A New Kind of Drug Antagonism: Evidence that Agonists Cause a Molecular Change in Acetylcholine Receptors

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SUMMARY

The effects of various curare-like compounds on the contractile responses to carbachol and suxamethonium of thin strips of chick biventer cervicis and of leech muscle have been studied. In chick muscle, suxamethonium and carbachol appeared to act on the same receptor, and were antagonized by tubocurarine, gallamine, and three substituted decamethonium derivatives. The actions of tubocurarine and gallamine deviated only very slightly from the conventional competitive model; in contrast, the decamethonium derivatives exhibited a qualitatively different kind of antagonism. Experiments with these compounds, including a 2-chloroethylamine derivative that caused an irreversible type of block, showed that the degree of antagonism produced was increased if the antagonist was applied at the same time as, or shortly after, a dose of agonist. This was confirmed in experiments with leech muscle, in which suxamethonium and carbachol appeared to act on different receptors; the substituted decamethonium compounds showed specificity for the suxamethonium receptors, and here the same kind of interaction with agonist as had been seen with chick muscle was observed. It is postulated that agonists cause a change in the molecular structure of the receptor that increases the affinity of the receptor for certain antagonists. This has been termed the metaphilic effect, and it is suggested that this molecular change may be related to the processes of stimulation and desensitization by agonists.

INTRODUCTION

It is generally accepted that acetylcholine and related agonists elicit responses from sensitive tissues by combining with specific receptor sites in the tissue. Furthermore, a number of specific antagonists of acetylcholine appear to combine with the same receptors, making them unavailable to the agonists. The quantitative effects of many reversible antagonists are compatible with drug-receptor association models based on the law of mass action, and are formally equivalent to the Michaelis-Menten approach to enzyme-substrate interactions (1–7).

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Various attempts have recently been made to use this predictable behavior in order to identify the binding of radioactively labeled antagonists to receptors in tissues (7–9). Studies by Paton and Rang (7) on the uptake of atropine by intestinal smooth muscle showed that the tissue contained only a very small amount of receptor material, and appeared rather unsuitable as a starting point for the chemical isolation of the receptor substance. We have therefore explored a number of other acetylcholine-sensitive tissues, in the hope of finding one richer in receptor material.

This paper describes experiments made on acetylcholine-sensitive "slow" muscle of the chick (10) and on leech body wall muscle. In addition to the familiar acetylcholine antagonists, tubocurarine and gallamine, we have studied some newly synthesized substituted decamethonium derivatives, including a 2-chloroethylamine

derivative designed to form a covalent bond with nicotinic receptors (see Fig. 1), analogous to the action of benzilylcholine mustard on muscarinic receptors (11).

These experiments revealed a new and unexpected type of interaction among antagonists, agonists, and receptors, not encompassed by the conventional theory, and the present paper deals with the elucidation of this phenomenon.

METHODS

Most of the experiments were carried out on strips dissected from the biventer cervicis muscle of the chick (10). Birds between 1 and 6 weeks old were used, and were killed by an injection of air into a wing vein. The biventer cervicis muscles were dissected free, the connective tissue sheath was removed under a low-power dissecting microscope, and a thin strand of muscle (about 0.5 mm in diameter) was separated from the edge of the caudal belly. This strip of muscle was mounted in an organ bath containing Krebs' solution, at 37°, of the following composition: NaCl, 113 mM; KCl, 4.7 mM; $CaCl_2$, 2.5 mM; KH_2PO_4, 1.2 mM; $MgSO_4$, 1.2 mM; $NaHCO_3$, 2.5 mM; and dextrose, 11.5 mM. This solution was bubbled with 95% O_2–5% CO_2. Isometric contractions were recorded by means of a transducer vacuum tube (RCA 5734) or a semiconductor strain gauge, operating a pen recorder. Strips were used in preference to whole muscles, because they responded much more rapidly to agonists and antagonists.

In other experiments, strips of leech dorsal muscle were cut with a pair of razor blades mounted 1 mm apart. The skin and overlying tissue were removed, and each strip was suspended at room temperature (20°) in a pH 7.3 solution of the following composition: NaCl, 112 mM; KCl, 5.6 mM; $CaCl_2$, 2.2 mM; $MgCl_2$, 2.05 mM; $NaHCO_3$, 12 mM; and dextrose, 11.5 mM. It was bubbled with 95% O_2–5% CO_2. This solution was selected after trying a number of variants of "frog Ringer's solution." The correct pH and the use of a CO_2-bicarbonate buffer were found to be important for consistent responses. Both the chick and the leech muscle preparations gave responses to suxamethonium or carbachol that reached a plateau in 60–90 sec and could be repeated every 4 min without desensitization (provided that the contractions were no more than about 50% maximal).

