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SULFUR AND SELENIUM COMPOUNDS RELATED TO ACETYLCHOLINE AND CHOLINE—VI.

EFFECTS OF HOMOCHOLINE DERIVATIVES ON THE ELECTROPLAX PREPARATION*

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Abstract—The depolarizing activities of homocholine, homocholinethiol, homocholineselenol, their acetyl esters, and their methyl derivatives were measured in the single-cell electroplax preparation and compared with the activities of the corresponding series of choline, cholinethiol, and cholineselenol derivatives. The possible basis for the striking inactivity of choline compared to closely related compounds is discussed.

IT HAS been suggested[6] that in the permeability changes of excitable membranes during electrical activity, a crucial role is played by a conformational change in a receptor protein brought about by the action of acetylcholine (ACh). The long-held concept that interactions of small molecules with proteins may induce conformational changes in the biopolymers has been confirmed in recent years by X-ray crystallographic and kinetic studies (see for instance Perutz *et al.*[7] and Monod *et al.*[8]). A considerable amount of indirect evidence in support of conformational changes of the three proteins reacting with ACh—ACh-esterase, choline acetylase, and ACh-receptor—has been obtained during recent years with the two enzymes in solution and with the receptor of the isolated electroplax of *Electrophorus electricus*. (For reviews see, for instance, Nachmansohn.[9–12])

In an endeavor to obtain additional information about the active site of the depolarizing receptor, a systematic investigation has been initiated of the relative depolarizing activities of oxygen, sulfur, and selenium isologs related to ACh and choline (Ch) on the monocellular electroplax of *E. electricus*. In isologs of this type, molecular size is affected relatively little when oxygen is replaced by sulfur and still less when sulfur is replaced by selenium. Thus, analogous oxygen, sulfur, and selenium compounds should have very similar abilities to fit receptor sites. On the other hand, electron distribution is greatly affected as oxygen is progressively replaced by sulfur and selenium.[13–15] For this reason, such isologs may be considered to be isosteric, but

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not isoelectronic, and might be expected to differ in their ability to be bound to receptor sites or to induce conformational changes in receptor proteins.

It was noted that replacement of the ether oxygen of ACh by sulfur or selenium greatly reduced the effects of the ester in the guinea pig ileum and frog rectus abdominis preparations.[2] A particularly favorable test material for evaluating the differences between the O, S, and Se isologs of ACh and related compounds proved to be the monocellular electroplax preparation of *E. electricus*. This preparation was first developed by Schoffeniels and Nachmansohn[16, 17] and greatly refined during the last few years by Higman and Bartels[18, 19] and Podleski.[20] Because of its ability to function over a relatively wide pH range, and the relative ease of obtaining both qualitative and quantitative information about the ability of compounds either to induce depolarization or to block depolarization, this preparation can yield—with unique precision—information about the effects of even minor modifications of chemical structure or electronic distribution of compounds acting on the ACh-receptor. Some of the results have already been reported.[4] These results confirmed and extended the earlier finding, with the guinea pig ileum and frog rectus abdominis preparations, that replacing the ether oxygen of ACh with sulfur or selenium progressively reduced the depolarizing activity of the ester. In contrast, it was found that replacement of the oxygen of choline by sulfur or selenium progressively increased depolarizing activity. It could be shown that cholinethiol was more active in the mercaptan than in the mercaptide form, while methylation of the oxygen, sulfur, and selenium of choline, cholinethiol, and cholineselenol increased the ability of these compounds to induce depolarization in the electroplax.[4]

In view of the observation that cholinethiol, cholineselenol, methoxycholine, methylthiocholine, methylselenocholine,[4] and a series of alkyl- and aryltrimethylammonium ions[21] were receptor activators in the electroplax, the almost total lack of depolarizing activity of choline was striking. For instance, while choline at a 1×10^{-1} M concentration induces only a small depolarization in the electroplax, methoxycholine at a 2×10^{-4} M and cholinethiol at a 5×10^{-5} M concentration depolarize to one-half maximum. Thus, among the rather numerous mono-onium compounds tested, choline was unique in not being a receptor activator.

