THE CHARACTER OF THE ANTAGONISM BETWEEN DRUGS OF THE "PACHYCURARE" GROUP AND CERTAIN DEPOLARIZING AGENTS IN FROG RECTUS ABDOMINIS MUSCLE

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The idea that competitive antagonism with acetylcholine underlies the mechanism of action of the "pachycurare" drugs* is not based on any substantial number of investigations devoted primarily to the quantitative study of the antagonism between the pachycurare drugs and depolarizing agents. Observations show that the antagonism for acetylcholine displayed by pachycurare drugs (gallamine triethiodide [5], d-tubocurarine, C-toxiferine II, and others [8]) does not entirely fit into the framework of concepts of competitive antagonism between these substances.

The present investigation was concerned with a study of the character of the antagonism between pachycurare drugs (d-tubocurarine chloride, Paramyon, and Diplacine) and such depolarizing agents as acetylcholine and succinylcholine.

METHOD

Experiments were carried out on the rectus abdominis muscle of the frog (males of species R. esculenta). The muscle was suspended in a 100-ml beaker in an isotonic sodium chloride solution, under conditions of constant aeration. In the first series of experiments we determined the concentrations of acetylcholine or succinylcholine required to evoke, in the presence of the given concentrations of pachycurare drugs, muscle contractions equal to those evoked by acetylcholine alone at a concentration of 1×10^{-6} moles/l or succinylcholine alone at a concentration of 4×10^{-7} moles/l. On the basis of the data so obtained, we calculated the difference $pA_2 - pA_{10}$, using the equation of H. Schild [7]; in cases of competitive antagonism this difference equals log 9, or 0.95.

In the second series of experiments we studied the magnitude of contraction† of the rectus muscle (in percent of maximum) as a function of the concentration of acetylcholine or succinylcholine, in the presence and absence of constant concentrations of pachycurare drugs.

It is well known that the equation describing the reaction of a drug with a specific receptor can be derived on the basis of the law of mass action as applied to monomolecular reactions [3]. In this case the relation between the magnitude of contraction of the rectus muscle and the concentration of acetylcholine (or succinylcholine) is expressed by the equation

$$y = \frac{100[\mathrm{ACh}]}{K + [\mathrm{ACh}]} \qquad (I)$$

where y is the response to acetylcholine (succinylcholine) in percent of the maximal response, which is taken as 100; [ACh] is the concentration of acetylcholine (succinylcholine) in moles per liter; and K is the dissociation constant for the acetylcholine-receptor (succinylcholine-receptor) complex.

In the presence of a competitive antagonist that reacts with the same receptors, also in a monomolecular reaction, the dependence of the magnitude of muscle contraction on the acetylcholine (succinylcholine) concentration will be expressed by the equation

$$y = \frac{100[\mathrm{ACh}]}{K\left(1 + \frac{[A]}{K_A}\right) + [\mathrm{ACh}]}, \qquad (II)$$

* The designation "pachycurare" is applied to true curariform drugs, which inhibit membrane depolarization and are supposed to have a competitive type of action, as distinguished from "leptocurare," comprising those relaxants, such as succinylcholine, having a cholinomimetic action and presumably exerting their effect through depolarization. Paramyon is listed in Lekarstvennye Sredstva (Drugs) as meso-3,4-diphenylhexane-bis-p-trimethylammonium iodide, and Diplacine as 1,3-di(β-platynecinium ethoxy)benzol chloride [Translator's note].

† Presumably, the height of isotonic contraction was studied, but the Russian original is not specific [Translator's note].

where [A] is the concentration of antagonist in moles per liter, and K is the dissociation constant of the antagonist-receptor complex.‡

Equations (I) and (II) may be converted into straight-line equations having the general form $y = a + bx$, by transforming them according to the method of double reciprocals:

$$\frac{1}{y} = \frac{1}{100} + \frac{K}{100} \cdot \frac{1}{[ACh]}, \qquad (Ia)$$

$$\frac{1}{y} = \frac{1}{100} + \frac{K}{100}\left(1 + \frac{[A]}{K_A}\right)\frac{1}{[ACh]} \qquad (IIa)$$

If we compare Equations (Ia) and (IIa), we see that in a Lineweaver-Burk plot [6], i.e., in plotting reciprocals, the dependence of magnitude of contraction of the rectus muscle on the concentration of acetylcholine (succinylcholine) ought to be represented by a straight line cutting the ordinate at 0.01, both in the presence and in the absence of a competitive antagonist, but the slope of the line increases when a competitive antagonist is added, to a value of $(1 + [A]/K_A)$.

