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DIFFERENTIATION OF ACETYLCHOLINE AND SUCCINYLCHOLINE RECEPTORS IN LEECH MUSCLE

BY

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In a study on the effects of some agonists on the leech muscle, Flacke & Yeoh (1968) showed that two groups of agonists, acetylcholine, carbachol and nicotine on the one hand, and succinylcholine and decamethonium on the other, could be differentiated by their potencies and the sizes of the maximal responses obtainable. It was thought that these differences could be explained on the basis of different sites of action of the two groups of agonists. This possibility was tested, and the present communication describes the interaction of the two groups of agonists with each other and with antagonist agents.

METHODS

The methods used have already been described (Flacke & Yeoh, 1968). The effects of different concentrations of an antagonist agent were determined after exposure of at least 30 min to each concentration. The antagonist was present throughout the test sequence—that is, during and between successive tests with an agonist. Exposure to the agonist was for a period of 30 sec. The shift in the concentration-response curves was determined with concentrations of the agonist causing 20–80% of the estimated maximum response, which was determined only at the end of the experiment by adding a supramaximal dose; this procedure was necessary because a maximum response was followed by a period of depressed sensitivity. The effect of an antagonist was measured by determining the concentration ratio of the agonist at 50% of the maximum response.

Drugs

The following drugs were used: acetylcholine chloride (Merck); carbaminoylcholine chloride (Merck); nicotine hydrogen tartrate (BDH); succinylcholine chloride (Burroughs Wellcome); decamethonium bromide (Burroughs Wellcome); physostigmine sulphate (Merck); (+)-tubocurarine chloride (Abbott); gallamine triethiodide (Davis and Greck). All drug concentrations are expressed as molar concentrations of the base.

RESULTS

Preliminary experiments confirmed earlier observations by several investigators (Minz, 1932; MacIntosh & Perry, 1950) that the contractile response of the leech muscle to

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acetylcholine (ACh) is effectively antagonized by (+)-tubocurarine. Atropine, hexamethonium, mecamylamine and procaine were found to have no significant antagonist effect in concentrations up to 10^{-4}M. (+)-Tubocurarine had no recognizable agonist effect.

Effect of (+)-tubocurarine

(+)-Tubocurarine displaced the concentration-response curve of ACh to the right. This effect is shown in Fig. 1 for concentrations of (+)-tubocurarine of 3×10^{-6}–3×10^{-5}M. Physostigmine (10^{-6}M) was present because, in the absence of any enzyme inhibitor, the muscle was very insensitive to ACh (Flacke & Yeoh, 1968). Carbachol and nicotine were found to be antagonized in the same way as ACh, but the effect on succinylcholine and decamethonium was quite different and will be described later.

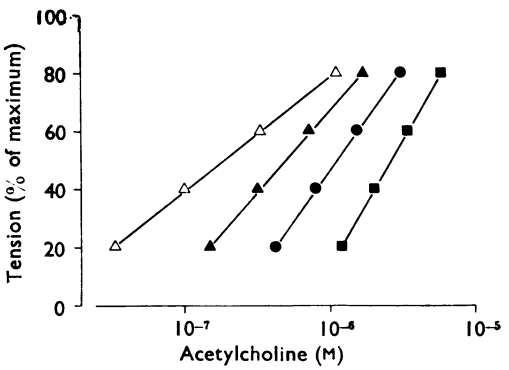

Fig. 1. Effect of (+)-tubocurarine on the contractile response of the leech muscle to acetylcholine. The concentration-response curves are the mean of five experiments and were obtained in the presence of physostigmine (10^{-6}M). Abscissa: concentration of acetylcholine (M); ordinate: tension developed in % of maximum. Concentrations of (+)-tubocurarine: △, nil; ▲, 3×10^{-6}M; ●, 10^{-5}M; ■, 3×10^{-5}M.

The quantitative aspects of the antagonism of ACh, carbachol and nicotine by (+)-tubocurarine are shown in Fig. 2. The effect of the antagonist was expressed as the concentration ratio, $[A']/[A]$, where $[A']$ is the concentration of the agonist causing 50% of the maximal contractions in the presence of the antagonist and $[A]$ is the concentration causing the same effect in its absence. The log (concentration-ratio–1) of each of the agonists was then plotted against the negative log of (+)-tubocurarine; if a straight line was obtained, the antagonism was likely to be competitive (Arunlakshana & Schild, 1959). The regression equations were estimated by the method of "least squares" and were of the form

$$\log (X-1) = \log K - n\, pA_x$$

where x is the concentration ratio of the agonist $[A']/[A]$, pA is the negative log of the concentration of the antagonist (Schild, 1947) and n and K are constants.

