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ANALOGS OF PARASYMPATHETIC NEUROEFFECTORS—II.

COMPARATIVE PHARMACOLOGICAL STUDIES OF ACETYLCHOLINE, ITS THIO AND SELENO ANALOGS, AND THEIR HYDROLYSIS PRODUCTS*

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Abstract—Acetylthiocholine and acetylselenocholine exert acetylcholine-like effects on the guinea pig ileum and frog rectus abdominis preparations. With the latter preparation, responses to acetylthiocholine and acetylselenocholine, in contrast to that to acetylcholine, are not enhanced by the addition of an anticholinesterase. This is attributable to the relatively high activity of the hydrolysis products, cholinethiol and cholineselenol; acetylthiocholine and acetylselenocholine undergo enzymatic hydrolysis at approximately the same rate as does acetylcholine. The hydrolysis products of acetylthiocholine and acetylselenocholine, which have effects on the guinea pig ileum comparable to those of the parent esters, are readily oxidized in air to the relatively inactive choline disulfide and choline diselenide, respectively. These observations are helpful in explaining many of the apparently contradictory statements in the literature regarding the actions of acetylthiocholine.

DURING the course of a systematic study of isologous oxygen, sulfur, and selenium compounds of biological interest, the analog of acetylcholine in which the ether-oxygen had been replaced by selenium (i.e. 'acetylselenocholine') was synthesized.[1] Since analogous selenium and sulfur compounds are generally similar in terms of molecular size but quite different in electron distribution,[2–4] and since acetylcholine is one of the rather small number of compounds with biological activities which can be considered in terms of molecular interactions,[5] a comparative pharmacological study of acetylcholine, acetylthiocholine, and acetylselenocholine (Fig. 1) was undertaken.

The first report of some of the pharmacological effects of the comparable sulfur analog of acetylcholine was published by Alexander *et al.*[6] in 1938, and this paper was followed almost immediately by a more detailed study by Renshaw *et al.*[7] These groups of workers were primarily concerned with the actions of 'acetylthiocholine' on the cat blood pressure, and their observations, although different in many respects, indicated that acetylthiocholine showed qualitatively many of the effects that are

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characteristic of acetylcholine. Alexander *et al.* stated that "the duration of action (of acetylthiocholine) indicates that it is probably destroyed rather rapidly by the blood" and that "when acetylthiocholine is placed in freshly-drawn blood at room temperature for one-half to one hour, its action is greatly reduced and in some cases reversed". This conclusion was apparently at variance with the observation noted earlier in the same paper that physostigmine did not augment the action of acetylthiocholine.

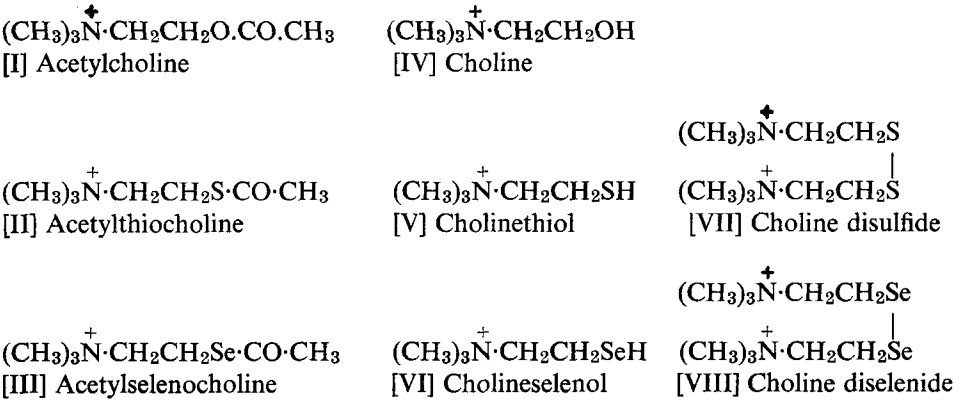

FIG. 1 Structural formulae of thio and seleno analogs of choline and acetylcholine.

More recently, Marquardt and Vogg[8] re-examined the actions of acetylthiocholine on the blood pressure and respiration in the cat. Their data generally agreed with those of the earlier workers, although they concluded that acetylthiocholine acts by releasing adrenaline. In addition, they examined the effects of acetylthiocholine on the isolated rabbit intestine, without presenting experimental results, and stated that on this preparation the analog acted in the same way as acetylcholine, but its action was slower in onset. Marquardt and Vogg investigated the hydrolysis of acetylthiocholine *in vitro* and found that nonspecific serum esterase hydrolyzed both acetylcholine and acetylthiocholine at the same rate, but that when "specific erythrocyte acetylcholinesterase" was employed, acetylthiocholine was hydrolyzed more slowly than acetylcholine; according to these authors, this was to be expected from the observations on the relative stability of thiol esters reported by Wieland *et al.*[9]

Liljestrand and Zottermann[10] compared the effects of close arterial injection of acetylthiocholine on the action potential in the sinus nerve deriving from the chemoreceptors of the carotid body. They reported that acetylthiocholine was "sometimes even more potent" than acetylcholine, but "in other experiments it displayed the same or even a somewhat smaller effect". It was characteristic, however, that the discharge evoked by acetylthiocholine was slower in onset and lasted much longer than those elicited by acetylcholine. They also found that, whereas pretreatment with an anticholinesterase caused a tenfold increase in the activity of acetylcholine and prolonged its effect, it caused acetylthiocholine to produce "considerably weaker effects". They reported that this effect was reproducible and in no way reduced the enhancement of the response to acetylcholine.