RESULTS

If we determine the values of pA_2 and pA_{10} in Fig. 1, we find that for the combination acetylcholine + tubocurarine $pA_2 - pA_{10} = 7.20 - 6.15 = 1.05$; for acetylcholine + Paramyon, $pA_2 - pA_{10} = 6.65 - 5.30 = 1.35$; for acetylcholine + Diplacine, $pA_2 - pA_{10} = 7.20 - 5.50 = 1.70$; for succinylcholine + Diplacine, $pA_2 - pA_{10} = 7.20 - 6.00 = 1.20$; and for succinylcholine + tubocurarine, $pA_2 - pA_{10} = 7.30 - 6.30 = 1.00$.

Examination of these calculated differences reveals that all of them are greater than 0.95, and consequently, the antagonism between pachycurare drugs and depolarizing agents is not strictly a competitive antagonism.

This conclusion is supported by the second group of experiments. As may be seen from Fig. 2, the curves representing the magnitude of contraction of the rectus muscle as a function of acetylcholine concentration, in the presence of tubocurarine at a concentration of 6×10^{-7} moles/l and Paramyon at 2×10^{-6} moles/l, conform completely to the theoretical concepts cited above as to the dependence of the magnitude of muscle contraction on the concentration of depolarizing agent in the presence of a competitive antagonist.

Thus, there is competitive antagonism between acetylcholine, on the one hand, and tubocurarine and Paramyon in these concentrations, on the other. But in the presence of tubocurarine in a concentration of 2×10^{-6} moles/l, or Paramyon in a concentration of 6×10^{-6} moles/l, the relation between the magnitude of contraction and acetylcholine concentration becomes nonlinear, and is expressed by curves approaching a parabola in form. A curvilinear relationship is also observed in the presence of Diplacine at a concentration of 6×10^{-6} moles/l.

Similar relationships are seen when pachycurare drugs are combined with succinylcholine: The reciprocal of the magnitude of muscle contraction is a linear function of the reciprocal of succinylcholine concentration, when the tubocurarine concentration is low, but as the tubocurarine concentration increases, this dependence becomes curvilinear (Fig. 3).

In addition, all the curves (see Figs. 2 and 3) intersect the ordinate at the value 0.01; it follows that the curvilinear relationship cannot be taken as proof that the antagonism between pachycurare drugs and depolarizing agents is not competitive in nature, since the slope and the intercept change in the presence of a noncompetitive antagonist.

The curvilinear relationship demonstrated in these experiments cannot be the result of a change in the value of K_A, since the affinity constants of quaternary ammonium bases, particularly tubocurarine, do not

‡Equations (I) and (II) are analogous to the equation of A. J. Clark [3] and J. H. Gaddum [4].

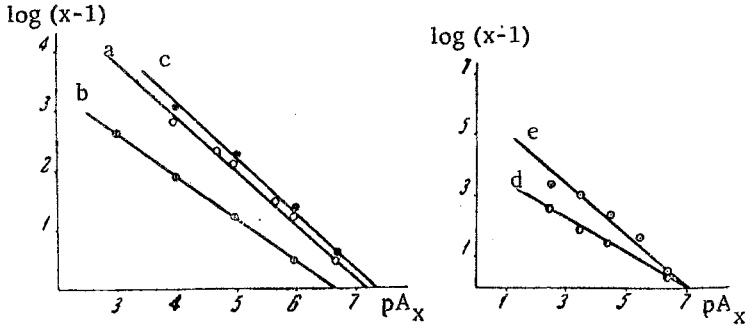

Fig. 1. The dependence of log (x–1) on the corresponding values of pA_X, for various pairs of drugs. a) Acetylcholine plus tubocurarine; b) acetylcholine plus Paramyon; c) succinylcholine plus tubocurarine; d) acetylcholine plus Diplacine; e) succinylcholine plus Diplacine.