The slopes of the lines for ACh, carbachol and nicotine are similar, with values of n close to 1. The regression lines for carbachol and nicotine fall within the 95% confidence limits of the line obtained for ACh. These observations indicate that in the leech muscle (+)-tubocurarine antagonizes the effect of ACh, carbachol, and nicotine to a similar extent.

The effect of (+)-tubocurarine on the contractile responses to succinylcholine and decamethonium was quite different. In a concentration of 10^{-5}M, (+)-tubocurarine did not antagonize the effect of the first exposure of the leech muscle to either of the

agonists. The responses declined—that is, tachyphylaxis occurred—however, when the muscle was exposed to the agonist at intervals of 10–15 min ; this effect is shown in Fig. 3 in an experiment with paired muscles. After a series of control responses to different concentrations of succinylcholine, (+)-tubocurarine (10^{-5}M) was added to one muscle. Thirty minutes later, the responses of this muscle to succinylcholine were compared with those of the untreated muscle. The contractions caused by 3×10^{-5}M succinylcholine were not reduced by the addition of (+)-tubocurarine, and there was little decline with repeated exposures. With concentrations of 10^{-4}M and 3×10^{-4}M succinylcholine, however, the responses to successive exposures at 10–15 min intervals declined. No decline occurred when the interval between exposures was increased from 15 to 60 min (Fig. 3A). In the absence of (+)-tubocurarine tachyphylaxis was never observed (Fig. 3B).

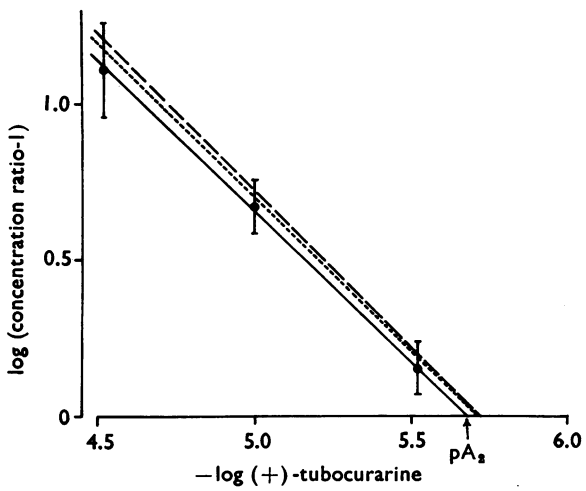

Fig. 2. Quantitative effect of (+)-tubocuarine on the responses of the leech muscle to acetylcholine, carbachol and nicotine. The regression lines were obtained from the equation log (concentration ratio–1)=log K–n log (+)-tubocurarine and are based on eight experiments with Ach (——), six experiments with carbachol (- - - -) and six experiments with nicotine (– – –). Abscissa: —log concentration of (+)-tubocurarine (M); ordinate: log (concentration ratio–1). The points represent the mean values for ACh with the 95% confidence limits indicated by the vertical lines. The values of n and pA_2 for ACh, carbachol and nicotine were, –0.96 and 5.68, –0.98 and 5.71, and –1.01 and 5.72 respectively.

Tachyphylaxis to succinylcholine or decamethonium was specific (Fig. 4). The upper tracing shows tachyphylaxis to succinylcholine (3×10^{-4}M). When the response to succinylcholine was almost abolished, administration of acetylcholine caused a normal response ; tachyphylaxis to acetylcholine was never observed. In the paired muscle from the same leech (lower tracings) no tachyphylaxis to succinylcholine occurred when (+)-tubocurarine was absent. The response to a second dose of succinylcholine, given 5 min after the first, was decreased, however, when (+)-tubocurarine was added to the bath immediately after washing out of the first dose. Thus the appearance of tachyphylaxis was not dependent on the presence of the antagonist during the " conditioning " contraction and was not a function of time of exposure to the antagonist.