For the purposes of measurement, the tension developed during the plateau of the contraction was used. The timing of the drug application cycle was arranged so that the agonist was washed out as soon as the plateau was reached. This cycle was controlled automatically, using a timing device and solenoid-operated syringes. When antagonists were studied, the drug was added to the bath in each cycle immediately after washing out the agonist, and was present throughout the cycle.

After a reversible antagonist was washed out, a second, control dose-response curve was always established. This was invariably within ±10% of the original, which gave confidence to quantitative measurements of the degree of *irreversible* block, when this final control could not be performed.

When dose-response curves were determined in the presence of a reversible antagonist or after application of an irreversible antagonist, the responses were kept within the range of the responses recorded for the control dose-response curve, by adjusting the dose of agonist. The antagonists studied usually caused a parallel shift to the right of the agonist log dose-response curve, enabling the effect to be expressed as a dose ratio (DR) from which fractional receptor occupancy by the antagonist (p) was calculated from the following equation (6).

$$p = \frac{DR - 1}{DR} \quad (1)$$

Where appropriate, the dissociation equilibrium constant (K_{eq}) was calculated from

$$K_{eq} = \frac{x}{DR - 1} \quad (2)$$

where x is the molar concentration of the antagonist used.

The drugs used were carbaminoylcholine chloride (carbachol) (Koch-Light), suxamethonium chloride (Allen & Hanbury), (+)-tubocurarine chloride (Burroughs

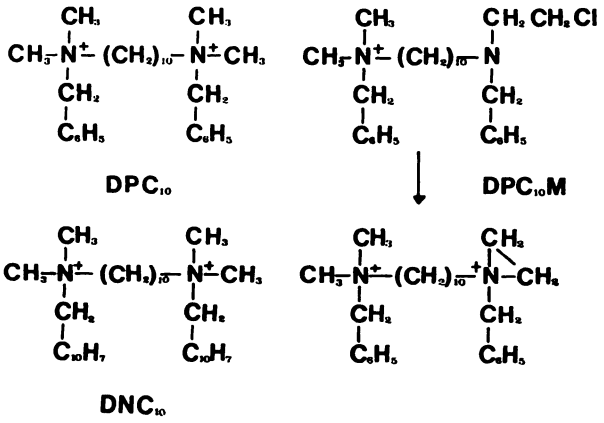

FIG. 1. *Structures of decamethonium derivatives studied*

The reversible antagonists, diphenyldecamethonium (DPC_{10}) and dinaphthyldecamethonium (DNC_{10}), are shown on the left. The alkylating derivative, diphenyldecamethonium mustard ($DPC_{10}M$), undergoes cyclization in solution at pH 7.3, from the 2-chloroethyl to the ethyleniminium form, as shown on the right.

Wellcome), gallamine triethiodide (May & Baker), caffeine (British Drug Houses), and physostigmine sulfate (Burroughs Wellcome). The decamethonium derivatives used were prepared by Dr. E. W. Gill, for the purposes of this study. These were decamethylene-1,10-bis[dimethylbenzylammonium bromide], decamethylene-1,10-bis[dimethyl-(1-naphthylmethylene)ammonium bromide], and decamethylene-1-(*N*-benzyl-2-chloroethylamino)-10-dimethylbenzylammonium chloride hydrochloride. The structural formulae of these compounds are shown in Fig. 1.

The symmetrical bisquaternary ammonium compounds, DPC_{10}[2] and DNC_{10}, were prepared by heating an excess of the appropriate tertiary amine with 1,10-decamethylene dibromide in dimethylformamide. $DPC_{10}M$ was prepared by combining 10-bromodecyldimethylbenzylammonium bromide with *N*-benzylethanolamine and, after anion exchange, allowing the resulting compound to react with thionyl chloride. The properties of this and related 2-haloalkylamines will be described in detail elsewhere.

Solutions of $DPC_{10}M$ were made at 1 mM concentration in 0.067 M sodium phosphate buffer (pH 7.3) at room temperature (20°) and left for 30 min; they were then stored for up to 3 hr on ice, appropriate dilutions for injection being made up in ice-cold 0.9% NaCl. Back-titration of unreacted thiosulfate against iodine (11) showed that this procedure gave a yield of approximately 75% ethyleniminium ion (EI^+). Concentrations of $DPC_{10}M$ are expressed throughout as concentrations of the ethyleniminium ion. Caffeine was prepared at a concentration of 100 mM in 70% ethanol. The volume usually added to the bath was 0.5 ml; addition of this volume of solvent to the bath caused a small contracture, but did not appear to affect the responsiveness of the tissue.

