulations and Gene Expression).

Second messenger systems interact; thus, the accumulation of cyclic GMP inhibits adenyl cyclase and the generation of cyclic AMP, and the generation of phosphatidyl inositol cascade inhibits the formation of cyclic AMP (Schimerlik 1990) and of the Ca^{++}-calmodulin system, as shown at the VIIIth ICS by Michael McKinney (Cuello, 1993). Many of these interactions regulate the Ca^{++} permeability and fluxes. Furthermore, the discoverer of protein kinase C, Yasutomi Nishizuka (1984) proposes that this kinase serves to regulate the interaction between several second messenger systems (Karczmar 1990).

Synthesis, Turnover, and Storage of Acetylcholine

There are four components of ACh synthesis. The first component, the generation of acetyl groups via the pyruvate and glucose metabolism and acetyl coenzyme A, was already studied in the 1940s by Mann, Quastel, and von Muralt (Karczmar 1967; Browning 1986; Tucek 1990).

The second component concerns choline. Following the studies of Birks and McIntosh (1961), the active neuronal uptake of choline, present in the blood via dietary sources as well as in the synaptic cleft following hydrolysis of the released ACh, was considered as a limiting step for both the generation of neuronal choline and for the synthesis of ACh; the classic results obtained by the Canadian investigators could not have been gathered without the previous development, by Fred Schueller and John Long, of hemicholinium, the specific inhibitor of the active transport of choline (Long 1963). Subsequently, the work of Bremer, Wecker, Greenberg, Wurtman, Blusztajn, Loffelholz, Ansell, and Tucek (Ansell and Spanner 1979; Tucek 1990) related the availability of choline and the synthesis of ACh to neuronal phospholipid metabolism and, particularly, that of phosphatidylcholine. How important the brain phospholipid metabolism is for the generation of choline is not clear at present (Tucek 1990); Richard Wurtman (Ulus et al. 1989; Nitsch et al. 1992b) opined that this metabolism is indeed very important and that, under certain stress circumstances, such as those occurring in SDAT, its obligatory maintenance may require "autocannibalism" of cholinergic neurons.

The third component of ACh synthesis is the acetylation of choline, mediated by choline acetyl transferase (CAT; E.C. 2.3.1.6.), an enzyme discovered by David Nachmansohn in the 1940s (with Machado). With the advent of molecular biology methods, the identification of the CAT gene in *Drosophila* (Greenspan 1980) and cloning and development of monoclonal CAT antibodies (Levey et al. 1981), the story of CAT became complex, as a number of transcriptional sites and exons were identified in animals and man (Strauss and his associates in Cuello 1993). For example, the 5′ flanking domain of CAT regulates on the transcriptional level the appearance of CAT in specific neurons as well as its ontogenesis, as described at the VIIIth ICS by Paul Salvaterra and his associates (1993). Transcriptional mechanisms also control the trophic effect of nerve growth factor (NGF) on CAT (see Bejanin, Mallet, and their associates in Cuello 1993; see also the section Trophic Factors, herein), and the compartmentalization of CAT within the cytosol and the membrane and the shifts between these two sites also contribute to the regulation of ACh synthesis (Rylett 1993). This new evidence suggests that CAT, rather than choline, is the limiting step in ACh synthesis (Rylett 1993). It must be added that second messengers, such as cyclic nucleotides and retinoic acid, activate ACh synthesis via their control of CAT activity and, perhaps, phospholipid metabolism (Blusztajn et al. 1993).

The fourth component of ACh anabolism is its accumulation and storage prior to its synaptic release, whether in the synaptic vesicles, according to the classical view on the matter (Whittaker 1990, 1992) or in the cytoplasm, as related to the cytoplasmic release of ACh (see the section Release of ACh). The vesicles are engaged in the process of recycling, as, following the vesicular uptake of ACh, the loaded vesicles move to the nerve terminal, fuse with the membrane, and release ACh, the empty vesicles coursing away from the membrane (Whittaker 1992). The vesicular uptake may include a high- and low-affinity mechanism, possibly characterizing the vesicular populations concerned with the readily releasable ACh and reserve ACh, respectively, as suggested at the VIIIth ICS by Brian Collier and his associates (Cuello 1993). Vesamicol is a specific inhibitor of the active, high-affinity vesicular uptake of ACh, acting at a proteoglycan receptor site, as shown at the VIIIth ICS by Stanley Parsons and his associates (Cuello 1993).

In the 1970s Steve (B.B.) Brodie and Erminio Costa stated that the turnover of a transmitter, that is, the steady state resulting from the dynamics of transmitter synthesis, uptake, hydrolysis, and release, is functionally more important than the levels of the transmitter, and Costa, Neff, Karlen, Hanin, Holmstedt, Jenden, and others developed the methodology needed for the measurement of ACh turnover (see, for example, Karlen et al. 1986). As pointed out at the VIIIth ICS by Stanislas Tucek, Don Jenden, Konrad Loffelholz, Oscar Scremin, and others (Cuello 1993), ACh turnover in the brain depends on the phospholipid metabolism and the generation and availability of several ACh precursors; the active, phosphorylation-dependent choline uptake across the blood-brain barrier; the arteriovenous difference in choline concentration that implies that there is a continuous choline loss from the brain; and such conditions as apnea and ischemia.

Cholinesterases

After Dale and Loewi established the existence and the role of ChEs (Augustinsson 1948), Stedman et al. (1932) and Mendel and Rudney (1943) described the "true," or "specific" or rbc, ChE and the "pseudo," or serum, ChE; today, these two forms are referred to as AChE and butyryl ChE (BuChE; see Karczmar 1967, 1970). Actually, we deal here with families of genetically diverse enzymes and isozymes rather than with two enzymes, as first demonstrated with respect to BuChEs by Werner Kalow (1959).

AChE is the synaptic enzyme, the rapidity of its action making the transmission possible. On the other hand, BuChE participates in the function of the smooth muscle, whether of the intestine (Koelle 1963) or of the trachea (Adler and Filbert 1990). BuChE probably does not control the pertinent transmission but acts as a "safety valve" for the parasympathetic system (Adler and Filbert 1990). These enzymes are also present at nonneuronal sites, sometimes at high concentrations, including the formed elements of blood and ephemeral organs, such as the placenta.

Further work with, particularly, organophosphorus anti-ChEs (see the section Anticholinesterases) and the analysis of the aminoacids abutting immediately on the phosphorylation sites of AChE treated with these drugs led to establishing the role of serine and imidazole noiety in the catalytic function of AChE (Usdin 1970). The subsequent research culminated with the description by Hermona Soreq and her associates of the human genome involved in the synthesis of several forms of AChE and BuChE (Soreq and Zakut 1990) and with Jean Massoulie's definition of physically and bindingwise different variants of AChE (Massoulie and Bon 1982; Sussman et al. 1991). As discussed at the VIIIth ICS by Hermona Soreq (Cuello 1993) the human genes for AChE and BuChE were mapped to chromosomes 22 and 26, respectively; these sites are subject to evolutionary and environmental mutagenesis, via exposure to anti-ChEs, and as result of such diseases as leukemia and motor activity (Gissiger and his associates in Cuello 1993). The six or more molecular forms of AChE distinguished by Jean Massoulie and his associates (Massoulie et al. 1993a, b) are generated by the H and T subunits, and they include symmetric, globular, whether amphiphilic or nonamphiphilic, forms on the one hand and asymmetric, collagen tailed forms on the other; these forms differ with regard to their cellular location and attachment to the cell membranes. Furthermore, Massoulie and Sussman (Sussman et al. 1991; Massoulie et al. 1993b) combined this approach with crystalographic determination to propose that there is within AChE "a deep and narrow gorge . . . for binding and hydrolyzing ACh . . . lined with the rings of 14 aromatic aminoacid residues," the quaternary group of ACh ligating the indole ring and the active site for hydrolysis of ACh being a serine-histamine-glutamate triad, a conclusion not too different from that reached in the 1960s. Finally, Massoulie found that anti-ChEs produce specific conformational changes in the gorge.

It became apparent in the 1960s that the classical notion of the unique association of AChE with the termination of cholinergic transmission may not be correct, as suggested by the presence of ChEs in nonneuronal tissues, including the preneurogenetic embryo (Koelle 1963; Karczmar 1963a; see also the section Cholinergic Ontogeny and Teratology). A relevant evidence concerns the novel phenomenon of the release of AChE that occurs upon physiological or presynaptic stimulation not only from cholinergic but also from the noncholinergic brain sites and the finding that exogenously applied AChE hyperpolarizes the dopaminergic neurons, acts on the auto- and heteroreceptive nerve terminal sites, and exhibits postsynaptic effects (Appleyard 1992).

Anticholinesterases

Currently, derivatives of physostigmine and other anti-ChEs are among most the widely used drugs, whether clinically (see the section Clinical Aspects) or in pharmacological analysis.

Following its purification, the structure of physostigmine was established in the 1910s and 1920s by Polonowski with Nitzburg and Stedman with Barger (Karczmar 1970; Holmstedt 1972). Following the synthesis of physostigmine, neostigmine, and some of their analogs by my late Oak Park, Illinois, neighbor, Percy Julian (see Karczmar 1970), countless derivatives of this carbamate anti-ChE were obtained, including many bisquaternaries, such as the very potent oxamides, and "simplified" compounds such as the hydroxyaniliniums (represented by edrophonium, a diagnostic agent for

myasthenia; see Long 1963). Although the duration of anti-ChE action of these compounds varies greatly, from just minutes or under 1 hour in the case of edrophonium and physostigmine, respectively, and several hours in that of such oxamides as ambenonium, all these carbamate and related compounds are classified together as reversible anti-ChEs; in their case, spontaneous or enzymic hydrolysis yields an unchanged inhibitor and unchanged ChE. (For the new reversible anti-ChEs, see the section Clinical Aspects.)