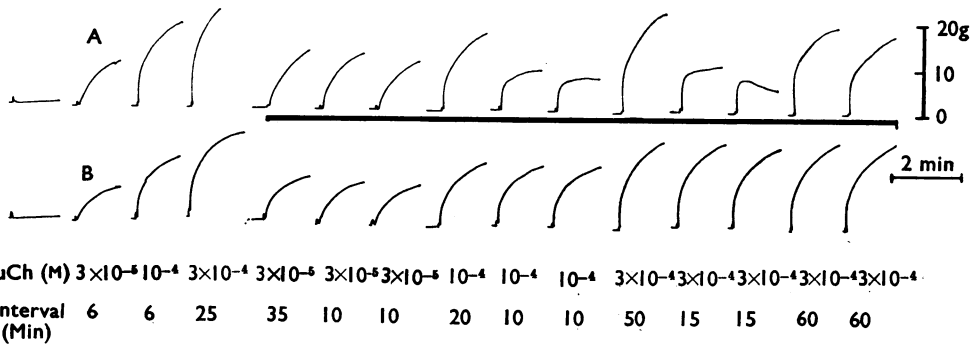

Fig. 3. Effect of (+)-tubocurarine on the responses of the leech muscle to succinylcholine. Paired dorsal muscles, A and B, from the same leech were simultaneously tested with the same concentrations of succinylcholine. The thick black line indicates the presence of (+)-tubocurarine 10^{-5}M). In the presence of (+)-tubocurarine (A), successive tests at 10 or 15 min intervals caused tachyphylaxis, but no tachyphylaxis occurred when the interval was prolonged to 60 min. In the absence of (+)-tubocurarine, no tachyphylaxis was seen (B). SuCh, Concentration of succinylcholine (M).

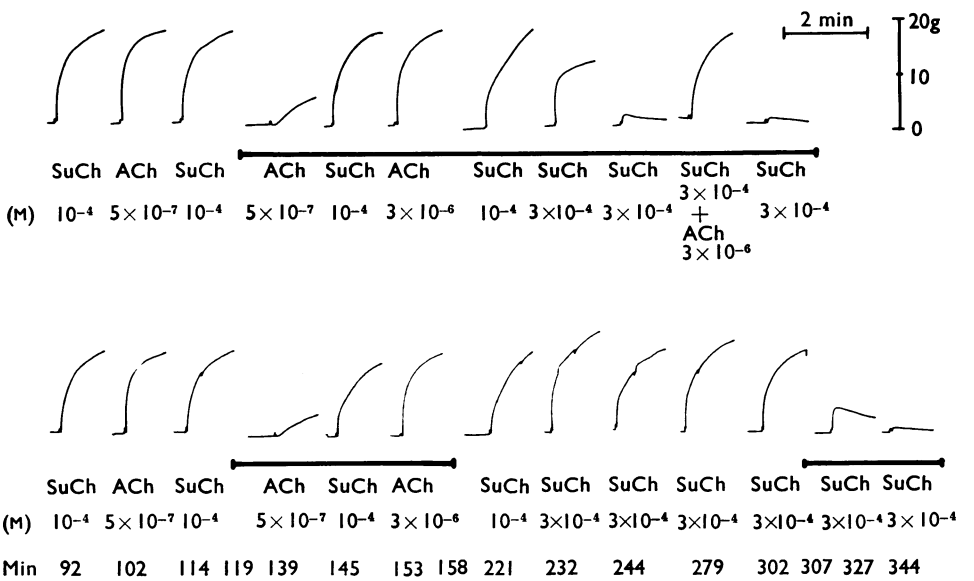

Fig. 4. Effects of (+)-tubocurarine on the responses of the leech muscle to succinylcholine or acetylcholine. The upper and lower records show the responses of paired dorsal muscles from the same leech. Exposure to (+)-tubocurarine, indicated by the thick black line, caused a decrease in sensitivity to ACh but not to succinylcholine. In the continued presence of (+)-tubocurarine (upper record) repeated tests with succinylcholine resulted in tachyphylaxis to succinylcholine but not to ACh. In the lower record, no tachyphylaxis occurred unless (+)-tubocurarine was added. The time intervals (min from the beginning of the experiments) are common to both records. SuCh, Succinylcholine (M); ACh, acetylcholine (M).

