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COMMENTARY

SOME ASPECTS OF EVOLUTIONARY PHARMACOLOGY

M. J. MICHELSON
Pharmacological Laboratory of the Sechenov Institute of Evolutionary Physiology and Biochemistry, Academy of Sciences of U.S.S.R., Leningrad, K-223, U.S.S.R.

THE MAIN aim of evolutionary pharmacology is to understand the development of the chemical sensitivity of tissues and organs during the evolution of the animal kingdom. Evolutionary pharmacology, similar to evolutionary physiology, is based on comparative pharmacology, ontogenetic pharmacology and the so-called "pathological pharmacology".[1–3]

Comparative pharmacology provides some evidence for the development of chemical sensitivity in phylogenesis. In the light of Haeckel's recapitulation law ontogenetic pharmacology allows this data to be checked. The most fruitful aspect of "pathological pharmacology", for evolutionary pharmacology, is the study of changes in the action of biologically active substances during different "experimental maladies", especially after denervation of some tissues. Orbeli[1] and Ginetsinsky[2,4] have shown that after denervation muscles especially acquire some features characteristic of earlier periods of development.

In this article some aspects of the evolutionary pharmacology of synaptic transmission have been considered.

1. THE TRANSMITTER SUBSTANCES

There is no data concerning changes in the quality of transmitter substances, that is their chemical structure, in the course of evolution. The same neurotransmitters are found in platyhelminths, arthropods, echinoderms and vertebrates; acetylcholine (ACh), catecholamines, 5-hydroxytryptamine, glutamic acid, GABA and glycine. "A primitive transmitter, from which another, perhaps more effective transmitter has developed, never existed: the cells of our brain produce the same transmitters as the nerve cells of lower worms".[5] Choline esters other than ACh are often found throughout the animal kingdom, but never act as transmitters.

One of the amazing enigmas is the diverse localization of the neurons producing the same transmitter in the nervous system on different phyletic lines. For example, cholinergic neurons in vertebrates are efferent but in arthropods only afferent (sensitive) and associated neurons are cholinergic; in annelids, both afferent and efferent neurons are cholinergic.[6] In different groups of animals therefore the neurons producing the same transmitter substance have quite different functions. Recently, Sakharov[7,8] put forward a hypothesis of polygenesis of neurons suggesting that the nerve cells arose during evolution repeatedly and from different sources. The type of biochemical organization was genetically conserved in the descendants of these neurons in spite of their different functions. Therefore, in different phyletic lines, the nerve

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cells of similar origin and with similar biochemical organization appear in different parts of the nervous system. Thus the neurons producing the same transmitter substance are homologous (have the same origin) but not analogous (have quite different functions).

2. CHOLINERGIC SYNAPSES

Acetylcholine (ACh) seems to be the most ancient transmitter and is a mediator in all animals in which chemical transmission is known.

The ability of the central nervous system to synthesize ACh was compared in different classes of vertebrates. Some quantitative differences in the activity of cholinacetylase were found[9,10] but its synthetic pathways were similar.[11]

Many cholinesterases (ChE) are known, and there is considerable interspecies variation in the properties of the enzymes ChE.[12] Until now no clear evolutionary tendency has been shown. Some peculiar properties are known to be characteristic of definite animal groups, for example, a bee-type and fly-type of acetylcholinesterase (AChE) but no progressive tendency, which could be recognized as a case of aromorphose,[13] was ever revealed.

There is evidence that the cholinoreceptors (ChRs) underwent great changes in the course of evolution. The variety of cholinoreceptive properties of postsynaptic membranes in cholinergic synapses in different animals is, perhaps, even greater than the variety of ChE's. Dale's classification[14] dividing the ChRs into muscarine sensitive (M-ChR) and nicotine-sensitive (N-ChR) has proved valid for the higher vertebrates, but it is obvious that this classification cannot be unconditionally applied to the invertebrate ChRs.

Some attempts were made to reveal evolutionary tendencies in the changes of ChRs.