Organophosphorus (OP) anti-ChEs were first synthesized in the middle of the last century, in France and Russia, as De Clermont and Moschnine developed tetraethylpyrophosphate (Holmstedt 1959, 1963; Karczmar 1970). Subsequent, vigorous synthesis of a multitude of OP drugs was carried out in Germany, as their toxic activity against parasites as well as animals was observed by Lange, Schrader, Krueger, and other German investigators (Holmstedt 1963). Their war gas potential was exploited at a large factory complex in Duhernfurt, East Germany, and Koelle (1981) contends that the negotiations after the Second World War between Churchill and Roosevelt on the one hand and Stalin on the other as to the exact position of the border between Poland and East Germany were long and difficult because Duhernfurt and its war gas factory became the bone of contention.

As the German war gas effort became known to the Allies in the 1930s, parallel research was initiated and carried out by the British team led by Lord Adrian and Saunders at Cambridge and elsewhere and the USA team at Edgewood Arsenal. This effort provided training to many prominent investigators such as, in the case of USA, Koelle, Gilman, Riker, Bodansky, Comroe and Wills (Koelle and Gilman 1949; Karczmar 1970). Among the well-known war gases that resulted from the German and Allied war effort are Tabun, Soman, and Sarin; the drug most frequently used in basic research is diisopropyl fluorophosphonate (DFP).

Contrary to the reversible inhibitors, the OP anti-ChEs cause, following "aging," that is, the allosteric change in the combination product, irreversible inhibition; indeed, the original substances, that is, the OP drug and the ChE, cannot be recovered after "aging." Limitless substitution are possible with respect to the parent substance, the phosphoric acid. These substitutions include inorganic ions such as halogens, and organic radicals such as CN; furthermore, sulfur may be substituted for the oxygen of the phosporic acid moiety (Holmstedt 1959, 1963). The substituted compounds include numerous insecticides such as ethoxy-4-nitrophenoxy-phenyl-phosphine sulfide (EPN), anthelminthics, and drugs potentially useful in SDAT therapy, such as metriphonate.

The anti-ChE effect depends on the reaction of the catalytic or esteratic site of ChE, which includes serine and one or more hydrophobic sites (Ishihara et al. 1991) with carbamate or related moiety of the reversible inhibitors and its phosphorylation in the case of the OP compounds. Quaternary grouping, when present, helps in the ligand action, as it reacts with the anionic site of the ChE molecule. Anti-ChEs differ with respect to their potency as inhibitors of AChE and BuChEs; this difference may be quite extensive, as in the case of the oxamide, ambenonium, and DFP, which inhibit preponderantly AChE and BuChE, respectively. Yet, even these compounds are not entirely specific, and most anti-ChEs exert significant actions on both enzymes. Furthermore, the anti-ChEs differ in their effects on the subtypes of ChEs that we described, and the mechanisms underlying these differences are not clear.

Originally, it was thought that particularly OP anti-ChEs, such as DFP, exert effects solely dependent on their inhibition of ChEs and the resulting accumulation of ACh; initially, these effects lead to the facilitation of cholinergic transmission sites, and then undue ACh accumulation blocks the sites, whether by "clogging" or desensitization (see the section Interactions, Modulations, and Gene Expression). This latter action results in anti-ChE toxicity, particularly respiratory failure (due to both central and peripheral block of the pertinent cholinergic transmission) and cardiovascular collapse. Yet, "direct" actions of anti-ChEs were already described in the 1940s (Karczmar 1967, 1970). Today, convincing evidence shows that anti-ChEs, particularly of the OP type, exert a number of "direct" postsynaptic actions on the receptor-channel macromolecule and/or its channel component (Albuquerque et al. 1984) and on carbohydrate metabolism; they also cause morphological, pathological (including muscle myopathies and neurotoxicity), and teratological actions (Karczmar 1984, 1985; see also the subsection Cholinergic Ontogeny and Teratology).

The delayed neurotoxicity, noticed for the first time in 1896 (Davies 1963), affects both peripheral and central axons, such as those of the selected spinal and supraspinal fasciculi; it is followed by demyelination. Many OP agents (such as Mipafox and DFP), exert this effect at large doses; phosphocreosote and triortho-tolyl phosphate (TOCP) are particularly neurotoxic, although they have a relatively weak anti-ChE action; their effect seems to be linked with the inhibition of a poorly defined enzyme, referred to as neurotoxic esterase (Johnson 1987; Abou-Donia and Lapadoula 1990).

Of particular interest are the delayed or chronic behavioral and electroencephalogram (EEG) actions (recorded in animals as well as in man) in industrial workers exposed to OP agents or volunteers used in the 1940s in pertinent research; these include nightmares and mood changes that persist for months or years (see Karczmar 1984).

The antidoting of the anti-ChE toxicity is important in view of the worldwide use of OP drugs as anthelminthics and insecticides, and their potential use as war

gases. Today, a combined prophylactic or antidotal treatment is employed that includes atropine or other atropinics to relieve or prevent central symptoms, including respiratory failure, reversible anti-ChE compounds that protect ChEs, and the oximes that work as reactivators of phosphorylated AChE, provided the latter did not have the time to "age." These interesting compounds, developed in the 1950s by Irving Wilson and David Nachmansohn (Karczmar 1970) force the release of the phosphoryl moiety of the blocked enzyme; the most potent oximes are quaternary in nature, hence they cannot antagonize the central OP toxicity, the newer tertiary oximes being of doubtful efficacy.

Release of ACh

The release of ACh was demonstrated in the 1930s by Dale, Feldberg, and Vogt (Feldberg 1987; Karczmar 1967) for peripheral cholinergic synapses; this constituted an important component of the proof of the cholinergicity of the pertinent synapses. It is very difficult to demonstrate the release of ACh from specific central synapses; using an ingenious microdialysis procedure to measure ACh outflow from selected brain area, Giancarlo Pepeu (Pepeu et al. 1990; Pepeu, 1993) came as near to this demonstration as possible.

The Ca^{++} dependence of ACh release at the periphery was already referred to; the validity of this dependence in the CNS was confirmed subsequently by Pepeu and others. The activation of protein kinase C, which abounds in the nerve terminals, appears to be necessary for ACh release via its regulation of Ca^{++} channels (Kaczmarek 1987). Several specific proteins present in the vesicles, such as synaptobrevin, and the presynaptic plasma membrane may contribute to the release via targeting the vesicles at the release sites and via other mechanisms (Betz 1992). Another protein that may be involved is synapsin; Paul Greengard and his associates (Greengard 1987) presented evidence that this protein regulates, via phosphorylations and dephosphorylations and additional second messenger mechanisms, ACh release; a specific non-ACh transmitter may be involved in the synapsin activation, although this and other aspects of Greengard's hypothesis are controversial (Karczmar 1990). Other proteins, the so-called neurotransmitter transporters that are involved in blocking neurotransmitter uptake, were identified for GABA and catecholamines. Although some reuptake of ACh occurs, this is minimal compared to the uptake of choline, and transporters for either ACh or choline have not been identified as yet.

The vesicular hypothesis of the release of ACh, based on the concepts of Katz, Miledi, DeRobertis, and, particularly, Victor Whittaker, was already described. Is this hypothesis an absolute tenet today? Actually, the cytoplasmic, vesicle-independent release of ACh was well documented by Maurice Israel, Yves Dunant, and N. Morel; they used the elegant, ultrarapid and ultrasensitive choline oxidase chemoluminescent method for ACh measurement, as well as cytoplasmal-proteoliposomal membrane complexes endowed with a "reconstituted ACh release mechanism" (Israel and Morel 1990; see however, Whittaker 1992). This release is mediated by a nerve terminal proteolipid, the mediatophore; the Kd subunit of the mediatophore was completely sequenced. The essential features of the non-vesicular release of ACh, such as Ca^{++} dependence, were preserved in the proteoliposomal model as well as in the oocyte loaded with mediatophore mRNA and the K_d protein. Furthermore, as shown at the VIIIth ICS by Dunant, Cavalli, and their associates (Cuello 1993), in the primed oocyte the rate of ACh release and the expression of the mediatophore were decreased in parallel by the antisense probes.

The auto- and heteroreceptor-mediated regulation of ACh release was first described in the 1950s. Koelle, Nishi, Szerb, and Polak (Karczmar 1990; Schuetze and Role 1987) demonstrated muscarinic and nicotinic effects on cholinergic nerve terminals and ACh release in the ganglia and CNS that are mediated by cholinergic muscarinic and nicotinic presynaptic autoreceptors; these findings were confirmed recently by means of appropriate antibodies (see for example, Dunant and Cavalli in Cuello 1993). The heteroregulation by ACh and cholinomimetics of the release of nonACh transmitters, posited first by Loffelholz and Muscholl (Loffelholz et al. 1967) for noradrenergic transmission, obtains as well for other transmitters. Whether in the case of the auto- or heteroregulation, both facilitatory and inhibitory mechanisms may occur, although the latter are more frequent.

The reverse takes place as well, as shown for the first time by Amadeo Marrazzi (Karczmar 1967) with respect to catecholamines. Besides catecholamines, indoleamine, aminoacid, and polypeptide transmitters—including galanin and its analogs (see Barfai and Consolo in Cuello 1993)—as well as prostaglandins and, as shown by Remi Quirion at the VIIIth ICS (Cuello 1993), interleukins affect the release of ACh. Again, inhibition of release is the prevalent phenomenon; opposite effects may occur at different sites and at different concentrations of the agents in question, as shown at the VIIIth ICS by Shirati, Consolo, and their colleagues (Cuello 1993).