A few other papers on the pharmacology of acetylthiocholine have been published[11,12] and many studies on its hydrolysis by cholinesterase have been made.[13–22] In the discussion of one of the early papers, Glick,[13] referring to the work of Alexander *et al.*, acknowledged that the reported short duration of action of acetylthiocholine was inconsistent with the observation that its action was not augmented by physostigmine. He suggested that "perhaps it is so because of the relatively large doses of thio compounds required compared with the oxygen analogs, in which case the rate of destruction of the drugs would be a less pertinent factor". It is not clear how this explains the short duration of action of acetylthiocholine on the cat blood pressure since, in molecular terms, the reported dose of acetylthiocholine required for a given effect was only about three times that of acetylcholine.

Koelle and Friedenwald[14] compared the hydrolysis of acetylthiocholine with that of acetylcholine, using enzymes from a variety of sources; their results are reported as the ratio of the hydrolysis rate of acetylthiocholine to that of acetylcholine. If these ratios are plotted against actual hydrolysis rate for acetylcholine (Fig. 2), it

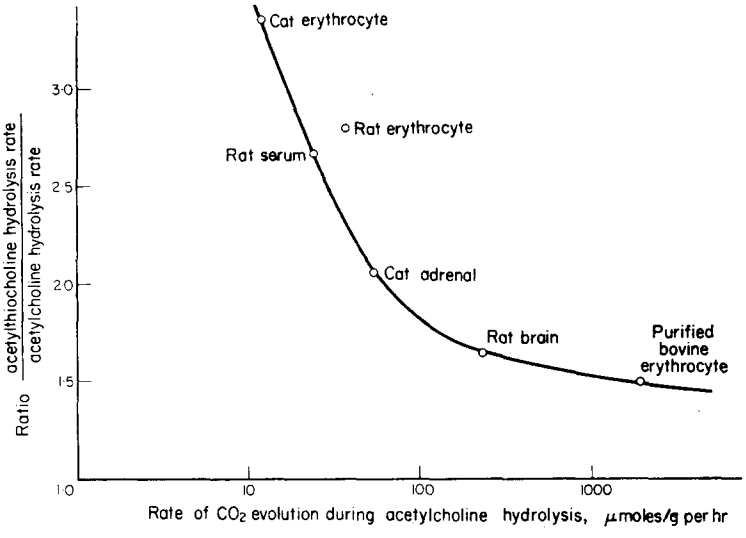

FIG. 2. Plot of the ratio of hydrolysis rates of acetylthiocholine and acetylcholine, using the same source of enzyme, against the actual rate of hydrolysis of acetylcholine obtained by using cholinesterase of varying specificity from six different sources (each represented by a point on the graph); from the data of Koelle and Friedenwald.[14]

seems evident that the more specific the cholinesterase, the smaller is the ratio of the rates; this tendency parallels that reported by Marquardt and Vogg.[8] Subsequently, Van Rossum and Hurtmans,[11] Heilbronn,[18] and Ellman *et al.*[20] found that the hydrolyses of acetylcholine and acetylthiocholine catalyzed by acetylcholinesterase proceed at approximately the same rate. Thus, it is generally agreed that the rates of hydrolysis of acetylcholine and acetylthiocholine are of the same order despite the impression reported by Alexander *et al.*

In addition to studies of the pharmacological effects of acetylthiocholine, the activity of 'thiocholine', a product of hydrolysis of acetylthiocholine, was measured on the isolated rabbit ileum by Wells and Mallov[23] and reported by them to be about three times that of choline. Accordingly, a study of the relevant literature reveals an apparent paradox. Thus, acetylcholine and acetylthiocholine are two structurally similar compounds having similar pharmacological effects on several preparations; they undergo enzymatic hydrolysis at about the same rate, but the effects of acetylcholine are markedly enhanced by the presence of a cholinesterase inhibitor, whereas those of acetylthiocholine are considerably diminished.

The purpose of this paper is to present the results obtained in a comparative study of acetylcholine, acetylthiocholine, and acetylselenocholine. These results will be restricted to those obtained with the isolated guinea pig ileum and the frog rectus abdominis preparation; work with several other systems is now in progress.

EXPERIMENTAL

Materials

Acetylcholine chloride was purchased from Merck and acetylthiocholine iodide and eserine sulfate from Nutritional Biochemicals Corp. Acetylselenocholine bromide[1] was kindly prepared in this laboratory by Dr. W. H. H. Günther. Cholinethiol (thiocholine, β-mercaptoethyltrimethylammonium iodide; (V) in Fig. 1), was prepared in solution and without isolation, by the hydrolysis of acetylthiocholine either by the acetylcholinesterase of the electric eel or, as was the case in the preliminary experiments, by the method of Böhme and Schlephack.[24] The purity of the material obtained by the latter method was determined by iodometric titration.