2.1 *The increase in the "specificity" of cholinoreceptors in the course of evolution*

Ginetsinsky[4] demonstrated that the highly selective sensitivity to the nicotinic action of ACh is characteristic of higher vertebrate fast skeletal muscles, but the amphibian tonic muscles react with a contracture, not only to ACh and nicotine, but also to arecoline and some other muscarinomimetic drugs. After denervation even mammalian skeletal muscles become sensitive to a great variety of cholinomimetics. In tissue culture of rabbit skeletal muscle the myosymplasts contracted under the influence of a wide range of cholinergic drugs including curare and atropine in addition to cholinomimetics.

A divergent development of the sensitivity to nicotinic and to the muscarinic action of ACh in vertebrate heart and skeletal muscles was followed.[16] In the lamprey (*Cyclostomata*) the ChR's of the heart muscle are nicotine-sensitive and do not differ in sensitivity from skeletal muscles (see also [17,18]). In all other vertebrates, however, the heart ChR's are selectively muscarine-sensitive, and the skeletal muscles selectively nicotine-sensitive.

The non-visceral muscles of some echinoderms (Holothuroidea) are very sensitive both to muscarinomimetics and to nicotinomimetics.[19] One can speculate that in echinoderms, occupying a comparatively low position in the Deuterostomia branch of the phylogenetic tree, the ChR's possess a low specificity compared with the corresponding receptors of vertebrate muscles. But the use of selective alkylating agents

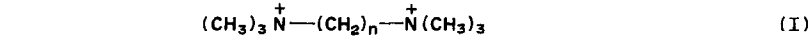
recently allowed us to show that the sensitivity of holothurian muscles, both to nicotinic and to muscarinic agents, is due not to a low specificity of its ChR's but to the presence of two types of ChR's, one resembling the M-ChR and the other the N-ChR of vertebrates.[20]

It is also difficult to reconcile the highly selective sensitivity of non-visceral (somatic, locomotor) muscles of many lower phyla of invertebrates (bivalve and gastropod molluscs, different phyla of worms) only to the nicotinic action of ACh with the suggestion that the specificity of ChR's increases in the course of evolution.[21–23] Nevertheless, this idea remains very attractive, and such facts as the highly unspecific sensitivity of denervated vertebrate skeletal muscles, of myosymplasts in tissue culture and some other data still await an adequate explanation.

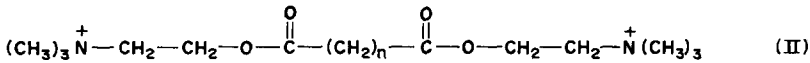
Another suggestion is based on the idea that the changes of cholinoceptive properties of postsynaptic membranes in the course of evolution are due not only, and sometimes not so much, to the changes in the properties of individual ChR molecules, as to the changes in the mutual disposition of these molecules, to their aggregation into olygomeric complexes.

2.2 *The changes in the mutual disposition of cholinoreceptors in the course of evolution*

2.2.1 *Skeletal muscles of higher vertebrates.* In mammalian skeletal muscles a sharp maximum of blocking activity has been revealed in the polymethylene-*bis*-trimethylammonium series (I) and in the series of dicholinic esters of dicarboxylic acids (II) at decamethonium (I, n = 10) and suxamethonium (II, n = 2).[24,25]



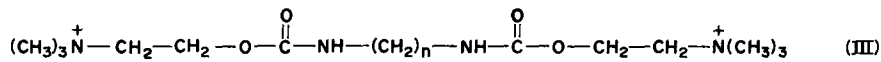
(I)



(II)

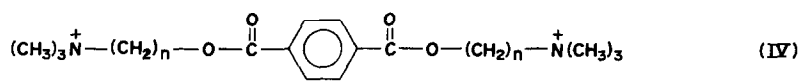
These results were the main reason for the suggestion that the mutual disposition of ChR's on the cholinoreceptive membrane is such that the distance between the anionic sites of two adjacent receptors is equal to the internitrogen distance in decamethonium or suxamethonium (about 14 Å). Further studies revealed both in series (I) and (II) a second maximum of blocking potency with an internitrogen chain containing from 14 to 18 atoms (about 20 Å)[26,27] (Fig. 1). The results with series II were obtained after prevention of the hydrolysis of the dicholinic esters by inhibition of ChE.