As is well known, neurotoxins derived from various sources, such as amphibian skin, snake venoms, insects, and bacteria, also affect ACh release. These proteins include the botulinum (clostridial) toxin that inhibits the ACh release and black spider venom that causes a massive release of ACh. These substances act not on the cholinergic nerve terminal receptors but on receptors concerned with Ca^{++} fluxes, microtubule

systems of the terminals, and/or the synaptosomal and vesicular membranes (Dolly 1993).

Central Cholinergic Pathways

Dale and Feldberg (Feldberg 1945) postulated the existence of central cholinergic pathways long before the Ecclesian demonstration of a central cholinergic synapse. Further development of this concept occurred when Curtis, Crawford, Krnjevic, Bradley, Himwich, and others demonstrated cholinoceptivity of specific CNS sites and, particularly, when George Koelle developed the histochemical staining method for AChE and applied it for the localization of AChE in the CNS (Koelle 1963). His work, that of Gerebtzoff (Koelle, o. c.), and of Shute and Lewis (1963) led to the concepts of the ascending cholinergic reticular system, cholinergic pathways to the hypothalamus, and the cholinergic limbic system.

CAT constitutes a better marker of cholinergic synapses and pathways than AChE, and this marking became possible with the development of immunochemical staining for CAT by Henry Kimura and the McGeers (McGeer et al. 1987). The use of this method by the McGeers, Butcher, Mesulam, and others, the employment of markers for cholinergic receptors, and the appropriate use of lesions led to the delineation of the important central cholinergic pathways. These pathways emanate from the medial forebrain cholinergic systems and radiate to the limbic system, cortex, and the hypothalamus; they include medioseptal nuclei and pathways, nuclei of the diagonal band of Broca, nucleus basalis of Meynert, medial habenular nuclei, and the neopontine tegmental tegmental nuclei. In addition, cholinergic networks are located in the retina and basal ganglia; presumably, these networks are independent of the cholinergic radiations described above. Less defined are the descending cholinergic pathways and the spinal circuitry concerning the cholinergic preganglionic and motor neurons (Karczmar 1990).

The employment of novel methodology, such as the localization of the mRNA for CAT and positron-emission tomography (PET) of radiolabeled ligands of pertinent proteins (Butcher et al., in Cuello, 1993) essentially confirmed this description of pathways. As already stressed, the pathways in question abut on both muscarinic and nicotinic sites, and the central distribution of these receptors begins to be defined (see the section Receptors).

Central Cholinergic Functions, Behaviors, and EEG

As already indicated, the cholinergic correlates of behavior were described long before the demonstration of cholinergic transmission: Christison in 1885 self-administered the calabar bean at, actually, a toxic dose (he lived, however, to describe vividly the mental and functional effects of the ingestion), and Bezold and Gotz in 1867 and others described the respiratory and convulsive effects of either the purified bean extract or physostigmine (Karczmar 1970; Holmstedt 1972). In the 1940s studies of conditioning (Funderburk and Case 1947), and multiple other functions were initiated, including those dependent on the hypothalamus, such as appetitive effects and thermocontrol (Myers 1974). Subsequently, Himwich, Bovet, Oliverio, Jouvet, Deutsch, Drachman, Stein, Russel, and others demonstrated the effects of atropinics, anti-ChEs, and cholinergic agonists and antagonists (whether given directly into localized CNS sites or given systemically in the case of drugs capable of crossing the blood-brain barrier) on learning and memory, arousal and sleep, nociception, aggression, fear, addiction, and reward-punishment behavior (Waser 1975; Karczmar 1967, 1970, 1981, 1990; Hintgen and Aprison 1976). The cholinergic correlates of these functions and behaviors were established not only by the nature of the drugs employed but also by the presence of the cholinergic pathways in the pertinent brain areas and the demonstration of the release of ACh following either the stimulation of these pathways or the evocation of these functions (Pepeu 1993).

Particularly interesting (see the section Clinical Aspects) was the demonstration that anti-ChEs and cholinergic agonists facilitate a number of conditioning paradigms while atropinics block learning, conditioning, and memory, both in animals and man; thus, Drachman (1978) showed quantitatively that, in man, atropinic amnesia is a close equivalent of senescent amnesia.

It must be stressed that no behavior is a one-transmitter affair, and catecholaminergic, serotonergic, and other systems participate in the functions listed; yet, frequently, the cholinergic system constitutes the significant correlate. This is true for learning, memory, and aggression; there are many forms of aggression, such as predatory and emotional aggression, and the aggression related to the defense of the pups or habitat, and so on, and certain transmitters affect one or another of these aggressions, yet only the cholinergic system regulates all these forms (Karczmar 1978; Eichelman 1990). Altogether, there is no measurable animal or human behavior that does not exhibit cholinergic correlates.

The EEG and related phenomena merit special comment. That the cholinergic system evokes EEG desynchronization and contributes significantly to the Magun–Moruzzi EEG and behavioral arousal and to the function of the reticular formation was demonstrated in the 1950s by Wescoe, Bremer, Chatonnet, Himwich, Rinaldi, Domino, Longo, and others (Karczmar 1967). Yet, subsequent research of the Killams, Bures, Gangloff, and, particularly, Abraham Wikler (Karczmar 1967)

showed that the cholinergic EEG arousal was not accompanied by behavioral arousal, there being a "divorce" between these two phenomena. This paradox was resolved, as it became apparent via the power spectrum analysis of the EEG that the cholinergic EEG arousal differs from the EEG arousal accompanying behavioral wakefulness (Karczmar 1979).

A special EEG-behavior relation concerns the rapid-eye movement (REM) sleep. In the 1960s, Hernandez-Peon and Michel Jouvet demonstrated cholinergic contribution to both the slow-wave and the paradoxical or REM sleep. Although Jouvet is known primarily for relating the norepinephrine-serotonin dipole and its anatomical equivalents, the locus ceruleus and the Raphe nuclei, to the slow-wave sleep–REM sleep system, he also demonstrated early that atropine blocks the REM sleep (Jouvet 1972). The cholinergicity of the REM sleep became clearer with the researches of Longo and his colleagues (Karczmar et al. 1970) and of Hobson, Steriade, Baghdoyan, McCorley, and their associates (Steriade et al. 1992). This research showed that the REM sleep may be evoked after the depletion of brain catecholamines and that it is generated by the cholinoceptive brainstem neurons. As shown by Gillin and his associates (Velazquez-Moctezuma et al. 1992), analogous cholinergic phenomena obtain in man, and the pharmacologic profiles of man and animal REM sleep are similar, in both cases M2 receptor being involved (Gillin et al. 1993). At the VIIIth ICS Allan Hobson and his associates (Cuello 1993) added a refinement to the matter, as they demonstrated that the cholinergic anterodorsal pontine tegmentum contributes to an "immediate but short lived REM sleep," whereas the cholinergic peribrachial pons evokes "long term," delayed REM sleep.

Now, the fast EEG and mental function of REM sleep, that is, dreams, seem to relate to alertness rather than sleep phenomena, and in 1971 Karczmar proposed that there is an EEG-behavior continuum that includes REM sleep and its analog generated in the course of wakefulness and that the latter constitutes a general cholinergic cognitive syndrome, the Cholinergic Alert Immobile Behavior (CANMB; Karczmar 1979, 1990), which includes the learning and memory phenomena. This notion is consistent with those of Allan Hobson, Micea Steriade, and their associates (Cuello 1993) and of McCormick (1992) that the cholinoceptive neurons of the tegmentum and the dorsolateral geniculate control the firing rates and the excitability of brainstem and cortical neurons and their EEG rhythms and that the pacemaker cells in the reticular formation on the one hand and those in the brainstem and basal forebrain neurons on the other constitute a dipole regulating the balance between the REM and slow sleep and between wakefulness and sleep.

Cholinergic Ontogeny and Teratology

In the 1930s such early investigators of the ontogeny of the cholinergic system as Nachmansohn, Youngstrom, and Bacq (Karczmar 1963a) attempted to prove the cholinergicity of the nervous system by demonstrating that AChE appears at the time of the ontogenetic onset of such functions as motility. An unforeseen phenomenon that was discovered at that time was that AChE and, as shown subsequently, ACh and CAT arise, whether in vertebrate or invertebrate ontogenesis, precociously, that is, before neurogenesis (Karczmar 1963a; Buznikov 1984). In fact, cholinergic components may appear in high concentrations in the two-cell stage or, even, in the unfertilized egg. This precocious appearance of the cholinergic components relates to their presence at noncholinergic sites. Taken together, these phenomena suggest that the cholinergic system must play a nontransmittive role, and its trophic role was suggested early (Karczmar 1946, 1963a).

In the course of ontogenesis the components of the cholinergic system increase significantly in concentration or activity at the onset of neurogenesis as well as subsequently; in fact, this increase continues postnatally, at least in animals. Yet, there are periods of diminution of cholinergic components, in parallel with the decrease in the number of nonneuronal cells in the case of the prenervous ontogeny or with the characteristic neuronal "death" that occurs during neurogenesis.

The cholinergic neurogenesis is characterized by a nonparallel development of CAT, ChEs, ACh synthesis, and cholinergic receptor-channel macromolecules (Karczmar 1963a, b; Rotundo 1987; Layer et al. 1987; see, however, Giacobini 1986). Thus, the cholinergic receptors bind early the ligands and yet are "silent" with respect to ACh and cholinomimetics, whereas CAT and ACh precede this event. The advent of their cholinoceptivity occurs prior to their morphological maturity, and, following the initiation of cholinoceptivity, the receptors change their structure, subunit composition, channel characteristics and kinetics, fluidity, and so on, both prenatally and postnatally, as their response assumes mature kinetics (Salpeter 1987; Giacobini 1986; Fambrough 1976).