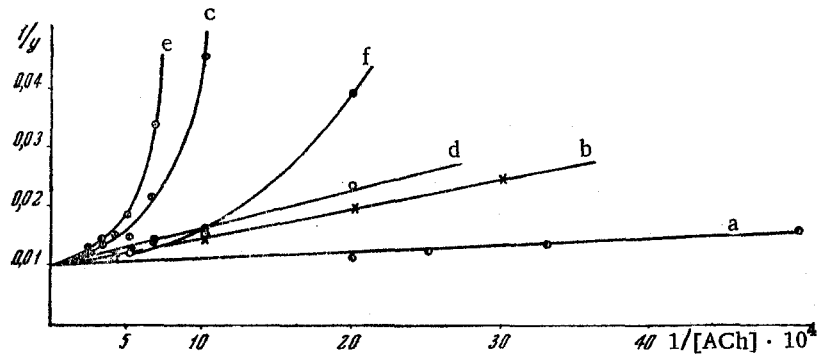

Fig. 2. Magnitude of rectus muscle contraction (on reciprocal scales) as a function of acetylcholine concentration, in the presence and absence of pachycurare drugs. a) Acetylcholine; b) acetylcholine plus tubocurarine (6×10^{-7} moles/l); c) acetylcholine plus tubocurarine (2×10^{-6} moles/l); d) acetylcholine plus Paramyon (2×10^{-6} moles/l); e) acetylcholine plus Paramyon (6×10^{-6} moles/l); f) acetylcholine plus Diplacine (6×10^{-6} moles/l).

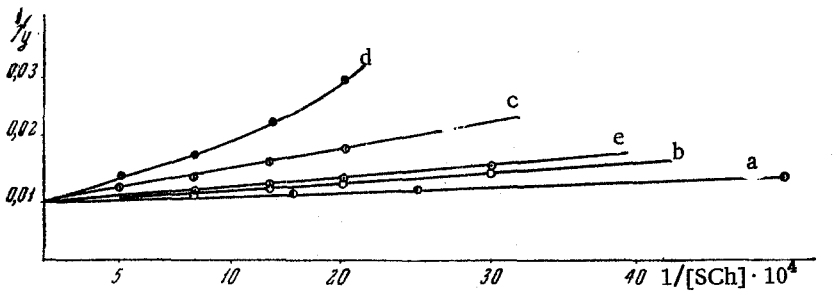

Fig. 3. Magnitude of muscle contraction (on reciprocal scales) as a function of succinylcholine concentration in the presence and absence of pachycurare drugs. a) Succinylcholine; b) succinylcholine plus Paramyon (2×10^{-6} moles/l); c) succinylcholine plus tubocurarine (2×10^{-6} moles/l); d) succinylcholine plus tubocurarine (4×10^{-6} moles/l); e) succinylcholine plus Diplacine (4×10^{-6} moles/l).

Certain Criteria Characterizing the Type of Antagonism Displayed by Pachycurare Drugs Toward Acetylcholine and Succinylcholine, Compared with Affinity Constants of Combined Substances