A maximum of blocking activity with 16–17 atoms in the internitrogen chain was revealed also in the series of polymethylene-*bis*-carbamoylcholines (III)[29] and in a series of diesters of terephthalic acid (IV) (Fig. 2).[30]



(III)

n = 6 - carbolonium



(IV)

maximum potency with n = 4

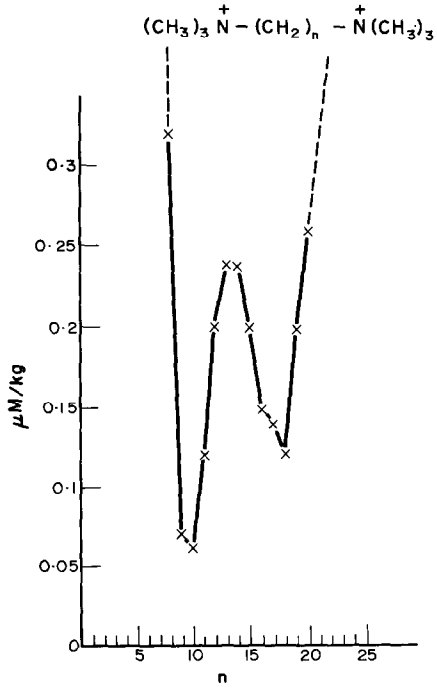
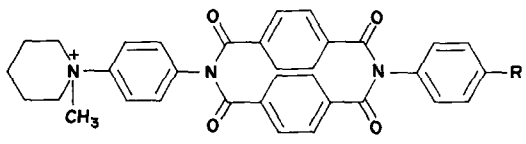


FIG. 1. Blocking activity of a series of polymethylene-*bis*-trimethyl-ammonium compounds. Cat tibialis muscle. Abscissa—number of methylene groups, n; ordinate—dose (μM/kg). Drawn with data calculated from refs 26 and 28.

Some compounds with quite rigid (inflexible) structure having two quaternary nitrogens separated by a distance of about 14 Å and 20 Å were synthesized and proved to possess a pronounced myorelaxant activity.[31,32] For example, for the compound (V) the blocking dose in the cat is 0·04 μmoles/kg i.v. and the withdrawal of one cationic head (VI) reduced the blocking activity 50-fold.[32]



These data suggest that on the subsynaptic membrane of mammalian skeletal muscle fibres the individual ChRs are aggregated into oligomeric complexes with a fixed mutual disposition. Two variants of this disposition can be called "C-10 structure" (about 14 Å between the anionic sites of adjacent ChRs), with which such compounds as decamethonium or suxamethonium can react, and "C-16 structure" (about 20 Å), with which such compounds as hexadecamethonium or carbolonium can interact.[33–35] The oligomeric structures can be arranged in tetrameric form (Fig. 3a). The C-16 structures are formed by the active centers of two adjacent receptors facing each other by their esterophilic sites and are disposed on the diagonals of the square. The C-10 structures are on the sides of the square.[23,31,36–38]

The scheme implies that a compound like sebacoyldicholine can interact with the anionic sites of the C-16 structure by their cationic heads and with the esterophilic

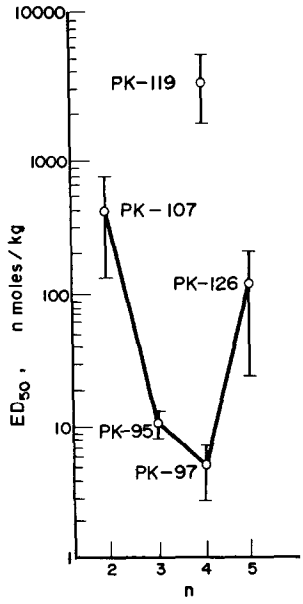
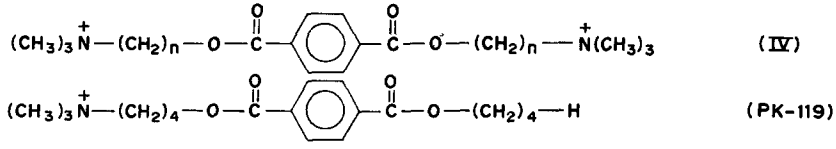