It is important in this context to note the cholinergic effects on development. Cholinergic agonists and antagonists cause in several animal species embryonic death or teratology; it is interesting that these effects were observed with pilocarpine by Matthews and Sollman as early as in 1902! The teratological effects may range from changes in "body proportions" and in the skeleton to micromelia, syndactilism, and phocomelia (Karczmar 1963b; Karczmar et al. 1973); most of these effects were obtained with anti-ChEs, including the OP drugs.

Only a few data are available about the teratological action of cholinergic agonists or antagonists in man. Controversial evidence was presented in the course of legal proceedings with respect to antihistaminic drugs that, although they are essentially antihistaminics, exert also atropinic effects; these drugs, such as doxylamine, are used in man as antiemetics or analgesics. Considerable controversy surrounds pertinent studies carried out in primates (Hendrickx et al. 1982; McBride 1985).

Trophic Phenomena

The matter of trophic factors relates to development, as these factors exert their primary role during the latter, and as the precocious ontogenetic presence of the cholinergic system may reflect its trophic function. A related speculation arose with respect to the nerve-dependent regeneration of cut limbs of urodeles (Karczmar 1946). Today, this kind of trophic effect would be termed *anterograde*, resulting from the forward movement, target-oriented, of a nerve-derived trophic substance (Hendry 1976). Another speculation presented by Cajal (1913) concerned the dependence of growing or regenerating nerve axons on a target-derived substance or, as suggested by Victor Hamburger (1952), the "remote milieu"; we deal, in this case, with a "retrograde" trophic action (Hendry 1976). Ultimately, Rita Levi-Montalcini, appropriately Victor Hamburger's student, demonstrated the presence and the trophic function of the NGF and pioneered the efforts leading to the purification and clarification of its polypeptide structure (Levi-Montalcini and Angeletti 1968). The presence of NGF was first established for mouse sarcoma, submaxillary gland, and glia. Although its function was first established with respect to the sympathetic ganglia, its presence in the CNS and its role in the development and maintenance of the cholinergic CNS were subsequently established by Hans Thoenen and his associates (1987). An important advance was made when it was shown that NGF and certain other neurotrophins antagonize the cholinergic neuron damage caused by appropriate lesions, whether surgical or chemical, in adult animals; this involves morphological changes, including nerve terminal recovery, as shown by Cuello and his associates (Cuello 1993), as well as the augmentation of choline uptake, CAT activity, AChE and ACh levels, and the activation of the phosphatidyl inositol cascade (see the results reported at the VIIIth ICS by Cuello, Hefti, and Williams in Cuello 1993). Other trophic factors, such as glycyl-l-glutamine, may exert trophic effects on specific components of the cholinergic system, such as AChE (Koelle 1988).

Some 25 trophic factors are recognized today; they range, chemically, from proteins and polypeptides to gangliosides, such as MM-1, and their relatively simple components, such as sialic acid, and to acetyl-L-carnitine; second messengers, including cAMP, may also exhibit trophic effects or act by stimulating the synthesis of trophins. The trophic actions are exerted via neurotrophin receptors that are specific for the factors, as it was demonstrated for NGF, ciliary neurotrophic factor (CNTP), and the brain-derived neurotrophic factor (BDNF; see, for example, Kaplan et al. 1991).

Some seven retrograde-acting trophins exert an effect on the cholinergic system, CAT, and AChE; some five have a possible effect, and many more were not studied with respect to the cholinergic system (Karczmar 1990). Furthermore, NGF and other trophins exert actions, primarily or secondarily, on neurons other than cholinergic neurons. Finally, cholinergic neurons may exert anterograde trophic action on other neurons or nonnervous cells, possibly by activating second messengers endowed with trophic action; in fact, ACh itself may exert such an action.

Significant progress was made in the molecular biology and biosynthesis of NGF and its interaction with the second messengers (Mocchetti 1991). Thus, its transcription was preliminarily characterized, and it was shown that protein kinases A and C, steroids, thyroxin, and certain hypothalamic hormones may induce NGF expression; it is of interest that neuronal activity, particularly at the beta adrenergic receptor, may exert a similar effect (Mocchetti 1991).

As the trophic phenomena apparently are not restricted to ontogenesis, they contribute to brain plasticity—a novel concept, replacing that of nonproliferation, stability, and degeneration of adult neurons. This property is exploited in Sweden and Italy in the treatment in man of neurodegenerative conditions, including SDAT, by means of trophic factors and/or gangliosides (see the section Clinical Aspects).

Interactions, Modulations, and Gene Expression

A cholinergic system does not exist in a vacuum; it is subject to interactions with and modulations by other bioactive, endogenous substances. It is easy to illustrate this interaction by demonstrating that cholinergic agonists and antagonists affect the levels and turnover of other transmitters, including catecholamine, indole amines, and aminoacids (Karczmar 1978). This should be expected, as the cholinergic radiations abut on the other transmitter systems, and it was shown early by Eccles (1963) that such couplings result in vectoral interactions between the pertinent responses, such as the E and I potentials. Thus, it was shown directly for the nigrostriatal and brainstem areas that specific excitation of cholinergic neurons affects catecholaminergic or GABAeric neurons of these areas (Straughan and James 1979). That every central function or behavior is affected

by several transmitter systems and their agonists and antagonists also illustrates this notion (Karczmar 1978).

Modulations constitute a more subtle example of the interaction between the cholinergic and other systems. The term *modulation*, defined first by Kyo Koketsu, Les Blaber, and this author (Karczmar et al. 1972; Karczmar 1990; Akasu et al. 1981), includes pre- and postsynaptic modulatory phenomena. Thus, ACh release may be modified via hetero- and autoreceptors present at the cholinergic nerve terminals (see the section Release of ACh); this results in a change in the postsynaptic response, decrease being the more common phenomenon.

Then, the postsynaptic cholinoceptive response may be modulated by a number of endogenous substances (such as ATP, certain polypeptides, histamine and 5-HTP) and certain drugs (such as the oxamide WIN8078, Prednisolone, and NaF). These drugs modulate through either their effect on the membrane potential or by changing the postsynaptic sensitivity without affecting the potential. The early example of this effect was provided for the neuromyal junction by Stephen Thesleff (1955) and Karczmar and Howard (1955). The effect in question was desensitization or receptor inactivation arising from prolonged application of depolarizers, including ACh, to the motor endplate. The opposite process is that of sensitization (Karczmar and Howard 1955; Karczmar 1957, 1987). Both processes are due to an allosteric receptor change (Akasu et al. 1981); in the case of desensitization, this change may be mediated by the activation of one or more kinases by a noncholinergic transmitter (Greengard 1987; see, however, Colquhoun et al. 1990).

The drugs acting as postsynaptic modulators, such as NaF and the oxamide WIN8078, facilitate transmission, including that of OP-bound synapses, even after the process of aging has intervened, and, uniquely, antagonize both the competitive and depolarizing neuromyal blockers (Karczmar 1957).

Postsynaptic modulation may also result from the so-called transsynaptic regulation, that is, postsynaptic activation of protein synthesis. This concept dates to the demonstration by Holger Hyden (1972) of learning-dependent activation of specific mRNAs and proteins in pertinent brain parts; subsequently, this process was shown to occur upon presynaptic stimulation (Axelrod 1971; Costa and Guidotti 1978). Perhaps the ultimate expression of transsynaptic regulation and the resulting modulation of postsynaptic responses is constituted by genetic induction; Hyden mentioned this phenomenon, without being able to demonstrate it directly, to explain his findings. The genetic induction may take form of either immediate, early, or late gene expression (Menetrey et al. 1989) and may be generated by transmitters and physiologic stimuli (Shen et al. 1992).

Clinical Aspects

As already alluded to, cholinergic or anticholinergic therapy is employed quite extensively today in a number of conditions, and only a few additional comments will be made now. The ophthalmic use of cholinergic agonists in glaucoma dates from the experimentation of the Edinburgh team; today, beta blockers appear to be more useful in this condition. Another early example is the use of anti-ChEs in myasthenia gravis. It must be stressed that, although the demonstration of the autoimmune character of this disease (Patrick and Lindstrom 1973) led to the effective employment of antiinflammatory and antibodal therapy of this condition, anti-ChEs are still used frequently in the initial phase of myasthenia. Another use of cholinergic drugs that has been abandoned is that of atropine in parkinsonian disease and of atropine coma in certain forms of depression. In view of the availability today of effective antagonists of atropine coma that renders this therapy relatively safe, and as, today, electroshock therapy seems to return to fashion, this treatment perhaps should be considered again.

There are several novel, experimental uses of cholinergic drugs today, including the use of certain neurotoxins in dystonias and atonias (Hirsch and Dougherty, in Cuello 1993; Jenden, 1990), of cholinergic precursors or anti-ChEs in anosmia, and of cholinergic agonists and anti-ChEs as antagonists of toxicity resulting from the overdose of tricyclic antidepressants; they are also used to shorten the recovery from anesthesia and in paralytic ileus. Then, there may be some exploitation of the cholinergic correlate of schizophrenia, although the nature of this correlate, negative or positive, is controversial (compare Tandon et al. in Cuello 1993; Levin et al. 1990; and Karczmar 1988).