Preparations

A. Guinea pig ileum. A guinea pig of either sex, usually weighing between 150 and 250 g, was killed by a blow on the head and bled. The mesenteric membrane was completely removed from the terminal 10-cm strip of ileum, which was then placed in a dish of Tyrode's solution at room temperature. The lumen of the gut was perfused by inducing peristalsis with the minimum hydrostatic pressure necessary, usually equivalent to about 2 cm of Tyrode's solution. The terminal 3 to 4 cm of ileum was then suspended in Tyrode's solution, in a bath of 4 ml capacity, at $37{\cdot}0° \pm 0{\cdot}02$, and attached to a light (total weight excluding load $= 2{\cdot}0$ g) isotonic frontal-writing lever giving a magnification of 4 and bearing a load of 0·5 g. The organ bath was connected to coils of glass tubing so that the bathing fluid could be replaced by upward displacement and overflow, with preheated Tyrode's solution. The coils were of such volume that sufficient solution could be run through the organ bath to effect a complete change of the bathing fluid without exposing the muscle to air and without cooling. Originally, drugs were added directly to the bath, a procedure which, when employed with a rapidly responding muscle such as gut, does not permit a high degree of reproducibility because of variations in the time needed for the mixing of the drug solutions *in situ*. In later experiments a system giving much more reproducible responses was employed; in this the drug solution was diluted before being added to the organ bath by perfusion and overflow. The apparatus employed is shown in Fig. 3. The modified Tyrode's solution used in all experiments contained $5{\cdot}4 \times 10^{-3}$ M potassium, which

is double the usual concentration. The agonist activity of the compounds tested was estimated by determining the equipotent molar ratios relative to acetylcholine by means of the usual (2 + 2) dose assay method. Throughout these assays a cycle of 90 sec with a contact time of 10 sec was employed.

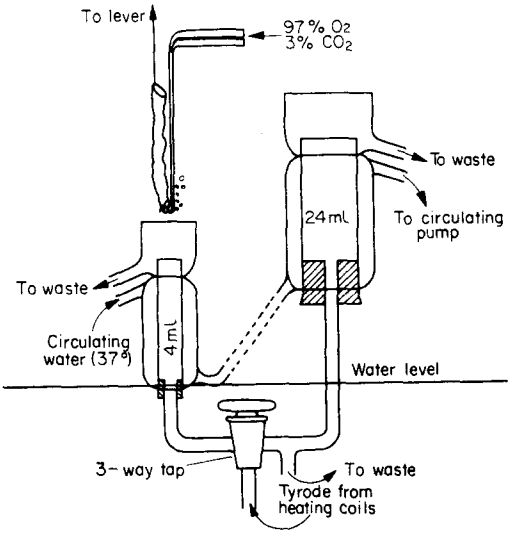

FIG. 3. Apparatus used for assays on guinea pig ileum. The drug solution is diluted in the larger bath before it is perfused into the smaller bath containing the preparation.

B. Frog rectus abdominis. The frogs (*Rana pipiens*) were kept at 4° for several days before use. The muscle was set up in a 4-ml organ bath maintained at 20°, under a tension of 5 g, which roughly corresponds to the resting tension *in vivo*. The muscle contracted whenever the tension was removed, even without the addition of any drug to the bath, and the effect of a dose of drug was measured by the difference between the height of the contraction it produced and the height of the contraction produced solely by the removal of the weight, the 'zero value'. The weight was removed smoothly by means of a lift device (Fig. 4) immediately before the drug was injected.

The contact time normally used was 2 min; at the end of this period the drug solution was displaced from the bath by fresh frog Ringer's solution and the muscle was washed for 10 sec. After 1 min the weight was slowly lowered until the muscle returned to its normally contracted length, equivalent to the 'zero value'. One minute later, after washing for 5 sec, the weight was slowly lowered to stretch the muscle to its original length. The contractions of the muscle were recorded on a smoked drum with an isotonic frontal-writing lever, and throughout the period of drug contact a 60-cycle vibrator was employed.

RESULTS

Preliminary experiments with the guinea pig ileum preparation suggested that acetylthiocholine and acetylselenocholine acted like acetylcholine itself although, as expected, these compounds were less active, having equipotent molar ratios of 320 ± 8·7* and 220 ± 14·7,* respectively, relative to acetylcholine. The log–dose response curves for all three compounds were parallel; the responses of the gut to

* Standard error of mean.