Effect of gallamine

Gallamine, which had no agonist activity, was similarly tested for its ability to antagonize the various agonists. The regression lines, correlating log (concentration ratio–1) with pA, show that ACh, carbachol and nicotine are antagonized to a lesser extent than succinylcholine and decamethonium (Fig. 5). There is an approximately ten-fold difference in the sensitivity of the two groups of agonists to the antagonist action of gallamine as indicated by their pA_2 values. The slopes of their regression lines do not differ from each other, the values of n being close to 1 (Table 1). The regression line for decamethonium is almost identical with that for succinylcholine and the lines for carbachol and nicotine fall within the confidence limits obtained for ACh (Fig. 5). No tachyphylaxis was seen in the presence of gallamine.

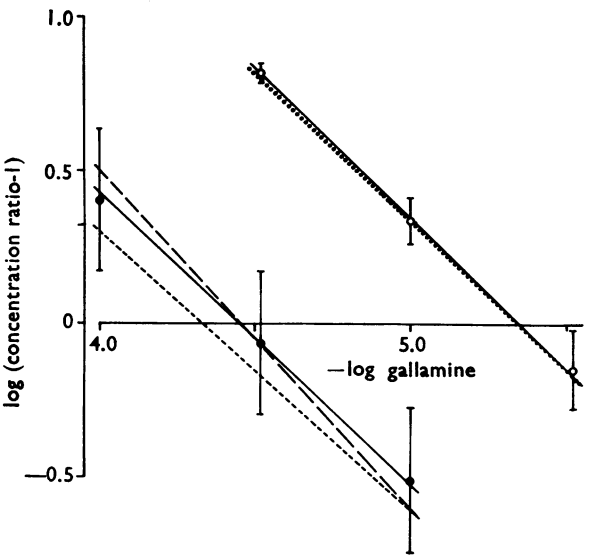

Fig. 5. Quantitative effect of gallamine on the response of the leech muscle to ACh, carbachol, nicotine, succinylcholine and decamethonium. The regression lines correlating log (concentration ratio–1) with the negative log of gallamine are the mean of four experiments in all cases. Abscissa: –log concentration of gallamine; ordinate; log (concentration ratio–1). Succinylcholine (○) and ACh (●) have uninterrupted regression lines; the vertical lines indicate the 95% confidence limits. Decamethonium, . . .; carbachol, - - - - ; nicotine – – –. The slopes of the regression lines and the pA_2 values are given in Table 1.

TABLE 1

pA_2 VALUES AND SLOPE (n) OF THE REGRESSION LINES CORRELATING LOG (CONCENTRATION RATIO–1) WITH THE NEGATIVE LOG OF THE CONCENTRATION OF GALLAMINE FOR TWO GROUPS OF AGONISTS

Agonist	pA_2	Slope (n)
Acetylcholine	4·44	0·91
Carbachol	4·32	0·90
Nicotine	4·45	1·03
Succinylcholine	5·35	0·93
Decamethonium	5·34	0·91

Interaction between carbachol and succinylcholine

It has been shown (Flacke & Yeoh, 1968) that, in the leech muscle, succinylcholine was capable of eliciting only about 70% of the maximum response obtained with carbachol. In a study of the interaction between these two drugs we have observed the following. When both drugs were given together in submaximal concentrations they always produced an effect greater than that caused by either drug alone. When the response to a supramaximal dose of succinylcholine had reached its maximum, addition of a maximal dose of carbachol to the bath increased tension by approximately a further 25%. Finally, when a dose of carbachol had caused a contraction of about 90% of the maximal response, a supramaximal dose of succinylcholine caused no further change in tension.

DISCUSSION

We have presented evidence (Flacke & Yeoh, 1968) that, in the leech muscle, acetylcholine, carbachol and nicotine form one group of agonists, and succinylcholine and decamethonium, the so-called long-acting depolarizers, form another. The grouping was based on the differences observed in potency and in size of the maximal responses.

These observations could be explained by the two groups of agonists acting on the same receptor but differing in affinity and intrinsic activity (Ariens, 1954) or efficacy (Stephenson, 1956) or, in Paton's terminology, in their dissociation rate constants (Paton, 1961). The results of the exploration of the interactions of the agonists with each other and with competitive antagonists, reported in the present paper, however, require a different interpretation. It is suggested that the two groups of agonists act on different receptors. The evidence supporting this hypothesis will now be reviewed.