To obtain information about the specifity of the teleologically very important inactivity of choline, a comparative investigation of the depolarizing activities of a series of homocholine isologs was undertaken:

$(CH_3)_3\overset{+}{N}CH_2CH_2\text{-B-R}$ $(CH_3)_3\overset{+}{N}CH_2CH_2CH_2\text{-B-R}$ B = O, S, Se

Choline isologs Homocholine isologs R = —H, —CH_3, —$COCH_3$

MATERIALS AND METHODS

The synthesis of the homocholine isologs has been described.[1, 3]

Single electroplax cells from the organ of Sachs of *E. electricus* were dissected as previously described.[16, 17] The cells were mounted between chambers of the type used previously,[16] except that the airlift was eliminated from the chamber on the innervated side, thus reducing the volume of that chamber to 1 ml. The cells were impaled with 3 M KCl-filled glass microelectrodes with resistances between 7 and 15 MΩ. By means of Ag–AgCl electrodes in Ringer–agar bridges on either side of the electroplax, in conjunction with a microelectrode inside the cell, the potential across either the

innervated or noninnervated membrane could be monitored. A Varian paper recorder was used to record slow changes; action and postsynaptic potentials were photographed from an oscilloscope. Direct and indirect spikes were distinguished from one another by the method of Podleski.[20] Details of impalement and solution-changing techniques are described elsewhere.[22] The Ringer's solution used was of the same composition and pH as that used previously.[18] A total of fifty-six cells from eleven eels was used in this study. A minimum of three cells from at least two eels was used to determine each depolarization curve or minimal blocking concentration.

RESULTS

As is true for most electrically excitable cells, the resting potential across the membrane of the electroplax is negative inside the cell with respect to the outside. For convenience this potential will be considered positive in this paper.

Most of the compounds tested depolarized the innervated membrane of the electroplax, presumably by reacting with synaptic receptor sites. A typical record of an experiment with a depolarizing compound is shown in Fig. 1. For each concentration

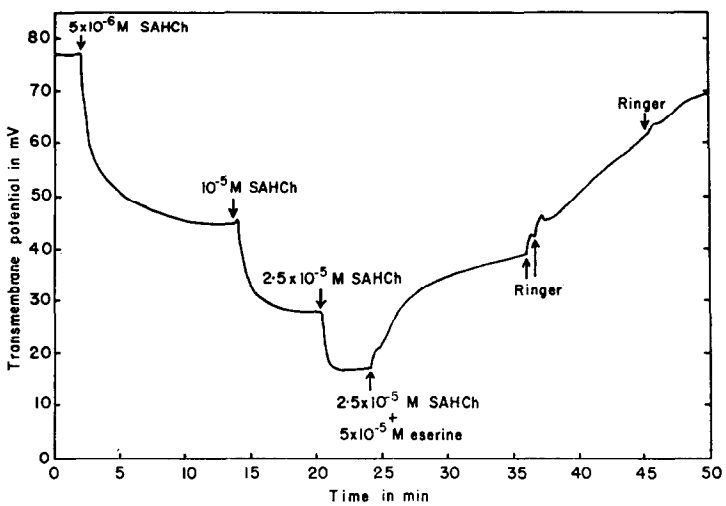

FIG. 1. The effect of acetylthiohomocholine (SAHCh) without and with eserine on the potential across the innervated membrane of an electroplax. The potential is positive outside the cell with respect to the inside.

of acetylthiohomocholine, the potential levels off in time to a steady value, referred to as the steady-state membrane potential for that concentration. This is typical for most depolarizing compounds. If the concentration of the compound is not changed, the lower-than-normal membrane potential will remain steady for more than 1 hr. After 24 min, 5×10^{-5} M eserine was added while the acetylthiohomocholine concentration remained at $2{\cdot}5 \times 10^{-5}$ M. This resulted in a repolarization, in contrast to the well-known potentiation by eserine of the action of ACh at synaptic junctions. As will be seen later, homocholinethiol depolarizes more strongly than does acetylthiohomocholine. Eserine prevents the formation of the more potent hydrolysis product by inhibiting ACh-esterase.