FIG. 2. Blocking activity of *bis*-quaternary derivatives of terephthalic acid (IV) (PK-107, PK-95, PK-97, PK-126), and the monoquaternary derivative PK-119. Cat tibialis muscle. Abscissa—number of methylene groups, n; ordinate—ED_{50} in nmoles (logarithmic scale).[29]



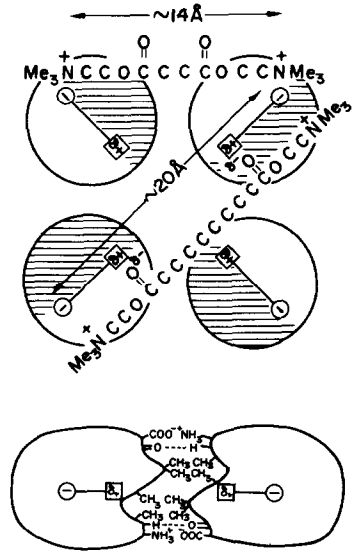


FIG. 3. Schemes of mutual disposition of cholinoreceptors. Modified after.[37] (a) Tetrameric scheme; the C-16 structures are on the diagonals of the square and the C-10 structures are on the sides of the square. (b) Scheme explaining the possible interaction between the sub-units of a dimeric cholinoreceptor (C-16 structure). Explanation in text.

sites by their carboxylic groups. In the C-10 structure there are no esterophilic sites between the anionic ones and the ester groups of suxamethonium cannot play an active role in the interaction with the C-10 structure.

The tetrameric scheme implies also that the C-10 structure (side of the square) is asymmetric inasmuch as the primary and secondary structures of protein molecules are asymmetric. In order to show this asymmetry one side of each receptor sub-unit is shaded (Fig. 3a). Consequently a drug molecule requires an asymmetric internitrogen chain to be complementary with the C-10 structure. Table 1 gives a striking example of this.

TABLE 1. EFFECT OF ASYMMETRY ON THE BLOCKING ACTIVITY OF BISQUATERNARY COMPOUNDS CONTAINING 10 ATOMS BETWEEN THE NITROGENS

Substance	Rat[39] diaphragm (μM/ml)	Rabbit[24] head drop dose (mg/kg)
$Me_3\overset{+}{N}-CH_2-CH_2-O-\overset{O}{\overset{\|}{C}}-CH_2-CH_2-\overset{O}{\overset{\|}{C}}-O-CH_2-CH_2-\overset{+}{N}Me_3$	0·07	0·2
$Me_3\overset{+}{N}-CH_2-CH_2-O-\overset{O}{\overset{\|}{C}}-CH_2-CH_2-\overset{O}{\overset{\|}{C}}-O-\overset{CH_3}{\overset{\|}{C}}H-CH_2-\overset{+}{N}Me_3$	0·002	
$Me_3\overset{+}{N}-CH_2-\overset{CH_3}{\overset{\|}{C}}H-O-\overset{O}{\overset{\|}{C}}-CH_2-CH_2-\overset{O}{\overset{\|}{C}}-O-\overset{CH_3}{\overset{\|}{C}}H-CH_2-\overset{+}{N}Me_3$		30·0

The concept of a tetrameric structure of the cholinoreceptors has received the support in recent years from a series of investigations carried out by quite different methods, i.e. by biochemical isolation of cholinoreceptor macromolecules.[40–42]