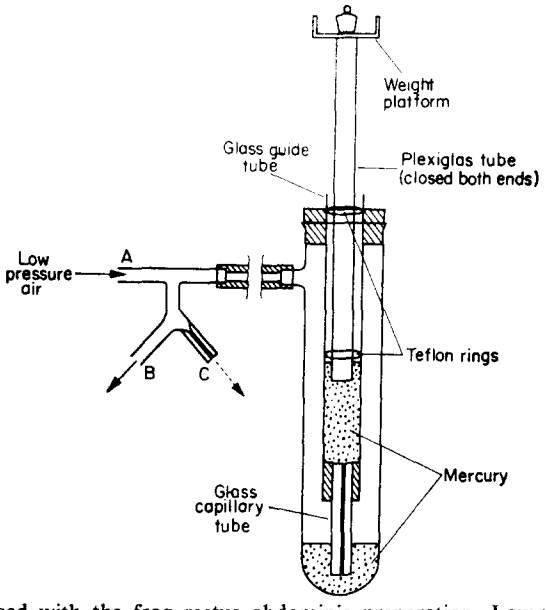

FIG. 4. Lift device used with the frog rectus abdominis preparation. Low-pressure air (approx. 1 lb/in^2) from a diaphragm-type regulator enters the multijunction tube at A, and its escape from B and C is prevented by clipped rubber tubing. The Plexiglas tube is raised by the mercury only until the head of mercury (together with the weight of the tube) is equal to the air pressure. Controlled release is obtained by removing the clip from the tubing over the capillary limb C, and return of the platform to its lowest position is achieved by removing the clip on B. The clip on C is then replaced, and the cycle may be started again by clipping B.

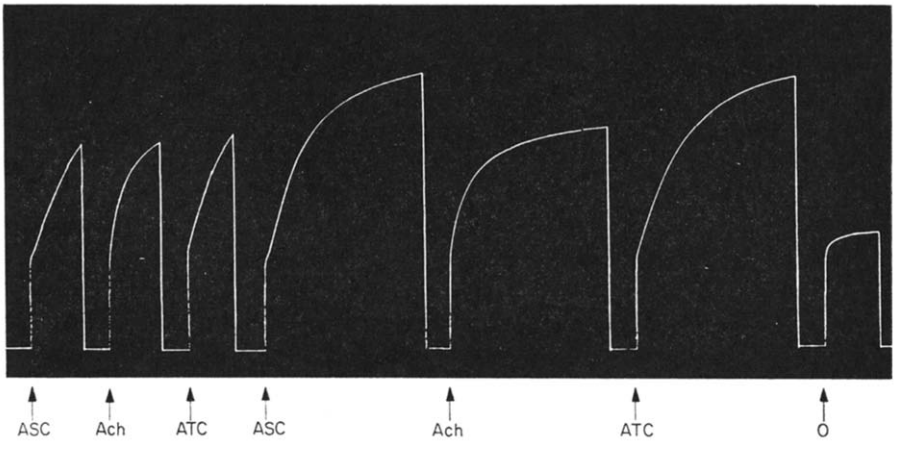

FIG. 5. Frog rectus abdominis preparation (no eserine present). Contact times of 2 min and 6 min were employed; 0 = zero value (i.e. tension removed but no drug added to bath).
ASC = 5×10^{-6} M acetylselenocholine. Ach = 10^{-6} M acetylcholine. ATC = $7{\cdot}5 \times 10^{-6}$ M acetylthiocholine. (Concentrations given were those actually present in the organ bath.)

acetylthiocholine and acetylselenocholine were as rapid as to acetylcholine itself, and no unusual slowness of relaxation was observed on washing out the drug.

By using the frog rectus abdominis preparation it was possible to study the effects of cholinesterase on the actions of these compounds *in situ*. Figure 5 shows some typical

results obtained with this preparation in the absence of eserine. It should be noted that the response to a concentration of drug is given by the difference between the height of the contraction it produces and the 'zero value'. Two features of this tracing are of particular significance. Firstly, the shape of the 'contractions': those of acetylthiocholine and acetylselenocholine are similar to each other and appreciably different from that produced by acetylcholine. Secondly, it is evident that when the contact time for the drug is extended from 2 to 6 min, the height of the acetylcholine contraction is not increased to any great extent, whereas that caused by either acetylthiocholine or acetylselenocholine does not reach a maximum even within 6 min. These observations seem to be in agreement with those of Liljestrand and Zottermann,[10] in indicating that the action of acetylthiocholine is slower in onset than that of acetylcholine and is more prolonged.

Figure 6 demonstrates directly the effects of eserine on equiactive concentrations of acetylcholine, acetylthiocholine, and acetylselenocholine. The first three contractions