TABLE 1. ACTIVITIES OF CHOLINE AND HOMOCHOLINE, THEIR SULFUR AND SELENIUM ISOLOGS, AND THEIR DERIVATIVES IN THE ELECTROPLAX PREPARATION

Compound	2-Carbon analogs			3-Carbon analogs		
	Equipotent molar ratio	No. of expts.	Average molar conc. required to depolarize to 45 mV	Equipotent molar ratio	No. of expts.	Average molar conc. required to depolarize to 45 mV
Acetylcholine (with eserine)	1	6	3×10^{-6}	67	8	2×10^{-4}
Acetylcholine	3,300*		1×10^{-2}	670	3	2×10^{-3}
Acetylthiocholine (with eserine)	17	7	5×10^{-5}	10	3	3×10^{-5}
Acetylthiocholine	17	6	5×10^{-5}	3	4	8×10^{-6}
Acetylselenocholine (with eserine)†	—	6	—	—	4	—
Acetylselenocholine	33	4	1×10^{-4}	33	4	1×10^{-4}
Choline	33,000‡	3	1×10^{-1}‡	1,000	3	3×10^{-3}
Cholinethiol	17	6	5×10^{-5}	3	3	8×10^{-6}
Cholineselenol	?§	5	—	33	4	1×10^{-4}
Methoxycholine	67	3	2×10^{-4}	20	3	6×10^{-5}
Methylthiocholine	7	3	2×10^{-5}	2	3	6×10^{-6}
Methylselenocholine	3	4	1×10^{-5}	2	3	6×10^{-6}

* Acetylcholine without eserine does not give a typical depolarization curve, as will be described in a forthcoming paper by Eva Bartels. The figure given here is only tentative.

† Acetylselenocholine and acetylselenohomocholine with eserine do not depolarize to as low as 45 mV, even at very high concentrations.

‡ Choline, even at 10^{-1} M, depolarized only to 65 mV, so the equipotent molar ratio should be even higher than 33,000.

§ This value was unobtainable because cholineselenol oxidizes so rapidly to choline diselenide.

The steady-state membrane potentials from all experiments with the depolarizing compounds tested were plotted against the concentrations applied. A membrane potential of 45 mV was chosen as a standard depolarization for comparing the potency, because it is roughly halfway between the usual resting potential of about 80 mV and a steady-state potential of about 15 mV which is the maximum depolarization obtainable with the usual depolarizing agents. In the case of ACh with eserine, the average molar concentration of ACh required to produce a steady-state potential. of 45 mV was 3×10^{-6} M (all averages were rounded to the nearest whole number) ACh with eserine was chosen as a standard of reference in calculating the equivalent molar ratios.

Table 1 shows the equipotent molar ratios obtained. Each value is the average of three or more cells from two or more eels. In the second, third, and fourth columns are the values that were reported earlier.[4] In the last three columns are the ratios for the three carbon analogs of these same compounds, the number of cells tested, and the average molar concentration required to produce a steady-state potential of 45 mV. Homocholine disulfide and diselenide acted as receptor inhibitors. These results will be presented in a future paper.

DISCUSSION

It was noted as early as 1911 by Hunt and de Taveau[23] that the acetyl esters of formocholine and homocholine were considerably less active than ACh. The importance of structural and stereochemical features in the action of depolarizing agents has been emphasized by Pfeiffer[24] and Ing,[25] among others. An ester grouping is not required for pharmacological action, as was established by the finding that not only esters but also various alkyl-, alkoxyalkyl-, and alkylcarbonylalkyl-trimethylammonium salts possessed activity in a variety of preparations,[26–28] this activity being quite sensitive to the length of the alkyl chains and the position of the ether and carbonyl oxygens.

It can be seen in Table 1 that, while acetylhomocholine is much less active than ACh, the ability of homocholine to act as a receptor activator greatly exceeds that of choline. Thus, while choline is almost ineffective even at a concentration 33,000 times as high as the concentration of ACh required to depolarize to one-half maximum, acetylhomocholine is only fifteen times more active than homocholine. Homocholine, in contrast to choline, can induce complete depolarization in the electroplax preparation (Fig. 2). For all compounds, except ACh, increasing the distance between the quaternary nitrogen and oxygen, sulfur, or selenium, resulted in an increase in depolarizing activity.