2.2.2 *Signs of the presence of oligomeric structures in skeletal muscles of lower vertebrates and in non-visceral muscles of invertebrates.* The tonic skeletal muscles of lower vertebrates and the non-visceral muscles of many invertebrates (echinoderms, molluscs, annelids) possess a high sensitivity to ACh but differ widely in their sensitivity to bisquaternary compounds. The muscles of animals low on the scale of evolutionary development (bivalves and gastropods among the molluscs; tunicates among the chordates) are completely insensitive to bisquaternary compounds, both when the chain between the nitrogen atoms comprises 10 atoms (decamethonium, suxamethonium) and when it contains 14 or 16 atoms (suberyldicholine, sebacoyldicholine, carbolonium). In the more highly organized animals (cephalopod molluscs or cyclostomes) the muscles are very sensitive to the compounds with a 16-atom chain, but rather insensitive to substances with 10 atoms between the nitrogens. The muscles of animals at the highest level of organization (mammals, birds) react to very low doses of both groups of bisquaternary drugs, those with 16 and those with 10 atoms in the internitrogen chain, the bisquaternary compounds appearing to be many times more potent than ACh.

These results have suggested that the grouping of ChRs into oligomeric structures has proceeded gradually in the process of evolution. For the lower stages of evolution an irregular, "accidental" arrangement of individual ChRs on the cholinoceptive

membrane is characteristic, but in the course of development a gradual grouping of the individual receptors into oligomeric structures has taken place. It seems that the first to appear was the C-16 structure, and then, at the highest stages of evolution, the C-10 structure was added to it[31,37,38,43] (see Table 2).

2.2.3 *Changes during ontogenesis and after denervation.* In immaturely born mammals (dog, rats and mice) the sensitivity to depolarizing myorelaxants with a 10-atom chain, such as suxamethonium, increases during postnatal ontogenesis. Clinical observations indicate that the new-born child needs more suxamethonium per kg of body weight for myorelaxation compared with an adult man.[31]

After chronic denervation the sensitivity of mammalian and avian skeletal muscles to suxamethonium decreases compared with the sensitivity to monoquaternary agents such as ACh or carbacholine.[31,44] We can regard these results as signs of "degradation" of the C-10 structure after denervation. This structure probably is the last to appear in the course of development and the first to degrade after denervation. It has been shown recently in annelids that after chronic denervation of body wall muscles their sensitivity to suxamethonium and decamethonium disappears but the sensitivity to sebacoyldicholine unaltered.[45] One can speculate that the C-16 structure which is the first to appear in the course of development, does not "degrade" after denervation as quickly as the C-10 structure.

2.2.4 *The occurrence of the C-16 structure.* The C-16 structure seems not only to be the first to appear during the course of evolution, but also to be more generally distributed. In the nicotine-sensitive heart of the lamprey (*Lampetra fluviatilis*) sebacoyldicholine and suberyldicholine are 3–10 times as potent as ACh, but succinyldicholine (suxamethonium) is 40 times less potent than ACh.[17] In the nicotine-sensitive heart of a bivalve mollusc *Anadonta cygnea*, sebacoyldicholine proved to be 20–30 times as potent as ACh. but suxamethonium and decamethonium possessed a very poor activity.[21]

The existence of the C-16 structure in some nerve cells has also been described.

2.2.5 *Nerve cells.* Hexadecamethylene-*bis*-trialkylammonium proved to be a very potent ganglioblocking agent, more potent than hexamethonium.[26] The study of nicotine-sensitive neurons of two gastropod molluscs, *Limnaea stagnalis* and *Planorbis corneus*, also revealed some signs of the existence of the C-16 but not of the C-10 structure. The properties of these ChRs are similar to those of vertebrate skeletal muscles (not to the ChR's of vertebrate ganglia). In *P. corneus* sebacoyldicholine proved to be a more potent depolarizing agent than ACh. Other bisquaternary compounds with a 16-atom chain were also very active in *P. corneus* and in *L. stagnalis*, but suxamethonium and decamethonium possess a very poor activity, and hexamethonium is quite ineffective.[21,46,47]