Particularly intense at this time is the experimental use of cholinergic drugs, including muscarinic agonists, precursors, and, particularly, anti-ChEs, in SDAT. This use is based on the so-called cholinergic hypothesis of SDAT that originated with the findings of the Perrys and of Whitehouse (Whitehouse 1981) of the loss in SDAT patients of the cholinergic neurons of the forebrain and the resulting loss of CAT; this loss was consistent with the memory impairment that is characteristic of SDAT, as well as with the strong cholinergic correlate of learning and memory (see the section Central Cholinergic Functions). Following the use of physostigmine in SDAT (Peters and Levin 1979; Thal 1991) and as its effectiveness appeared dubious, the second generation of anti-ChEs were developed. These include physostigmine and pyridine derivatives, such as heptylphysostigmine and the huperzines; tetrahydroaminoacridine (tacrine, THA), today the most intensely used anti-ChE; and even OP drugs, such as metrifonate (Giacobini 1991). It is interesting that, like physostig-

mine, the huperzines are naturally occurring compounds, their source being the Chinese clubmoss (Giacobini 1991; Hanin et al. 1991). The aim was to obtain compounds with longer action, better penetration into the CNS, and more specific anti-AChE action, and this aim persists, as still newer anti-ChEs are being developed, such as the Sandoz drugs.

Some investigators contend that anti-ChEs delay the progress of SDAT; however, the consensus seems to be that, even with the newest anti-ChEs the results are limited: Certain aspects of memory seem to benefit to a limited degree, and there is little if any restoration of other aspects of cognition and social, domestic, or professional function. Theoretically, several reasons may underlie this situation. First, anti-ChEs facilitate the function of existing cholinergic neurons; yet, in late SDAT very few such neurons may remain intact; second, anti-ChEs exhibit many cholinergic and noncholinergic effects that are counterproductive in this context, the "therapeutic window" of anti-ChE therapy is narrow, and centrally effective doses of these compounds may not be achievable as they would produce untoward side effects (Karczmar and Dun 1988). More importantly, the nature of SDAT as perceived today should limit the expectations of success that may be achieved with anti-ChEs. In a nutshell, SDAT appears to be a multifactorial condition that embraces diffuse degenerative changes of the neuronal cytoskeleton, including undue beta-amyloid synthesis and formation of senile plaques, neurofibrillatory tangles and heavy protein; these changes affect cholinergic and noncholinergic neurons and, besides ACh, other transmitters and peptides. The related problem is that many animal models used today concern more the "cholinergic hypothesis" of SDAT than the actual disease, as these models rely on chemical or surgical damage inflicted on the cholinergic neurons; in fact, these models differ from SDAT in several respects (Karczmar 1991).

As any area of the cholinergic field, this particular area is not devoid of controversies. Contrary to the sense of the views described, there is sporadic information that some of the cholinergic animal models of SDAT may exhibit abnormal amyloid formation (Wallace et al. 1991); also, Richard Wurtman and his associates (Nitsch et al. 1992a) claim that cholinergic therapy may prevent the formation of amyloid depositions. Altogether, it is this author's present belief that the animal and tissue culture models that rely on molecular engineering or other means to evoke the pertinent cytoskeleton changes (see, for example, Price 1993) appear to be more appropriate in the present context than the models based on the cholinergic hypothesis of SDAT. It must be noted that the arguments raised here militate not only against the effectiveness in SDAT of anti-ChEs but against the effectiveness of cholinergic agonists and precursors as well; yet considerable development of these compounds continues, as discussed at the VIIIth ICS (see for example, Davis 1993).

It must be stressed that the story of the clinical use and effectiveness of cholinergic and anticholinergic drugs has barely begun. In this context, certain issues raised by Don Jenden (1990) are pertinent. Jenden stresses that, as many subtypes of cholinergic receptors are being identified and their localization is becoming better known, appropriate synthesis of specific receptor agonists and antagonists will yield better therapeutic agents that would, it is hoped, be devoid of deleterious side actions; as important, the interplay between intrinsic efficacy and tissue distribution of the compounds in question will dictate the specificity and organ or tissue localization of these compounds, hence, the therapeutic specificity of their action.

ENVOI

The cholinergic studies that brought to us so many crucial findings in the past, will indubitably bring new, no less crucial findings in the future. These will lead to a better understanding of the second messenger and G proteins cascade as activated by the cholinergic system, and of its relationship to specific, cholinergically engendered currents; better definition of and addition to the present list of the nicotinic and muscarinic receptors; clarification of the interaction between the cholinergic and other systems and of the modulatory and early gene processes; further identification of trophic factors and their role in the ontogenesis and maintenance of the cholinergic system; increased availability of new synthetic therapeutic agents; and, above all, specific descriptions of the molecular biology of the components of the cholinergic system and of its ontogeny. However, to use the facts related to these and other factors for the "prediction of the postsynaptic . . . cholinergic . . . outcome . . . requires a computer program which does not begin to exist"; predicting the "ultimate . . . behavioral outcome may be beyond any potential computer capacity" (Karczmar 1993), as the system in question is complex enough to be, on the basis of Goedel's theorem and the theory of chaos, never fully consistent and never predictable.

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REFERENCES

Abou-Donia MB, Lapadula DM (1990): Mechanisms of organophosphorus ester-induced neurotoxicity: Type I and Type II. Annu Rev Pharmacol Toxicol 30:405–440

Adams PR, Brown DA (1982): Synaptic inhibition of the M-current: Slow excitatory synaptic potential mechanism in bullfrog sympathetic neurones. J Physiol (Lond) 332:263–272

Adler M, Filbert MG (1990): Role of butyrylcholinesterase in canine tracheal smooth muscle function. FEBS Lett 267:107–110

Akasu T, Hirai K, Koketsu K (1981): Increase in acetylcholine-receptor sensitivity by adenosine triphosphate: A novel action of ATP on ACh-sensitivity. Br J Pharmacol 74:505–507

Albuquerque EX, Akaike A, Shaw, K-P, Rickett DL (1984): The interaction of anticholinesterase agents with the acetylcholine receptor-ionic channel complex. Fund Appl Toxicol 4:S27–S33

Ansell GB, Spanner S (1979): Sources of choline for acetylcholine synthesis in the brain. In Barbeau A, Growdon JH, Wurtman RJ, (eds), Choline and Lecithin in Brain Disorders. New York, Raven Press, pp 35–56

Appleyard M (1992): Secreted acetylcholinesterase: Non-classical aspects of a classical enzyme. Trends Neurosci 15:485–490

Ariens EJ (1960): Ph.D. Dissertation. Utrecht, Netherlands, University of Utrecht

Augustinsson K-B (1948): Cholinesterases: A study in comparative anatomy. Acta Physiol Scand 15 S52:1–182

Axelrod J (1971): Noradrenaline: Fate of control of its biosynthesis. Science 173:598–606

Barnard EA, Beeson DM, Cockroft, VB, Darlison, MG, Hicks AA, Lai FA, Moss SJ, Squire, MD (1987): Molecular biology of nicotinic acetylcholine receptors from chicken muscle and brain. In Dowdall MJ, Hawthorne NJ, (eds), Cellular and Molecular Basis of Cholinergic Function. Chichester, England, Ellis Horwood, pp 15–32

Barron DH, Matthews BHC (1936): Electrotonus in ventral roots of the spinal cord. J Physiol (Lond) 87:26P–27P

Berridge MJ (1987): Inositol triphosphate and diacylglycerol: Two interacting second messengers. Annu Rev Biochem 56:159–193

Betz H (1992): Transmitter release from nerve terminals: A Ca^{++-} regulated, multiprotein process. Neurosci Facts 3:2–3

Birdsall HJ, Hulme EC (1983): Muscarinic receptor subclasses. Trends Pharmacol Sci 4:459–463

Birks R, MacIntosh FC (1961): Acetylcholine metabolism of a sympathetic ganglion. Can J of Biochem Physiol 39:787–827

Blusztajn JK, Schuller U, Venturini A, Jackson DA, Lee HJ, Wainer BH (1993): Stimulatory effects of cAMP and retinoic acid on acetylcholine synthesis in murine septal cell line are additive. In Cuello AC, (ed), Cholinergic Neurotransmission: Function and Dysfunction. Amsterdam, Elsevier (In press)

Bradley PB, Wolstencroft JH (1962): Excitation and inhibition of brain-stem neurones by noradrenaline and acetylcholine. Nature 196:840–873

Brooks MMcC, Eccles JC (1947): Electrical investigation of monosynaptic pathway through the spinal cord. J Neurophysiol 10:251–274

Brown JH (ed) (1989): The Muscarinic Receptors. NJ, Humana Press

Brown JH, Masters SB (1986): Differential effects of carbachol and oxotremorine on muscarinic receptors, cyclic AMP formation, and phosphoinositide turnover in chick heart muscle. In Hanin I (ed), Dynamics of Cholinergic Function. New York, Plenum Press, pp 939–946

Browning ET (1986): Acetylcholine synthesis: Substrate availability and synthetic reaction. In Goldberg AM, Hanin I (eds), Biology of Cholinergic Function. New York, Raven Press, pp 187–201

Buznikov GA (1984): The actions of neurotransmitters and related substances on early embrogenesis. Pharmacol Ther 25:23–59

Cajal S, Ramon Y (1913): Estudios Sobre la Degeneration y Regeneracion del Sistema Nervioso. Madrid, N. Moya

Chagas C, Paes De Carvalho A (eds) (1961): Bioelectrogenesis. Amsterdam, Elsevier

Changeux J-P (1993): Compartmentalized expression of acetylcholine receptor genes during motor-endplate development. Neurosci Facts 4:1–3

Christison R (1885): On the properties of the ordeal bean of old calabar Western Africa. Monthly Journal of Medical Science, London and Edinburgh 20:193–204

Colquhoun D, Cachelin AB, Marshall CG, Mathie A, Ogden DC (1990): Function of nicotinic synapses. In Aquilonius S-M, Gillberg PG (eds), Cholinergic Neurotransmission: Functional and Clinical Aspects. Amsterdam, Elsevier, pp 43–50

Costa E, Guidotti A (1978): Molecular mechanisms mediating the trans-synaptic regulation of gene expression in adrenal medulla. In Lipton MA, Dimascio A, Killam KF (eds), A Generation of Progress. New York, Raven Press, pp 235–246