The observation that ACh-receptors and ACh-esterase display similar stereospecificity when presented with some optically active compounds has been used to suggest the "near identity of these two bioreceptors for ACh".[29] This proposal is incompatible with the following four observations. (1) While ACh, acetylthiolcholine, and acetylselenolcholine are hydrolyzed by electric eel ACh-esterase at very similar rates,[30] they have widely different depolarizing activities in the electroplax[4] and in other preparations.[2] (2) Molecules devoid of anything resembling ester groupings were as effective as receptor activators as ACh itself. (3) After blocking electrical activity with DFP without depolarizing the membrane, addition of carbamyl

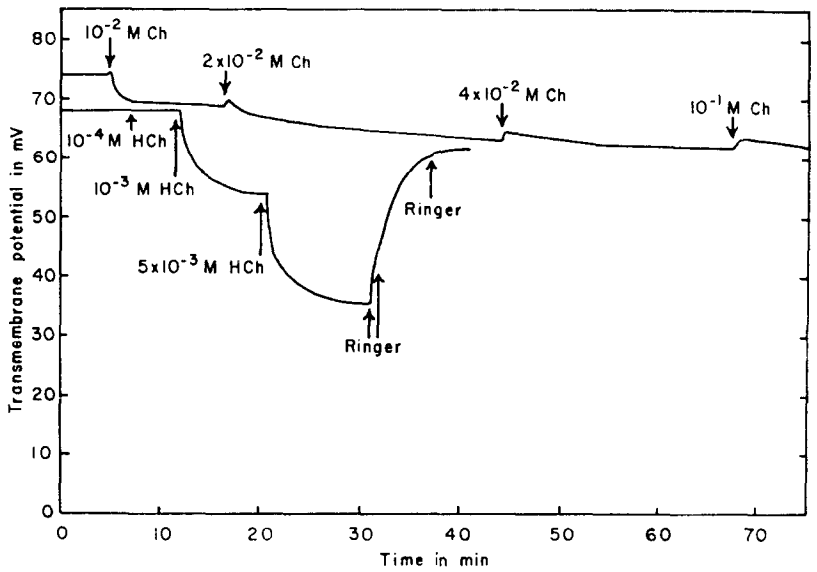

FIG. 2. The effect of choline chloride (Ch) and homocholine iodide (HCh) on the potential across the innervated membrane of the electroplax. Experiments with two different cells are shown. The homocholine was dissolved in normal eel Ringer's solution. The choline was dissolved in a special Ringer's solution in which the sodium concentration was reduced by the amount of choline added, so that the osmolarity would remain constant. Therefore, the 10^{-1} M choline solution contained slightly less than half the normal concentration of sodium. The small depolarization seen with choline may be caused by the low sodium. T. R. Podleski (personal communication) has found that low sodium solutions (sucrose replacement) have little effect on the resting potential of the electroplax.

choline-induced depolarization.[31] (4) Different relative orders of affinity are exhibited by inhibitors reacting with either enzyme or with receptor.[21]

While much evidence has accumulated that the receptors of the electroplax and the active site of ACh-esterase both possess an anionic site, the active groups in the receptor inducing depolarization equivalent to the esteratic site of the enzyme must be different.[4, 22] Hydrophobic bonding has been postulated as playing a major role in the induction of depolarization.[4, 32]

The results summarized in Table 1 emphasize again the unique lack of depolarizing activity of choline. Replacement of the hydroxy group of this compound by thiol or selenol groups or lengthening of the alkyl chain between the quaternary nitrogen and the alcohol group restores depolarizing action. It is well established that alcohols have a considerably greater capacity to form hydrogen bonds than have analogous thiols.[33] Oxygen will form hydrogen bonds more effectively than sulfur whether it acts as a hydrogen donor[33] or as a hydrogen acceptor.[14, 33] Thus, it seems reasonable to assume that the unique lack of depolarizing activity of choline may be correlated to the much higher capacity of this compound to form hydrogen bonds through its hydroxy group than that of any of the nonalcoholic quaternary ammonium compounds considered here. High hydrogen-bonding capacity, in turn, would suggest a high degree of hydration for the hydroxy group, which may interfere with the induction of a conformational change. In homocholine, hydration would also be expected to occur. Lengthening of the alkyl chain, however, would be expected to enable a

methylene group rather than the hydrated hydroxy group to interact with the active site.

The striking precision and specificity in the action of ACh at the synapse is, of course, partly dependent on the extremely great difference in the abilities of ACh and of its hydrolysis product to induce depolarization. The findings reported here emphasize the great structural specificity in the inactivity of choline in view of the considerable activity of very closely related compounds.

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