Combination	K_{aff} (antagonist-receptor)	K_{aff} (antagonist-receptor)	pA_2—pA_{10}	Inhibition index at a pachycurare drug conc. of the order of 10^{-7}	Inhibition index at a pachycurare drug conc. of the order of 10^{-4}	Reduction factor
Acetylcholine	$0,65 \cdot 10^6$	—	—	—	—	—
Succinylcholine	$1,22 \cdot 10^6$	—	—	—	—	—
Acetylcholine plus tubocurarine	—	$3,7 \cdot 10^6$	1,05	15	6,29	2,3
Acethylcholine+Paramyon	—	$1,64 \cdot 10^6$	1,35	3,8	0,84	4,5
Acethylcholine+Diplacine	—	$0,75 \cdot 10^6$	1,70	7,5	0,23	32,6
Succinylcholine + tubocurarine	—	$2,17 \cdot 10^6$	1,00	7,5	4,18	1,8
Succinylcholine + Diplacine	—	$0,33 \cdot 10^6$	1,20	3,63	0,85	4,2

change as their concentration increases [8]. Since acetylcholine and succinylcholine react with choline-reactive structures according to the model of a monomolecular reaction (see Figs. 2 and 3), the curvilinear relation observed between the magnitude of muscle contraction and concentration of depolarizing agent in the presence of high concentrations of pachycurare drugs is the result of a change in the monomolecular character of the reaction of pachycurare drugs with these choline-reactive structures. Certain histamine antagonists react with specific receptors in similar fashion [9]. In this case, Equation (II) must be given in a more general form:

$$y = \frac{100\,[\mathrm{ACh}]}{K\left(1 + \frac{[A]^n}{K_A}\right) + [\mathrm{ACh}]},$$

where n is the number of molecules of pachycurare drug associated with a single receptor.

For acetylcholine plus tubocurarine (2×10^{-6} moles/l), n = 0.968; for acetylcholine plus Paramyon (6×10^{-6} moles/l), n = 0.987; and for succinylcholine plus tubocurarine (4×10^{-6} moles/l), n = 0.875.

These values of n can be understood on the assumption that pachycurare drugs at high concentrations block receptors in choline-reactive structures not only by chemical reaction but also by adsorptive binding.

The possibility that a number of substances undergo both chemical and adsorptive reactions with protoplasmic cell structures can be regarded as established [2].

Since adsorption bonds are less stable than chemical bonds, smaller concentrations of depolarizing agents are required to overcome the antagonistic action of high concentrations of pachycurare drugs than would be expected if only chemical reactions (of the monomolecular reaction type) took place between pachycurare drugs and choline-reactive structures. This is confirmed by a calculation of the inhibition indices, whose values decrease as the concentration of pachycurare drug increases [1] (see table).

If pachycurare drugs react with choline-reactive structures by both chemical and adsorptive mechanisms, the affinity constants (K_{aff}) determined in these experiments are over-all constants reflecting the rate of chemical and adsorption reaction. We should therefore expect that the greater the affinity constants of pachycurare drugs and the depolarizing agents (and consequently, the greater the specific weight of the chemical reaction of these substances with receptors), the more the antagonism of pachycurare drugs toward the depolarizing agents will approach a true competitive antagonism.

Examination of the data in the table bears out this supposition completely.

The role of the adsorptive component in the reaction of pachycurare drugs with the choline-reactive structures becomes greater as the concentration of pachycurare drug increases. At low concentrations of pachycurare drug (of the order of 1×10^{-7}-1×10^{-6} moles/l), the adsorption mechanism is practically absent, and the antagonism between pachycurare (in these concentrations) and the depolarizing agents is a true competitive antagonism.

SUMMARY

The author studied the antagonism between pachycurare-type drugs (d-tubocurarine chloride, Paramyon, and Diplacine) and depolarizing agents (acetylcholine and succinylcholine) in experiments on the frog rectus abdominis muscle. It was shown that pA_2-$pA_{10} > 0.95$ for all the combinations studied, and therefore, the antagonism between pachycurare drugs and depolarizing substances is not strictly competitive. In studying the relationship between the magnitude of muscle contraction and the concentration of depolarizing substance, the author established that pachycurare drugs in concentrations of about 10^{-7}-10^{-6} moles/l act as a competitive antagonist of the depolarizing agents. As the concentration of pachycurare drugs increases, the competitive nature of the antagonism changes; this is due to a change in the monomolecular character of the interaction between pachycurare drugs and choline-reactive structures. This change may be caused by simultaneous chemical and adsorptive blocking of specific receptors.

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