Cuello AC (ed) (1993): Cholinergic Neurotransmission: Function and Dysfunction, Proceedings of the Eighth International Cholinergic Symposium. Amsterdam, Elsevier

Dahlbom R, Ringdahl B, Resul B, Jenden DJ (1986): Stereoselectivity of some muscarinic and antimuscarinic ganets related to oxotremorine. In Hanin I (ed), Dynamics of Cholinergic Function. New York, Plenum Press, pp 385–394

Dale HH (1914): The action of certain esters and ethers of choline and their relation to muscarine. J Pharmacol Exp Ther 6:147–190

Dale HH (1935): Pharmacology and nerve endings. Proc R Soc Med 28:319–322

Davies DR (1963): Neurotoxicity of organophosphorus compounds. In Koelle GB (ed), Cholinesterases and Anticholinesterase Agents. Berlin, Springer-Verlag, pp 8860–8862

Davis RE (1933): Subtype selective muscarinic agonists: Potential therapeutic agents for Alzheimer's disease. In Cuello AC (ed), Cholinergic Neurotransmission: Function and Dysfunction. Amsterdam, Elsevier (In press)

DeRobertis E (1967): Ultrastructure and cytochemistry of the synaptic region. Science 156:907–914

Dolly, JO (1993): Mechanism of inhibition of transmitter release by Botulinum and Tetanus toxins. Neurosci Facts 4:8

Dorje F, Wess J, Lambrecht G, Tacke R, Muttschler E, Brann MR (1991): Antagonist binding profiles of five cloned human muscarinic receptor subtypes. J Pharmacol Exp Ther 256:727–733

Drachman DA (1978): Central cholinergic system and memory. In Lipton MA, DiMascio A, Killam KF (eds), Psychopharmacology: A Generation of Progress. New York, Raven Press, pp 651–662

Dunlap K, Holz GG, Rane SG (1987): G proteins as regulators of ion channel function. Trends Neurosci 10:241–244

Eccles JC (1963): The Physiology of Synapses. Berlin, Springer-Verlag

Eccles JC (1969): Historical introduction. Fed Proc 28:90–94

Eccles JC (1987): The story of the Renshaw cell. In Dun NJ, Perlman RL (eds), Neurobiology of Acetylcholine, A Symposium in Honor of Alexander G. Karczmar. New York, Plenum Press, pp 189–194

Eccles RM, Libet B (1961): Origin and blockade of the synaptic responses of curarized sympathetic ganglia. J Physiol (Lond) 157:484–503

Eccles JC, Katz B, Koketsu K (1953): Cholinergic and inhibitory synapses in a central nervous pathway. Aust J Sci 16:50–54

Eichelman BS (1990): Neurochemical and psychopharmacological aspects of aggressive behavior. Annu Rev Med 41:149–158

Fambrough DM (1976): Development of cholinergic innervation of skeletal, cardiac, and smooth muscle. In Goldberg AM, Hanin I (eds), Biology of Cholinergic Function. New York, Raven Press, pp 101–160

Fatt P, Katz B (1952): Spontaneous subthreshold activity at motor nerve endings. J Physiol 117:109–128

Feldberg W (1945): Present views on the mode of action of acetylcholine in the central nervous system. Physiol Rev 25:596–642

Feldberg W (1987): On the distribution of cholinergic neurons: personal reminiscences of the thirties. In Dowdell MJ, Hawthorne JN (eds), Cellular and Molecular Basis of Cholinergic Function. Chichester, England, Ellis Horwood, pp 1–10

Fuhner H (1917): Untersuchungen über die periphere Wirkung des Physostigmins. Arch Exp Pathol Pharmakol 82:205–220

Funderburk WH, Case TJ (1947): Effect of parasympathetic drugs on the conditioned responses. J Neurophysiol 10:179–188

Giacobini E (1986): Development of peripheral parasympathetic neurons and synapses. In Gootman PM (ed), Developmental Neurobiology of the Autonomic Nervous System. Clifton, NJ, The Human Press, pp 29–67

Giacobini E (1991): The second generation of cholinesterase inhibitors: Pharmacological aspects. In Becker R, Giacobini E (eds), Cholinergic Basis for Alzheimer Therapy. Boston, Birkhauser, pp 247–262

Gillin JC, Salin-Pascual R, Velazquez-Moctezuma J, Shiromani P, Zoltoski R (1993): Cholinergic receptor subtypes and REM sleep in animals, normal controls, and psychiatric disorders. In Cuello AC (ed), Cholinergic Neurotransmission: Function and Dysfunction. Amsterdam, Elsevier (In press)

Gilman AG (1987): G Proteins: Transducers of receptor-generated signals. Annu Rev Biochem 55:515–649

Greengard P (1987): Neuronal phosphoproteins. Neurobiology 1:81–119

Greenspan RJ (1980): Mutations of choline acetyltransferase and associated neural defects in *Drosophila melanogaster*. Comp Physiol 137:83–92

Hamburger V (1952): Development of the nervous system. Ann NY Acad Sci 55:1178–1132

Hamill OP, Marty A, Neher F, Sakmann B, Sigworth FJ (1981): Improved patch-clamp techniques for high resolution current recording from cells and cell-free membrane patches. Pflugers Arch 391:85–100

Hanin I, Tang Xi Can, Kozikowski AP (1991): Clinical and preclinical studies with huperzine. In Becker R, Giacobini E (eds), Cholinergic Basis for Alzheimer Therapy. Boston, Birkhauser, pp 305–313

Heilbronn E, Winter A (eds) (1970): Drugs and Cholinergic Mechanisms in the CNS. Stockholm, Forvarfets Forksskningsanstalt

Hendrickx AG, Prahalada S, Rowland JM (1982): Embryotoxicity studies on benedectin in Cynomolgus monkeys (*Macaca fascicularis*). Teratology 25:47A

Hendry IA (1976): Control in the development of the vertebrate sympathetic nervous system. Rev Neurosci 2: 149–194

Hintgen JN, Aprison MH (1976): Behavioral and Environmental aspects of the cholinergic system. In Goldberg AM, Hanin I (eds), Biology of Cholinergic Function. New York, Raven Press, pp 515–566

Hodgkin AL, Huxley AF (1952): Currents carried by sodium and chloride ions through the membrane of the giant axon of Loligo. J Physiol (Lond) 116:449–472

Holmstedt B (1959): Pharmacology of organophosphorus cholinesterase inhibitors. Pharmacol Rev 11:567–688

Holmstedt B (1963): Structure-activity relationships of the organophosphorus anticholinesterase agents. In Koelle GB (ed), Cholinesterases and Anticholinesterase Agents. Berlin, Springer-Verlag, pp 428–425

Holmstedt B (1972): The ordeal bean of Old Calabar. In Swain T (ed), Plants in the Development of Modern Medicine. Cambridge, MA, Harvard University Press, pp 303–360

Holmstedt B, Wassen SH, Chanh P-H, Clavel P, Lasserre B (1984): Alleged native antidote of curare. Goteborg Etnografiska Museum, Annals 1983/1984:19–25

Hulme EC, Birdsall NJM, Buckley NJ (1990): Muscarinic receptor subtypes. Annu Rev Pharmacol Toxicol 30:633–673

Hyden H (1972): Some brain protein changes reflecting neuronal plasticity at learning. In Karczmar AG, Eccles JC (eds), Brain and Human Behavior. Berlin, Springer-Verlag, pp 94–110

Ishihara Y, Kato K, Goto G (1991): Central cholinergic agents.

I. Potent acetylcholinesterase inhibitors. Chem Pharm Bull (Tokyo) 39:3225–3235

Israel M, Morel N (1990): Mediatophore: A nerve terminal membrane protein supporting the final step of acetylcholine release process. In Aquilonius S-M, Gillberg P-G (eds), Cholinergic Neurotransmission: Functional and Clinical Aspects. Amsterdam, Elsevier, pp 101–110

Jankowska E, Lindstrom S (1971): Morphological identification of Renshaw cells. Acta Physiol Scand 81:428–430

Jenden DJ (1990): Achievement in cholinergic research, 1969–1989: Drug development. In Aquilonius S-M, Gillberg P-G (eds), Cholinergic Neurotransmission: Functional and Clinical Aspects. Amsterdam, Elsevier, pp 479–486

Johnson MK (1987): Acetylcholinesterases and neuropathy target esterase (NTE) compared and contrasted with the aid of organophosphorus esters. In Dowdall MJ, Hawthorne JN (eds), Cellular and Molecular Basis of Cholinergic Function. Chichester, England, Ellis Horwood, pp 556–568

Jouvet M (1972): Some monoaminergic mechanisms controlling sleep and wakening. In Karczmar AG, Eccles JC (eds), Brain and Human Behavior. Berlin, Springer-Verlag, pp 131–160

Kaczmarek, LK (1987): The role of protein kinase C in the regulation of ion channels and neurotransmitter release. Trends Neurosci 10:30–34

Kalow W (1959): Cholinesterase types. Ciba Foundation Symposia in Biochemistry Human Genetics. pp 39–56

Kaplan DR, Martin-Zanca D, Parada LF (1991): Tyrosine phosphorylation and tyrosine kinase activity of the trk protooncogene product induced by GNF. Nature 350:158–160

Karczmar AG (1946): The role of amputation and nerve resection in the regressing limbs of urodele larvae. J Exp Zoo 103:401–427

Karczmar AG (1957): Antagonism between a bis-quaternary oxamide, WIN 8078, and depolarizing and competitive blocking agents. J Pharmacol Exp Ther 116:39–47

Karczmar AG (1963a): Ontogenesis of cholinesterases. In Koelle GB (ed), Cholinesterases and Anticholinesterase Agents. Berlin, Springer-Verlag, pp 799–832