RESULTS

Effects of tubocurarine and gallamine on chick muscle. Tubocurarine and gallamine behaved as conventional reversible antagonists in that they caused a parallel shift to the right of the agonist log dose-response curve (Fig. 2a); they did not affect the desensitization caused by a given dose of agonist. Table 1 shows dose ratios measured in the presence of different concen-

[2] The abbreviations used are: DPC_{10}, decamethylene-1,10-bis[dimethylbenzylammonium bromide]; DNC_{10}, decamethylene-1,10-bis[dimethyl-(1-naphthylmethylene)ammonium bromide]; $DPC_{10}M$, decamethylene-1-(*N*-benzyl-2-chloroethylamino)-10-dimethylbenzylammonium chloride hydrochloride.

trations of tubocurarine and gallamine, using both carbachol and suxamethonium as agonists, and also the apparent K_{eq}, calculated from Eq. 2. The two agonists were indistinguishable (Fig. 2b), consistent with the view that they were acting on the same receptor. This is in contrast to the behavior of leech muscle, described by Flacke and Yeoh (12), in which tubocurarine discriminates sharply between these two agonists.

Quantitatively, the results fit the conventional theory quite well, but not perfectly; logarithmic plots of (dose ratio -1) against antagonist concentration should have a slope of unity, and an intercept on the abscissa equal to the equilibrium constant, K_{eq} (4). Figure 2 shows such plots for our data; since there was no suggestion that the points on the logarithmic plots were heteroscedastic, they were fitted by least squares regression lines. The slopes were 0.95 ± 0.009 for tubocurarine, and 0.88 ± 0.006 for gallamine. These slopes both differed significantly from unity ($p < 0.001$), which means that the estimate of equilibrium constant given by Eq. 2 varies with antagonist concentration, though only to a very small degree. Nevertheless, this parameter has been calculated for each experiment and tabulated as "apparent K_{eq}"