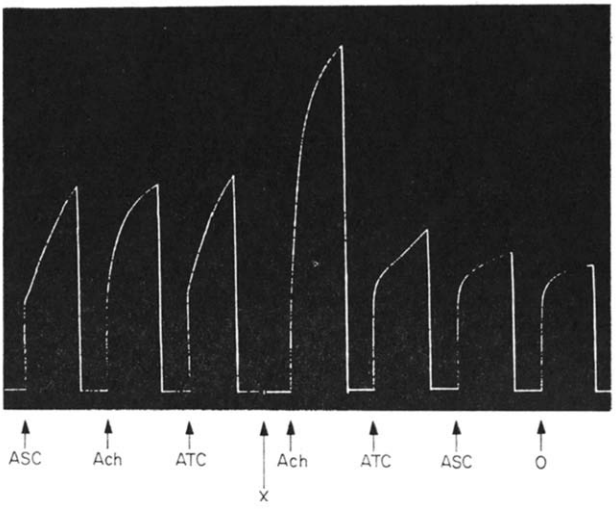

FIG. 6. Frog rectus abdominis preparation. 0 = Zero value. ASC = 5×10^{-6} M acetylselenocholine. Ach = 10^{-6} M acetylcholine. ATC = $7{\cdot}5 \times 10^{-6}$ M acetylthiocholine. At X, Ringer's solution was replaced by one containing eserine sulfate ($2{\cdot}5 \times 10^{-5}$ M).

were produced with no eserine present; again, the marked difference in the shapes of the contractions may be seen. At X, the Ringer's solution was replaced by one containing eserine, and the preparation was allowed to equilibrate for 30 min. The enhancement of the response to acetylcholine and the reduction of the responses to both acetylthiocholine and acetylselenocholine after eserine are clearly demonstrated. This result on the frog rectus preparation is compatible with results obtained by previous workers using other preparations.[8, 10]

Acetylcholinesterase modifies the actions of acetylcholine by hydrolyzing it to the relative inactive and stable choline (IV). Several experiments with freshly prepared cholinethiol (β-trimethylammonium ethanethiol, 'thiocholine'; [V] in Fig. 1) showed that this product of the hydrolysis of acetylthiocholine is, rather surprisingly, more active than its parent ester and that it is unstable in solution, being readily oxidized[25] in air to the corresponding choline disulfide [*bis*(β-trimethylammonium ethyl)

disulfide; [VII] in Fig. 1], which is relatively inactive. These points are demonstrated in Fig. 7. By preparing the cholinethiol [V] immediately before adding it to the organ bath (by hydrolyzing acetylthiocholine with a relatively large excess of acetylcholinesterase), it was possible to minimize the loss caused by oxidation.

The relatively low biological activity of 'thiocholine' reported by Wells and Mallov[23] can presumably be attributed to their use of a commercial preparation of this compound, likely to be in the disulfide form.

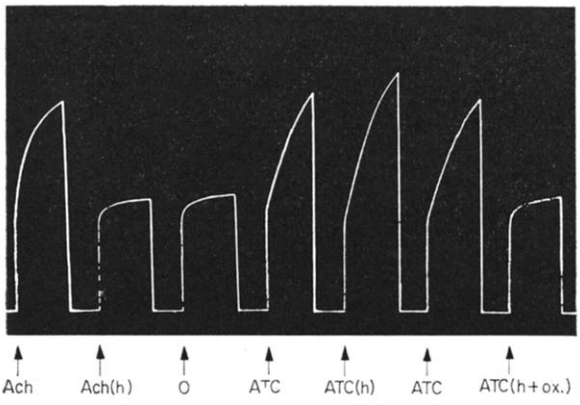

FIG. 7. Frog rectus abdominis preparation (no eserine present). 0 = Zero value. Ach = 10^{-6} M acetylcholine. ATC = $7{\cdot}5 \times 10^{-6}$ M acetylthiocholine. The ester was hydrolyzed (h) by acetylcholinesterase immediately before adding to the bath; (h+ox) denotes that, after enzymatic hydrolysis and before biological testing, sufficient 0·01 N iodine solution was added to effect complete oxidation.

Figure 7 shows concentrations of acetylcholine ($1{\cdot}0 \times 10^{-6}$ M) and acetylthiocholine ($7{\cdot}5 \times 10^{-6}$ M) that are equiactive on the frog rectus preparation, in the absence of eserine. After enzymatic hydrolysis of acetylcholine, the response is reduced to zero. In the case of acetylthiocholine, however, prior hydrolysis leads to an enhancement of the response. If the theoretical amount of 0·01 N iodine solution is added to cholinethiol [V] from hydrolyzed acetylthiocholine to convert it completely to the choline disulfide [VII],[22] the activity disappears. Although not shown here, it was later confirmed that the addition of 0·01 N iodine solution to nonhydrolyzed acetylthiocholine did not affect the response to the thiolester.

Cholineselenol (β-trimethylammonium ethaneselenol; [VI] in Fig. 1), produced by the enzymatic hydrolysis of acetylselenocholine, is more active than its parent ester, which in turn is more active than choline diselenide [VIII] (to which the selenol is converted exceedingly rapidly). The relatively high activity of the acetylselenocholine on the noneserinized frog rectus may be attributed to the steady production of cholineselenol [VI] immediately adjacent to the cholinergic receptors, where it acts before being oxidized. The characteristic shapes of the acetylthiocholine and acetylselenocholine contractions on the noneserinized rectus may be accounted for in terms of the steady conversion of the weakly active esters to their more active hydrolysis products under conditions which delay their oxidation. Figure 8 shows the effects on an eserinized rectus muscle of acetylthiocholine and acetylselenocholine pretreated with acetylcholinesterase. Although the hydrolysis *in vivo* of acetylselenocholine led to

an enhancement of the response (Fig. 6), this effect was not seen with hydrolysis *in vitro* (i.e. treatment with acetylcholinesterase prior to addition to the preparation; Fig. 7). This result most probably is attributable to the fact that, despite all the precautions taken to prevent it, enough of the active cholineselenol [VI] was oxidized