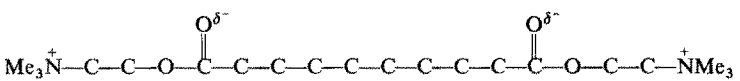
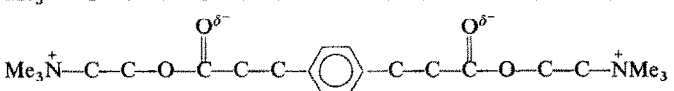
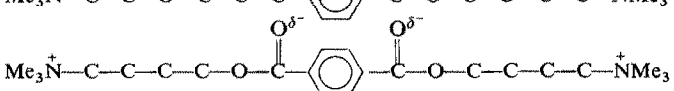
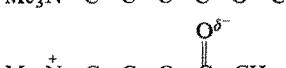
As it has been stated a compound like sebacoyldicholine is supposed to interact with the C-16 structure in at least four points: two cationic heads with two anionic sites, and two ester group with two esterophilic sites (Fig. 3a). Table 3 shows that hexadecamethylene-*bis*-trimethylammonium has little depolarizing activity, being 25 times less potent than ACh. The introduction of two carboxylic groups in the internitrogen chain increases the potency 56-fold (sebacoyldicholine). This result is consistent with the suggestion that the carboxyls of sebacoyldicholine can interact with the esterophilic sites of the C-16 structure. The compound PK-154 differs from sebacoyldicholine in containing a benzene ring in the central part of the molecule. PK-154

TABLE 2. THE ACTIVITY OF SOME MONO- AND BISQUATERNARY COMPOUNDS IN NON-VISCERAL MUSCLES OF DIFFERENT SPECIES RELATIVE TO ACETYLCHOLINE (POTENCY OF ACh = 1)

	ACh EC_{50} (M)	Bisquaternary compounds with internitrogen chain containing the following number of atoms				
		10 Decamethonium	10 Suxamethonium	14 Suberyldicholine	16 Sebacoyldicholine	16 Carbolonium
		A. Deuterostomia				
Phylum echinodermata						
Sea urchin, *Strongylocentrotus dr.*						
m. *retractor dentis*	8×10^{-8}	0	0	2·3	0·5	
Holoturia, *Cucumaria frondosa*						
m. *protractor pharingis*	3×10^{-7}	0	0	4	3	
Phylum chordata						
Larval-chordates (Tunicates)						
Ascidia, Tethyum aurantium						
Body wall muscles	$1{\cdot}7 \times 10^{-5}$	0	0	0	0	0
Vertebrata						
Cyclostomata, *Lampetra fluviat.*						
m. *retractor linguae*	4×10^{-6}	0	0·04	4		
Amphibia, *Rana temporaria*						
m. *rectus abdominis*	3×10^{-7}	0·06	0·15	17	28	
m. *sartorius*						
blocking concentration	1×10^{-5}	0·5	2	20	7	2
Reptilia, *Testudo horsfieldi*						
m. *testocervicalis*	5×10^{-7}	0·14	0·1	6	12	0·25
Agama caucasica						
m. *rectus abdominis*	7×10^{-7}	0·17	0·25	15	25	3·5
Aves, chick, m. *biventer cervicis*	$1{\cdot}7 \times 10^{-6}$	4·5	1	29	26	22