Karczmar AG (1963b): Ontogenetic effects. In Koelle GB (ed), Cholinesterases and Anticholinesterase Agents. Berlin, Springer-Verlag, pp 799–832

Karczmar AG (1967): Pharmacologic, toxicologic and therapeutic properties of anticholinesterase agents. In Root WS, Hoffman FG (eds), Physiological Pharmacology. New York, Academic Press, pp 163–322

Karczmar AG (1970): History of the research with anticholinesterase agents. In Karczmar AG (ed), Anticholinesterase Agents, Vol. 1. Oxford, Pergamon Press, pp 1–44

Karczmar, AG (1971): Possible mechanism underlying the so-called "divorce" phenomena of EEG desynchronizing actions of anticholinesterases. Abstract, Regional Midwest EEG Meeting, Hines, IL, Hines VA Hospital

Karczmar AG (1978): Multitransmitter mechanisms underlying selected functions, particularly aggression, learning and sexual behavior. In Deniker P, Radouco-Thomas C, Villeneuve A (eds), Neuropsychopharmacology, Proceedings of the 10th Congress of the CINP, Vol. 1. Oxford, Pergamon Press, pp 581–608

Karczmar, AG (1979): Brain acetylcholine and animal electrophysiology. In Davis KL, Berger PA (eds), Brain Acetylcholine and Neuropsychiatric Disease. New York, Plenum, pp 265–310

Karczmar AG (1981): Basic phenomena underlying novel use of cholinergic agents, anticholinesterases and precursors in neurological including peripheral and psychiatric disease. In Pepeu G, Ladinsky H (eds), Cholinergic Mechanisms. New York, Plenum, pp 853–869

Karczmar AG (1985): Present and future of the development of anti-OP drugs. Fundam Appl Toxicol 5:S270–S279

Karczmar AG (1986): Conference on dynamics of cholinergic function: Overview and comments. In Hanin I (ed), Dynamics of Cholinergic Function. New York, Plenum, pp 1215–1259

Karczmar AG (1987): Introduction to the session on modulators. Neuropharmacology 26:1019–1026

Karczmar AG (1988): Schizophrenia and cholinergic system. In Sen AG, Leed T (eds), Receptors and Ligands in Psychiatry. Cambridge, Cambridge University Press, pp 29–63

Karczmar AG (1990): Physiological cholinergic functions in the CNS. In Aquilonius S-M, Gillberg P-G (eds), Cholinergic Neurotransmission: Functional and Clinical Aspects. Amsterdam, Elsevier, pp 437–466

Karczmar AG (1991): SDAT models and their dynamics. In Becker R, Giacobini E (eds), Cholinergic Basis for Alzheimer Therapy. Boston, Birkhauser, pp 141–152

Karczmar AG (1993): Introduction: Electrophysiological aspects of cholinergic mechanisms. In Cuello AC (ed), Cholinergic Neurotransmission: Function and Dysfunction. Eighth International Cholinergic Symposium. Amsterdam, Elsevier (In press)

Karczmar AG, Dun NJ (1988): Effects of anticholinesterases pertinent for SDAT treatment but not necessarily underlying their clinical effectiveness. In Giacobini E, Becker R (eds), Current Research in Alzheimer Therapy. New York, Taylor and Francis, pp 15–29

Karczmar AG, Howard JW (1955): Antagonism of d-tubocurarine and other pharmacological properties of certain bisquaternary salts of basically substituted oxamides (WIN8077 and analogs). J Pharmacol Exp Ther 113:30

Karczmar AG, Longo VG, De Carolis A, Scotti (1970): A pharmacological model of paradoxical sleep: the role of cholinergic and monoamine systems. Physiol Behav 5:175–182

Karczmar AG, Nishi S, Blaber LC (1972): Synaptic modulations. In Karczmar AG, Eccles JC (eds), Brain and Human Behavior. Berlin, Springer-Verlag, pp 63–92

Karczmar AG, Srinivasan R, Bernsohn J (1973): Cholinergic function in the developing fetus. In Boreus LO (ed), Fetal Pharmacology. New York, Raven Press, pp 127–176

Karlen B, Lundgren G, Lundin J, Holmstedt B (1986): Acetylcholine turnover in mouse brain: Influence of cholinesterase inhibitors. In Hanin I (ed), Dynamics of Cholinergic Function. New York, Plenum, pp 781–790

Katayama Y, Nishi S (1987): Peptidergic transmission. In Karczmar AG, Koketsu K, Nishi S (eds), Autonomic and Enteric Ganglia. New York, Plenum, pp 181–200

Katz B, Miledi R (1965): The measurement of synaptic delay, and the time course of acetylcholine release at the neu-

romuscular junction. Proc R Soc London [Biol] 161: 483–495

Katz B, Miledi R (1970): Membrane noise produced by acetylcholine. Nature 226:962–963

Koelle GB (1963): Cytological distributions and physiological functions of cholinesterases. In Koelle GB (ed), Cholinesterases and Anticholinesterase Agents. Berlin, Springer-Verlag, pp 187–298

Koelle GB (1981): Organophosphorus poisoning—An overview. Fund Appl Toxicol 1:129–134

Koelle GB (1988): Enhancement of acetylcholinesterase synthesis by glycyl-l-glutamine: An example of a small peptide that regulates differential transcription? Trends Pharmacol Sci 9:318–321

Koelle GB, Gilman A (1949): Anticholinesterase drugs. J Pharmacol Exp Ther 95(II):166–216

Krnjevic K (1969): Central cholinergic pathways. Fed Proc 28:115–120

Kuba K, Nishi S (1987): General characteristics and mechanisms of nicotinic transmission in sympathetic ganglia. In Karczmar AG, Koketsu K, Nishi S (eds), Autonomic and Enteric Ganglia. New York, Plenum, pp 107–135

Kubo T, Fukuda H, Mikami A, Maeda A, Takahashi H, Mishina M, Haga T, Haga K, Ichiyama A, Kangawa K, Kojima M, Matsuo H, Hirose T, Numa S (1986): Cloning sequencing and expression of complementary DNA encoding the muscarinic acetylcholine receptor. Nature 323:411–416

Lambert DG, Nahorski SR (1990): Second-messenger responses associated with stimulation of neuronal muscarinic receptors expressed by a human neuroblastoma SH-SY5Y. In Aquilonius S-M, Gillberg P-G (eds), Cholinergic Neurotransmission: Functional and Clinical Aspects. Amsterdam, Elsevier, pp 31–42

Layer PG, Alber R, Sporns Q (1907): Quantitative development and molecular forms of acetyl- and butyrylcholinesterase during morphogenesis and synaptogenesis of chick brain and retina. Neurochemistry 49:175–182

Levey AI, Aoki M, Fitch FW, Wainer BH (1981): The productional of monoclonal antibodies reactive with bovine choline acetyltransferase. Brain Res 218:383–387

Levi-Montalcini R, Angeletti PU (1968): Nerve growth factor. Physiol Rev 48:534–569

Levin ED, McGurk SR, Rose JE, Butcher LL (1990): Cholinergic-dopaminergic interaction in cognitive performance. Behav Neurol Biol 54:271–299

Lloyd DPC (1946): Facilitation and inhibition of spinal motoneurons. J Neurophysiol 9:421–438

Loewi O (1923): Ueber die humorale Uebertragbarkeit der Herznervenreizungswirkung. Bemerkungen zu dem Aufsatz von H. J. Hamburger in Jg. 2, Nr. 28 dieser Wochenschrift. Klin Wochenschr 2:1840–1841

Loewi O, Navratil E (1926): Ueber humorale Uebertragbarkeit der Herzenswirkung. X. Uber das Schicksal des Vagusstoffes. Pflugers Arch 214:678–688

Loffelholz K, Lindmar R, Muscholl E (1967): Der Einfluss von Atropin auf die Nordrenalin-Freisetzung durch Azetylcholin. Naunyn-Schmiedebergs Arch Pharmakol Exp Pathol 257:308

Long, JP (1963): Structure-activity relationships of the reversible anticholinesterase agents. In Koelle GB (ed), Cholinesterases and Anticholinesterase Agents. Berlin, Springer-Verlag, pp 374–427

Massoulie J, Bon S (1982): The molecular forms of cholinesterase and acetylcholinesterase in vertebrates. Annu Rev Neurosci 5:57–106

Massoulie J, Bon S, Duval N, Legay C, Krejci E, and Coussen F (1993a): Expression of Torpedo and rat acetylcholinesterase forms in transfected cells. In Cuello AC (ed), Cholinergic Neurotransmission: Function and Dysfunction. Amsterdam, Elsevier (In press)

Massoulie J, et al. (1993b): Molecular and cell biology of cholinesterases. Progr Neurobiol 41:31–91

McBride WG (1985): Doxylamine succinate induced dysmorphogenesis in the marmoset (*Callithrix jacchas*). IRCS Med Sci 13:225–226

McCormick DA (1992): Cellular mechanisms underlying cholinergic and adrenergic modulation of neuronal firing mode in the cat and guinea pig dorsal lateral geniculate nucleus. J Neurosci 12:278–289

McGeer PL, McGeer EG, Mizukawa K, Tago H, Peng JH (1987): Distribution of cholinergic neurons in human brain. In Dun NJ, Perlman RL (eds), Neurobiology of Acetylcholine, A Symposium in Honor of Alexander G. Karczmar. New York, Plenum, pp 3–16

Mendel B, Rudney H (1943): Studies on cholinesterase. I. Cholinesterase and pseudo-cholinesterase. Biochem J 37:59–63

Menetrey D, Gannon A, Levine JD, Basbaum AI (1989): The expression of c-fos protein in presumed nociceptive interneurons of the rat spinal cord: Anatomical mapping of the central effect of noxious somatic, articular and visceral stimulation. J Comp Neurol 285:177–195