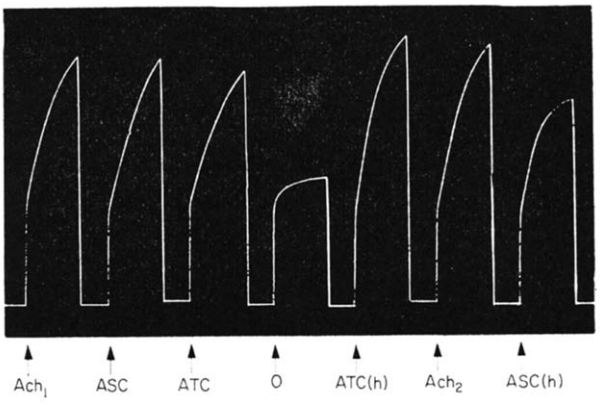

FIG. 8. Frog rectus abdominis preparation. 0 = Zero value. Ringer contained $2{\cdot}5 \times 10^{-5}$ M eserine, $Ach_1 = 4 \times 10^{-7}$ M. $Ach_2 = 4{\cdot}5 \times 10^{-7}$ M acetylcholine. ASC = $2{\cdot}2 \times 10^{-5}$ M acetylselenocholine. ATC = $1{\cdot}5 \times 10^{-5}$ M acetylthiocholine. The ester was hydrolyzed (h) by acetylcholinesterase immediately before being added to the bath.

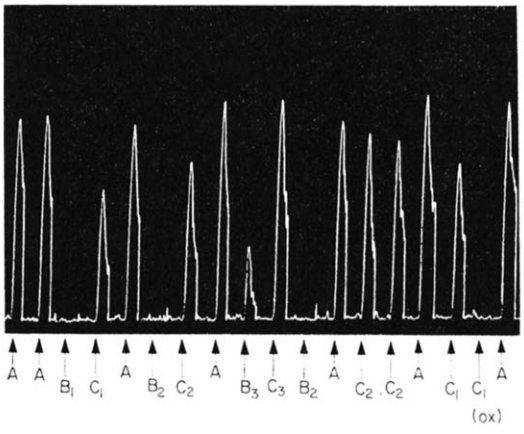

FIG. 9. Guinea pig ileum preparation. Cycle of drug administration = 90 sec, drug contact time = 10 sec. A = 5×10^{-6} M acetylselenocholine. B = 5×10^{-6} M acetylcholine to which acetylcholinesterase was added before injection. C = 5×10^{-6} M acetylselenocholine to which acetylcholinesterase was added before injection. Subscripts refer to times allowed for hydrolysis: 1 = 60 sec; 2 = 45 sec; 3 = 30 sec; (ox) denotes the addition of sufficient 0·01 N iodine to effect complete oxidation prior to injection.

to the relatively inactive choline diselenide, [*bis*(β-trimethylammonium ethyl) diselenide; [VIII] in Fig. 1 to reduce the response to less than that induced by acetylselenocholine. Since cholinethiol [V] is less readily oxidized than its selenium analog, hydrolysis of acetylthiocholine *in vitro* did produce the expected increase in response.

Figure 9 shows a similar effect with the guinea pig ileum. A solution of acetylcholine, equimolar with that of the acetylselenocholine used in the experiment, was

employed to indicate the completion of hydrolysis *in vitro* of the selenolester, since acetylcholine and acetylselenocholine undergo enzymatic hydrolysis at about the same rate.[26]

Because the active product of the enzymatic cleavage of acetylselenocholine undergoes rapid oxidation, resulting in a loss of activity, an attempt was made to reduce the time allowed for hydrolysis and so reduce, as far as possible, the time available for oxidation of the hydrolysis product. This is shown in C_1, C_2, and C_3 of Fig. 9. The time was not reduced to less than 30 sec for, as B_3 shows, the hydrolysis of acetylcholine was incomplete in this time. The last four contractions illustrate that acetylselenocholine follows a reaction sequence similar to that of acetylthiocholine.

Attempts to obtain cholineselenol [VI] by reduction of the stable choline diselenide [VIII] with sodium borohydride merely confirmed the ready oxidizability of the selenol. An aliquot of a 1% solution of the diselenide (diiodide) in water was treated with the theoretical amounts of hydriodic acid and sodium borohydride. The same reagents also were added to a volume of water equal to that of the diselenide solution and, after 3 min, the three solutions: (a) nonreduced diselenide, (b) reduced diselenide, and (c) reducing agent plus water, were diluted with Tyrode's solution and tested for activity on the frog rectus preparation. When freshly prepared, i.e. within 2 min of diluting the reduced solution, (b) produced a response greater than that from (a); but within 10 min the two responses had become identical. The response to (c) was the same as the 'zero value' and thus the enhancement of the diselenide response after reduction could not be attributed to the presence of any of the substances used to bring about the reduction.