The interaction between a partial agonist and a full agonist, both acting on the same receptor, should result in an antagonism of the full agonist by the partial agonist in that part of the concentration-response curve in which the response to the full agonist is greater than the response to the partial agonist (Ariens, 1954). This was not the case in the interaction between carbachol and succinylcholine. Succinylcholine, even in supramaximal concentrations, did not depress the response to carbachol, regardless of the sequence of administration, and if both were in submaximal concentrations, the effect was always additive.

The characterization of a receptor type provided by the quantitative interaction of an agonist with a competitive antagonist (Schild, 1957) indicated a difference between the acetylcholine group of agonists on the one hand, and the long-acting depolarizers on the other. With gallamine as the antagonist agent, the pA_2 values for the acetylcholine group agreed closely, as did the pA_2 values for succinylcholine and decamethonium. However, there was a difference of one log unit between the two groups.

The effect of (+)-tubocurarine on the responses of the leech muscle to succinylcholine and decamethonium was quite different from that of gallamine in that the responses were not antagonized by (+)-tubocurarine at a time when the responses to equi-effective concentrations of ACh, carbachol or nicotine were effectively antagonized. Although (+)-tubocurarine in the concentration employed in these experiments did not antagonize the depolarizing agents, however, repeated administration of these agents in

the presence of (+)-tubocurarine resulted in tachyphylaxis. When this occurred, there was no cross-tachyphylaxis to acetylcholine. This indicates that there must be different sites of action for the two groups of agonists.

Indirect evidence for pharmacological differences between the sites of action of (+)-tubocurarine and decamethonium in the mammalian motor endplate has been provided by Waser (1962, 1966) who investigated the distribution of labelled (+)-tubocurarine and decamethonium in mouse diaphragm. While (+)-tubocurarine was present almost exclusively in the endplate region of the muscle, decamethonium was much more widely distributed. The uptake kinetics of the two agents also indicated that the site of uptake of (+)-tubocurarine was saturated more easily than that of the depolarizing agents. Waser concluded that there are two types of receptors, " curare " receptors and receptors for the long-acting depolarizing agents. It is obvious that, in view of the competitive antagonism between acetylcholine and (+)-tubocurarine, the " curare " receptors also act as receptors for the transmitter, acetylcholine. Waser also pointed out, however, that the receptor specificity, as judged from uptake studies, is only relative and the difference found by him may imply no more than different affinity (or rate) constants. In this study, relative rather than absolute differences are suggested by the comparison of the effects of (+)-tubocurarine and gallamine (Figs. 2 and 5).

Recently, Paton & Waud (1967) have reported experiments in which succinylcholine was used to determine receptor occupancy by (+)-tubocurarine at the motor endplate. Their experiments were based on the explicit assumption that succinylcholine, decamethonium and octamethonium act on the same receptor as acetylcholine and (+)-tubocurarine. Although the experiments reported here do not refer to the vertebrate motor endplate, the pharmacological similarity of the leech muscle to the endplate is sufficient to warrant renewed examination of receptor specificity in the motor endplate.

SUMMARY

1. The interactions between two groups of agonists, namely, acetylcholine, carbachol and nicotine, on the one hand, and succinylcholine and decamethonium, on the other, and their antagonism by gallamine and (+)-tubocurarine were studied in the isolated dorsal muscle of the leech.

2. The maximal responses to succinylcholine and decamethonium were only 70% of those obtained with agents of the acetylcholine group.

3. The interaction between members of the two groups was not that of full and partial agonists acting on the same receptor.

4. With gallamine as antagonist, the pA_2 values obtained for the agonists agreed closely within each group but differed by one log unit between the two groups.

5. The antagonism between (+)-tubocurarine and the members of the acetylcholine group was competitive. Although (+)-tubocurarine did not antagonize succinylcholine or decamethonium, it caused tachyphylaxis to high concentration of these compounds, repeated at short intervals. Tachyphylaxis to acetylcholine was never observed.

6. These observations are interpreted as evidence that, in the leech muscle, the receptors for the acetylcholine group of agonists differ from those for the succinylcholine

group. The close pharmacological similarity between the vertebrate motor endplate and the leech muscle suggests the possibility that similar receptor differences may be present in the motor endplate also.

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