Mocchetti I (1991): Theoretical basis for a pharmacology of nerve growth factor biosynthesis. Annu Rev Pharmacol Toxicol 32:303–328

Mukaiyama O, Takeuchi A, Kimura T, Satoh S (1991): Effects of pirenzepine and AF-DX 116 on ganglionic transmission in the cardiac sympathetic nerves of the dog: Interaction of M1 and M2 receptors with nicotinic receptors. J Pharmacol Exp Ther 256:525–529

Myers RD (1974): Handbook of Drug and Chemical Stimulation of the Brain. Oradell, NJ, Van Nostrand Reinhold

Nitsch R, Slack BE, Wurtman RJ, Growdon JH (1992a): Processing of Alzheimer amyloid precursors stimulated by activation of muscarinic acetylcholine receptors. Science 258:304–307

Nitsch R, Blusztajn JK, Pittas A, Slack BE, Growdon HH, Wurtman RJ (1992b): Evidence for a membrane defect in Alzheimer diseased brain. Proc Natl Acad Sci USA 89:1671–1675

Nishizuka Y (1984): The role of protein kinase C in cell surface signal transduction and tumour production. Nature 308:693–697

Ogden DC, Colquhoun D, Marshall CG (1987): Activation of nicotinic ion channels by acetylcholine analogs. In Dowdall MJ, Hawthorne NJ (eds), Cellular and Molecular Basis of Cholionergic Function. Chichester, England, Ellis Horwood, pp 134–151

Palay SL, Palade GE (1955): Fine structure of the neurons. J Biophys Biochem Cytol 1:69–88

Patrick J, Lindstrom J (1973): Autoimmune response to acetylcholine receptor. Science 180:871–872

Pepeu G (1993): Overview and future directions on CNS cholinergic mechanisms. In Cuello AC (ed), Cholinergic Neurotransmission: Function and Dysfunction. Amsterdam, Elsevier (In press)

Pepeu G, Casamenti F, Giovannini MG, Vannucchi MG, Pedata F (1990): Principal aspects of the regulation of acetylcholine release in the brain. In Aquilonius S-M, Gillberg P-G (eds), Cholinergic Neurotransmission: Functional and Clinical Aspects. Amsterdam, Elsevier, pp 273–278

Peters B, Levin HS (1979): Effects of physostigmine and lecithin on memory in Alzheimer's disease. Ann Neurol 6:219–221

Price DL (1993): The Macaca mulatta model of Alzheimer's disease. Neurosci Facts 4:17–18

Ratnam M, Le Nguyen D, Rivier J, Sargent PB, Lindstrom J (1986): Transmembrane topography of nicotinic receptor: Immunochemical tests contradict theoretical predictions based on hydrophobicity profiles. Biochemistry 25:2633–2643

Rettig J, Wunder F, Stocker M, Lichtinghagen Mastiaux F, Bockh S, Kues W, Pederzani P, Schroter KH, Ruppersberg JP, Veh R, Pongs O (1992): Characteristics of a Shaw-related potassium channel family in rat brain. EMBO J 11:2473–2486

Rotundo RL (1987): Biogenesis and regulation of acetylcholinesterase. In Salpeter MM (ed), The Vertebrate Neuromuscular Junction. New York, Alan Liss, pp 247–248

Rylett RJ (1993): Synthesis and storage of acetylcholine. In Cuello AC (ed), Cholinergic Neurotransmission: Function and Dysfunction. Amsterdam, Elsevier (In press)

Salpeter MM (1987): Development and neural control of the neutromuscular junction and of the junctional acetylcholine receptor. In Salpeter MM (ed), The Vertebrate Neuromuscular Junction, New York, Alan Liss, pp 25–115

Salvaterra P, Kitamoto T, Ikeda K (1993): Molecular genetic specification of cholinergic neurons. In Cuello AC (ed), Cholinergic Neurotransmission: Function and Dysfunction. Amsterdam, Elsevier (In press)

Sargent PB (1993): The diversity of neuronal nicotinic acetylcholine receptors. Annu Rev Neurosci 16:403–443

Schimerlik MI (1990): Structure and function of muscarinic receptors. In Aquilonius S-M, Gillberg P-G (eds), Cholinergic Neurotransmission: Functional and Clinical Aspects, Amsterdam, Elsevier, pp 11–19

Schuetze SM, Role LW (1987): Developmental regulation of nicotinic acetylcholine receptors. Ann Rev Neurosci 10: 403–457

Shen E, Dun SL, Ren C, Dun NJ (1992): Hypovolemia induces Fos-like immunoreactivity in neurons of the rat supraoptic and paraventricular nuclei. J Auton Nerv Syst 37:227–230

Shute CCD, Lewis PR (1963): AChE distribution following lesions in the brain fibre tracts: Several tract systems in the upper brain stem of the rat are cholinergic. Nature 199:1160–1164

Soreq H, Zakut H (1990): Expression and in vivo amplification of the human acetylcholinesterase and butyrylcholinesterase genes. In Aquilonius S-M, Gillberg P-G (eds), Cholinergic Neurotransmission: Functional and Clinical Aspects. Amsterdam, Elsevier, pp 51–61

Stedman E, Stedman E, Easson LH (1932): Choline-esterase. An enzyme present in the blood serum of the horse. Biochem J 26:2056–2066

Steriade M, Curro Dossi R, Nunez A (1992): Network modulation of a slow intrinsic oscillation of cat thalamocortical neurons implicated in slow delta waves: Cortical potentiation and brainstem cholinergic suppression. J Neurosci 10:2541–2559

Straughan DW, James TA (1979): Microphysiological and pharmacological studies on transmitters in the substantia nigra. In Simon P (ed), Neurotransmitters, vol. 2, Advances in Pharmacology and Therapeutics, Proceedings of the 7th International Congress of Pharmacology. Oxford, Pergamon Press, pp 87–96

Sussman JL, Harel M, Frolow F, Oefner C, Goldman A, Toker L, Silman I (1991): Atomic structure of acetylcholinesterase from *Torpedo californica*: A prototypic acetylcholine-binding protein. Science 253:872–879

Szentagothai J (1983): The modular architectonic principle of neural centers. Rev Physiol Biochem Pharmacol 98:11–61

Takeuchi A, Takeuchi N (1960): Further analysis of relationship between end-plate potential and end-plate current. J Neurophysiol 23:397–402

Thal LJ (1991): Physostigmine in Alzheimer's disease. In Becker R, Giacobini E (eds), Cholinergic Basis for Alzheimer Therapy. Boston, Birkhauser, pp 209–215

Thesleff S (1955): The mode of neuromuscular block caused by acetylcholine, nicotine, decamethonium and succinylcholine. Acta Physiol Scand 34:218–231

Thoenen H, Auburger G, Hellweg R, Heumann R, Korsching S (1987): Cholinergic innervation and levels of growth factor and its mRNA in the central nervous system. In Dowdall MJ, Hawthorne NJ (eds), Cellular and Molecular Basis of Cholinergic Function. Chichester, England, Ellis Horwood, pp 379–388

Tucek S (1990): The synthesis of acetylcholine: Twenty years of progress. In Aquilonius S-M, Gilberg P-G (eds), Cholinergic Neurotransmission: Functional and Clinical Aspects. Amsterdam, Elsevier, pp 467–477

Ulus I, Wurtman RJ, Mauron C, Blusztajn JK (1989): Choline increases acetylcholine release protect against stimulation induced decrease phosphatide level within membranes of rat's corpus striatum. Brain Res 44:217–227

Usdin E (1970): Reactions of cholinesterases with substrates, inhibitors and reactivators. In Karczmar AG (ed), Anticholinesterase Agents, Vol. 1. International Encyclopedia of Pharmacology Therapy. Oxford, Pergamon Press, pp 47–354

Velazquez-Moctezuma J, Shiromani PJ, Gillin JC (1990): Acetylcholine and acetylcholine receptor subtypes in REM sleep generation. In Aquilonius S-M, Gillberg P-G (eds), Cholinergic Neurotransmission: Functional and Clinical Aspects. Amsterdam, Elsevier, pp 407–413

Wallace WC, Bragin V, Robakis NK, Sambamurti K, Vanderputten D, Merrit RC, Davis KL, Santucci AC, Haratounian V (1991): Increased biosynthesis of Alzheimer amyloid precursos protein in the cerebral cortex of rats with lesions of the nucleus basalis of Meynert. Mol Brain Res 10:173–178

Waser PG (ed) (1975): Cholinergic Mechanisms. New York, Raven Press

Weight FF (1968): Cholinergic mechanisms in recurrent inhibition of motoneurons. In Efron DH (ed), Psychopharmacology—A Review of Progress. Washington, DC, Public Health Service Publication No. 1836, pp 69–76

Whitehouse PJ, Price D, Clark A, Coyle JK, DeLong (1981): Alzheimer's disease evidence for a selective loss of cholinergic neurons in the nucleus basalis. Ann Neurol 10:122–126

Whittaker VP (ed) (1988): The Cholinergic Synapse. Berlin, Springer-Verlag

Whittaker VP (1990): The cell and molecular biology of the cholinergic synapse: Twenty years of progress. In Aquilonius S-M, Gillberg P-G (eds), Cholinergic Neurotransmission: Functional and Clinical Aspects. Amsterdam, Elsevier, pp 419–436

Whittaker VP (1992): The Cholinergic Neuron and Its Target: The Electromotor Innervation of the Electric Ray "Torpedo" as a Model. Boston, Birkhauser

Yamane HK, Fung BK-K (1993): Covalent modifications of G-proteins. Annu Rev Pharmacol Toxicol 33:201–241