The oxidation of cholinethiol [V], although much slower than cholineselenol [VI], does proceed fairly rapidly, as is shown in Fig. 10. In this experiment the cholinethiol [V] was prepared by hydrolyzing the ester with an excess of eel acetylcholinesterase in a completely filled and sealed flask. After allowing sufficient time for complete

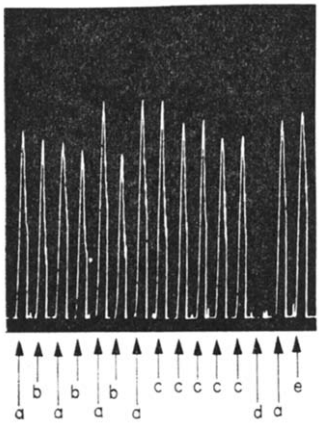

FIG. 10. Guinea pig ileum preparation. Cycle = 90 sec, drug contact time = 10 sec; a = 10^{-5} M acetylthiocholine; b = 10^{-5} M acetylthiocholine, hydrolyzed with acetylcholinesterase in a sealed flask and then exposed to atmosphere in open beaker. c = Same as b, except freshly removed from sealed flask; d = 10^{-5} M acetylthiocholine, hydrolyzed with acetylcholinesterase and oxidized by adding 0·01 N iodine solution; e = 10^{-5} M acetylthiocholine to which was added a volume of 0·01 N iodine equal to that used in d.

hydrolysis, some of the solution was placed in an open beaker, and aliquots of this were added to the organ bath, alternating with nonhydrolyzed acetylthiocholine solution of the same concentration. The steady decline in the response to 'cholinethiol' [V], (b), as compared to acetylthiocholine (a), is evident. The variations in response to the ester undoubtedly arise from the fact that the concentrations employed were at the lower limit of the linear part of the log dose response curve. Replacement of the cholinethiol solution exposed to the air in the beaker with fresh solution from the sealed flask (c) led initially to an increase in the response of the gut, the magnitude of which then steadily declined. At (d) the cholinethiol was oxidized completely, immediately prior to injection, by adding the theoretical quantity of 0·01 N iodine solution. That this amount of iodine had in itself no effect on the preparation was demonstrated by the addition of the same dose of acetylthiocholine to which iodine had been added (e).

Both acetylthiocholine and acetylselenocholine produce what Ariëns *et al.*[27] have called "non-competitive auto-inhibition"; that is, when increasing doses of the drug are added to the eserine-treated preparation, the magnitude of the contractions increased to a maximum, which is the same maximal response as that produced by acetylcholine. Increasing the drug concentration beyond that required for maximal contraction led to a progressively decreasing response, the drug appearing to act as its own antagonist (Fig. 11). Similar observations with the *n*-butyl and *n*-pentyltrimethylammonium salts on this particular preparation have been reported by Ariëns

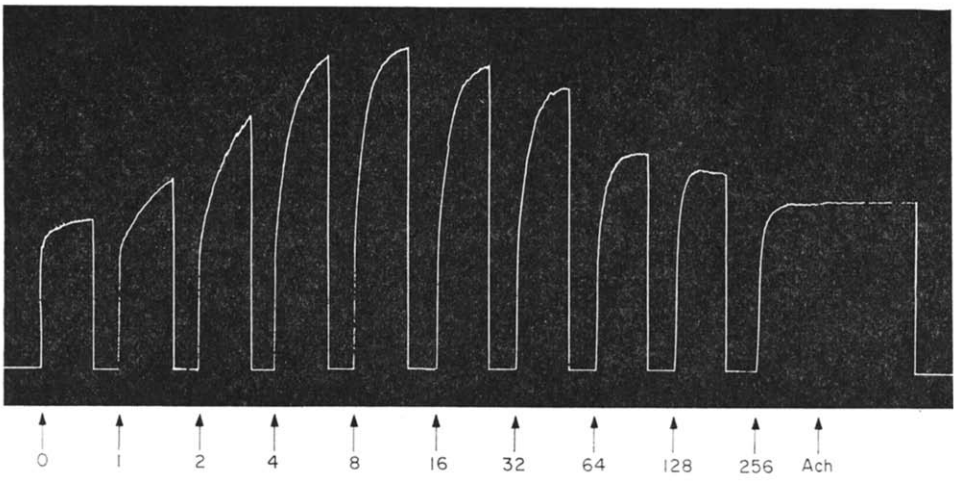

FIG. 11. Frog rectus abdominis preparation. Ringer contained $2{\cdot}5 \times 10^{-5}$ M eserine. Dose–response curve of acetylselenocholine. Figures refer to concentrations of acetylselenocholine ($\times 10^{-5}$ M). At Ach, 10^{-5} M acetylcholine was added; this concentration had previously been found to produce approximately 90% of the maximal contraction in the absence of acetylselenocholine.

et al. Such behavior might be explained by analogy with the well-known phenomenon of substrate inhibition of an enzyme.[28] Thus, the acetylthiocholine or acetylselenocholine molecules may be capable of acting as true agonists i.e. able to evoke the maximal response of which the tissue is capable, as distinct from a 'partial agonist',[29]

when entering into at least a two-point attachment with the receptor. At higher concentrations the number of drug molecules in the vicinity of the receptors may be sufficient to impose a one-point attachment of the quaternary nitrogen atom to the 'anionic site' and, when so linked, it is conceivable that the same molecule may act as an antagonist.