Mammalia, rat diaphragm, blocking concentration	7×10^{-6}	0·23	1·4	20		0·7
Cat, m. *gastrocnemius*, blocking dose in μmol/kg i.v. relative to carbachol	0·16	2·7	8	32	8	20
		B. Protostomia				
Bivalvia, *Mutilus edulis*, m. *retractor bissi ant.*	3×10^{-6}	0	0	0		
Gastropoda, *Tritonia diomedia*, m. *retractor radulae*	3×10^{-6}	0	0	0	0	0
Rapana bezoar, m. *retractor radulae*	7×10^{-7}	0	0	0		
Neptunea constricta, m. *retractor radulae*	3×10^{-6}	0·01	0·01	0·02	0·02	0
Cephalopoda, *Ommatostrephes sloanei pac.*, m. *retractor infundibuli*	5×10^{-6}	0	0	1	1	0·5
Phylum sipunculida *Physcosoma japonicum*, m. *retractor strombii*	4×10^{-7}	0	0	0·23	0·24	0·06
Phylum annelida Hirudinea, *Hirudo medicinalis*, m. *dorsalis*	1×10^{-6}	0·08	0·02	0·2	0·05	
Polychaeta, *Serpula vermicularis*, body wall muscles	$1·3 \times 10^{-6}$	0·1	0·01	0·5	1	
Oligochaeta, *Allolobophora longa*, body wall muscles	1×10^{-6}		1		10	

TABLE 3. DEPOLARIZING POTENCY OF *bis*-QUATERNARY COMPOUNDS IN THE NEURONS OF A GASTROPOD MOLLUSC *Planorbis corneus* RELATIVE TO ACETYLCHOLINE (POTENCY OF ACh=1)*

		Relative potency
Hexadecamethylene-*bis*-trimethyl-ammonium		0·04
Sebacoyldicholine		2·2
PK-154		10
PK-97		0·33
Acetylcholine		1 (3×10^{-6} M)

* Data of E. V. Zeimal.

The scheme at the top of the Table represents the C-16 structure; the central shaded part—the hypothetical hydrophobic area. The concentrations causing 10-mV depolarization were compared.

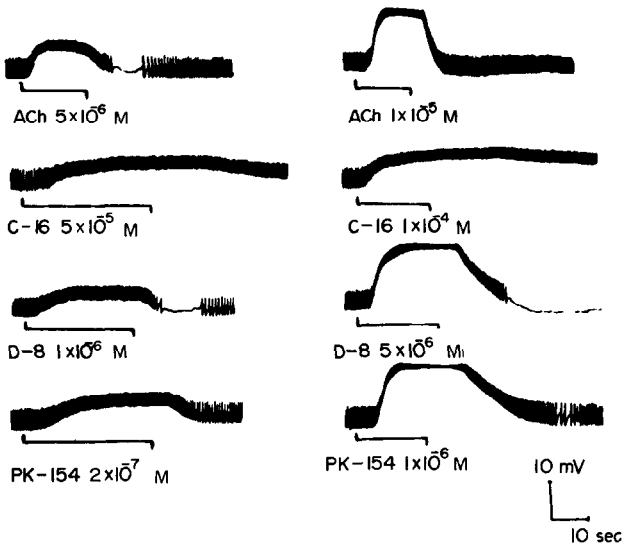


FIG. 4. Depolarizing activity of compounds with 16 atoms between quaternary nitrogens. Neuron of the *Planorbis corneus* pedal ganglion (P-2). Explanation in text. Data of E. V. Zeimal.

proved to be even more potent than sebacoyldicholine. This indicates that in the central part of the C-16 structure there is a hydrophobic area (the shaded area in the scheme in the top of Table 3) with which the benzene ring can interact. These results are illustrated in Fig. 4. The importance of the "correct" position of the ester groups is illustrated by comparison of PK-154 and PK-97 (Table 3). In PK-154 the position of the carboxylic groups is "correct" (the ester groups are separated from the nitrogen atoms by two methylenes as in ACh) and the ester groups can interact with the esterophilic sites of the C-16 structure. PK-154 is 30 times more potent than its isomer PK-97 in which there are four methylenes between the ester groupings and the nitrogens. In PK-97 the position of the ester groups is "incorrect" and their interaction with the esterophilic sites of the C-16 structure is impossible. The importance of carboxylic groups and their "correct" position for the interaction of drugs with the C-16 structure in vertebrate skeletal muscles has also been shown.[48]