The phenomenon of the bell-shaped dose–response curve also may be accounted for in terms of Paton's rate theory[30] by assuming that, in the case of the sulfur and selenium analogs, the drug molecules are more firmly bound to the receptors than is acetylcholine itself. A high concentration of the drug in the biophase, together with the low values of K_2 (the constant for the rate of dissociation of the drug-receptor complex), would serve to reduce the rate of drug-receptor dissociation to such a level that this type of antagonism would result.

The results are summarized in Fig. 12. The rates of enzymatic hydrolysis *in vitro* have been found to be of the same order, with a variety of sources of enzyme, including the frog rectus muscle itself.[25] The nature of the compounds makes it difficult to obtain reproducible quantitative values for the activities of either cholinethiol or cholineselenol and the values for the activities of acetylthiocholine and acetylselenocholine on the noneserinised frog rectus are of little significance for, as has been shown, under these conditions the contraction is produced by a mixture of three different substances, that is, the ester, its hydrolysis product, and the final oxidation product.

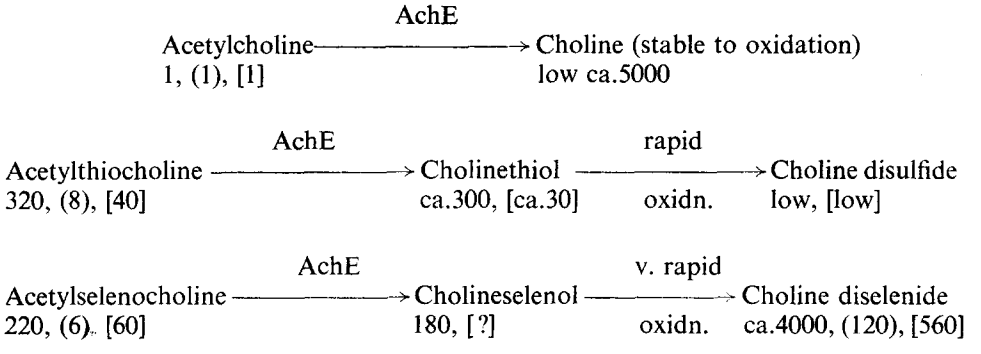

FIG. 12. Summary of results. Activities are given as equipotent molar ratios relative to acetylcholine (Ach = 1). Unbracketed values refer to guinea pig ileum and all others to frog rectus preparation (without eserine) and [with eserine].

DISCUSSION

It is interesting to speculate on the possible reasons for the unusually high activity of cholinethiol [V] and cholineselenol [VI], relative to their parent esters. The acetylcholine receptor[31–38] is believed to involve an 'anionic site' to which the quaternary nitrogen atom is attached, and an 'esteratic site' linked to the highly polarized ester group. Deacylation, of course, removes this latter center of high electron density in the molecule, and the low polarity of the residual hydroxy group is sometimes held to account for the low activity of choline itself.

There is some doubt whether the postulated esteratic site of the cholinergic receptor is anionic or cationic in character; the evidence presented here lends support to the latter view.

While, at physiological pH, choline exists in the alcohol form, there is evidence that the corresponding thiol and selenol molecules are predominantly in the mercaptide and selenomercaptide forms, respectively. The p*K*a of cholinethiol [V] (thiocholine) has been reported to be about 7·7;[17] this accounts for the fact that in the titrimetric estimation of acetylthiocholine by hydrolysis at pH 8, more base was consumed than was required for the neutralization of the liberated acetic acid, as a result of the simultaneous production of titratable cholinethiol [V].[18]

The high acidity of hydrogen selenide,[39] relative to that of hydrogen sulfide, suggests that selenols should be stronger acids than are the corresponding mercaptans which, in turn, are stronger acids than are the corresponding alcohols.[40] It may be assumed, therefore, that at physiological pH a selenol will be at least as highly ionized as is the corresponding thiol and probably considerably more so. Thus, while the cationic heads of choline [IV], cholinethiol [V], and cholineselenol [VI] should have the same ability to bind to the anionic site of the receptor, presumably the sulfur and selenium of the cholinethiomercaptide and cholineselenomercaptide ions could be attached to the esteratic site much more effectively than is the uncharged hydroxy group of choline.

In view of the pharmacologically important differences between 'mono-onium' and 'bis-onium' compounds, it should be noted that the oxidation of cholinethiol [V] and cholineselenol [VI] represents a convenient method of converting the former into the latter class of compounds.

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