2.2.6 *Possible biological significance of the grouping of individual receptors into oligomeric structures in the course of evolution.* It appears highly probable that the oligomeric structures have some advantages by comparison with monomers.[49] With an oligomeric structure there is a possibility of allosteric co-operative interaction between its sub-units as is known from the study of self-regulating proteins (some enzymes, haemoglobin). For instance when one ACh molecule interacts with the active center of one sub-unit of the dimeric C-16 structure (Fig. 3b), it induces conformational changes of the whole submolecule. Owing to intermonomeric bonds the conformational changes of one sub-unit will induce changes in the conformation of the other one. Changes in the conformation of the second sub-unit may enhance the interaction of its active center with the next ACh molecule and thus facilitate the action of the transmitter. This principle of positive feedback may be one of the goals of the oligomerization of receptor molecules.

3. THE POSSIBLE CHANGES IN THE MECHANISM OF TERMINATION OF THE ACTION OF ACETYLCHOLINE IN THE COURSE OF EVOLUTION

The hydrolysis of the synaptic ACh by AChE is the main but not the only mechanism of cessation of the transmitter action.

(1) Simple diffusion of ACh from the synaptic cleft is, probably, the most ancient mechanism of termination of its action. Diffusion is, probably, the only mechanism of cessation of the action of ACh in neuromuscular synapses of the body wall muscles of *Ascidia* (larval chordata) which are fully deprived of cholinesterases. The contraction of all body wall muscles leading to a reduction of the body volume seems to be the only defence reflex of these primitive sedentary and nearly immobile animals. The rate of relaxation of these muscles cannot play an important role in the life of the animal and the slow relaxation ensured by simple diffusion of the transmitter is quite adequate. It is interesting to mention that at the larval stage when the animal possesses a high mobility there are significant quantities of ChE in the tail muscles.[19]

Diffusion probably plays an important role in the termination of the action of ACh in the synapses of higher vertebrates rich in cholinesterase, for example in mammalian ganglionic synapses where the AChE is located chiefly on the presynaptic membrane.

In mammalian quick skeletal muscles the postsynaptic membrane is highly folded. Some recent calculations led to a suggestion that the diffusion of ACh from the primary synaptic cleft (where the ChRs are thought to be located) into the postsynaptic folds (the secondary synaptic cleft) occurs very quickly and constitutes the first step in lowering the concentration of ACh near the ChRs. The second step in bringing down the concentration of ACh in the synapse is performed by AChE.[50]

It is possible that in the first moment after the release of ACh by the nerve impulse its concentration in the synapse is so high that a substrate inhibition of AChE occurs. In this case the first, diffusional, step in lowering the concentration of ACh is necessary to stop the substrate inhibition and to follow the enzymatic hydrolysis, which is the second step of the process.

In the synapses of slow phasic muscles of lower vertebrates the postsynaptic folds are less developed and the postsynaptic membrane of amphibian tonic muscles is quite unfolded. Ger[50] suggested that the folding of the subsynaptic membrane is one of the mechanisms ensuring the quick function of the neuromuscular synapse in the evolution of vertebrate skeletal muscles. There is a parallel between the degree of subsynaptic folding and the duration of postsynaptic potential and postsynaptic current. With the folding of the postsynaptic membrane the duration of the postsynaptic potential and of the postsynaptic current becomes much less and the synapse acquires the ability to reproduce more frequent stimuli.

(2) *Allosteric action of high-energy phosphates on the cholinoreceptors.* A new mechanism of cessation of the action of ACh was recently discovered in the hearts of bivalve molluscs which are deprived of cholinesterases. It had been shown that the release of ACh from the nerve as well as the application of synthetic ACh induces a release from the heart muscle of an ATP-like substance which lowers the sensitivity of ChRs to ACh, probably by an allosteric interaction. This mechanism still subsists in the hearts of more developed animals; it has been demonstrated even in the heart of the frog. But in most cholinergic synapses of higher classes, both of molluscs

(cephalopods) and vertebrates (birds, mammals) which are rich in acetylcholinesterase the enzymatic hydrolysis of ACh is the main mechanism of stopping its action.[51]

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