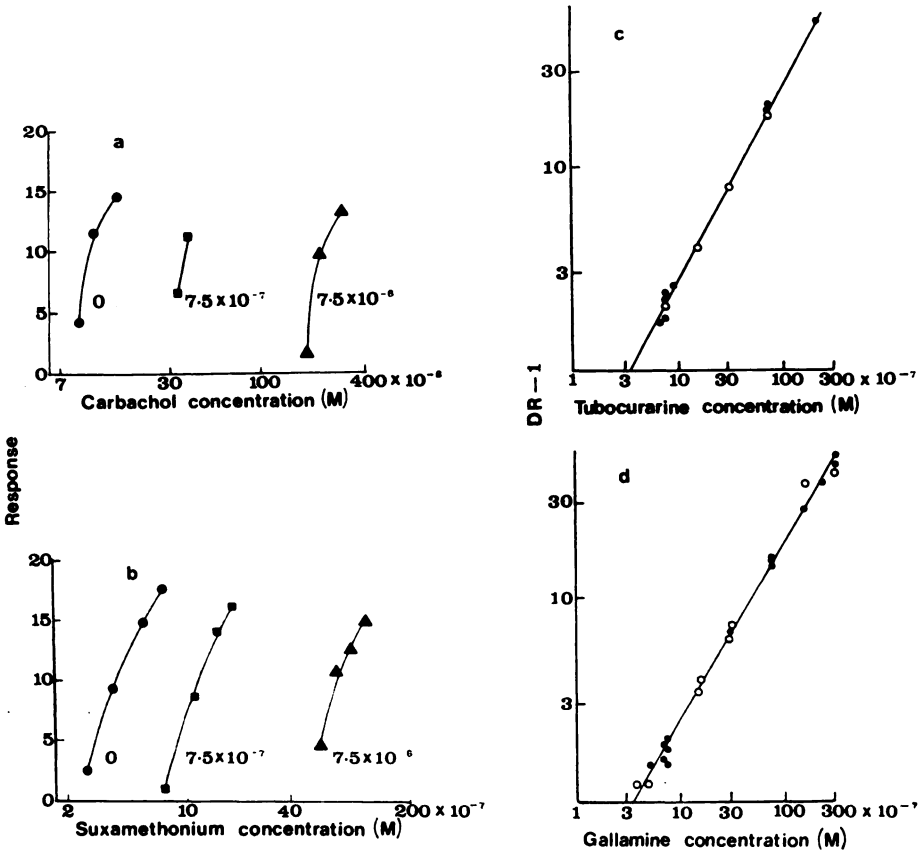

FIG. 2. *Action of tubocurarine and gallamine on chick muscle*

The left-hand panels show the log dose-response curves for (a) carbachol and (b) suxamethonium, obtained with a single preparation in the presence of different concentrations of tubocurarine as shown by the figures next to the curves. Each point represents a single measurement or the mean of two closely similar values. In this and subsequent figures, the ordinate represents tension, expressed in arbitrary units. The right-hand panels show log-log plots of (dose ratio $-$ 1) against antagonist concentration for (c) tubocurarine and (d) gallamine. Each point represents a single measurement, filled circles being obtained with carbachol as agonist, and open circles with suxamethonium.

TABLE 1
Action of tubocurarine and gallamine on chick muscle at 37°

Expt.	Antagonist	Antagonist concentration	Agonist	Dose ratio	Apparent K_{eq}
		$\times 10^{-7}$ M			$\times 10^{-7}$ M
1	Tubocurarine	7.5	Carbachol	2.8	4.2
		75	Carbachol	19.5	4.0
		225	Carbachol	53	4.3
		9.1	Carbachol	3.6	3.5
		7.5	Carbachol	3.2	3.4
2		7.5	Carbachol	3.4	3.1
		75	Carbachol	21	3.8
		7.5	Suxamethonium	3.1	3.6
		75	Suxamethonium	19	4.2
3		3.3	Carbachol	2.0	3.3
		6.7	Carbachol	2.7	3.9
4		15.7	Suxamethonium	5.0	3.9
		31.4	Suxamethonium	9.0	3.9
	Mean ± SE				3.78 ± 0.10
1	Gallamine	7.5	Carbachol	2.8	4.2
		75	Carbachol	16	5.0
		225	Carbachol	38.5	6.0
		75	Carbachol	15.5	5.2
		75	Carbachol	16	5.0
5		300	Carbachol	45.5	6.8
		7.5	Carbachol	2.5	5.0
		7.5	Carbachol	3.0	3.7
		75	Carbachol	16.0	5.0
		150	Carbachol	28.2	5.5
6		6.7	Carbachol	2.9	3.5
7		6.7	Carbachol	2.6	4.2
8		5.0	Carbachol	2.5	3.4
		5.0	Suxamethonium	2.2	4.2
9		15.7	Suxamethonium	4.9	4.0
		31.4	Suxamethonium	8.3	4.3
		157	Suxamethonium	37.5	4.3
		14.7	Suxamethonium	4.4	4.4
		3.7	Suxamethonium	2.2	3.1
		29.4	Suxamethonium	7.4	4.6
		73.5	Suxamethonium	16.0	4.9
		294	Suxamethonium	42.0	7.2
	Mean ± SE				4.70 ± 0.22

(Table 1). The mean value for tubocurarine, 3.8×10^{-7} M, is similar to the figure of 4.3×10^{-7} M obtained by Jenkinson (5) for the equilibrium constant of tubocurarine on the frog motor end plate.

We have not investigated further the reason for the deviation of our results from Eq. 2; several general explanations can be put forward. Operationally, it appears that gallamine (and, to a lesser extent, tubocurarine) combines more readily when all of the receptors are free than it does when a large fraction are already occupied. One possibility is that there is a mosaic of receptors, and that gallamine, with three quaternary groups, combines most favorably when three adjacent receptors are vacant. As the occupancy increases, the probability of a vacant receptor having vacant neighbors would decrease, and the "affinity" of the vacant receptors for gallamine would appear to be reduced. The

finding that tubocurarine, with only two quaternary groups, showed the same phenomenon to a much less marked degree is consistent with this interpretation.

A second possibility is that the receptors are not all identical, the population showing a scatter in its affinity for different antagonists. The antagonist would combine first with those receptors for which it had high affinity, and the apparent K_{eq} would increase as the fractional occupancy increased.

A third general possibility is that there is some degree of interaction between adjacent receptors, whereby occupation of one site decreases the probability of occupation of neighboring sites. It is not possible at present to choose among these general mechanisms, any of which might contribute to the anomalous behavior of gallamine.

Paton and Rang (7) showed that measurement of the dose ratio produced by two antagonists applied simultaneously could be used to test whether or not both antagonists compete at a single receptor site. If the two drugs, applied separately, give dose ratios DR_1 and DR_2, then, assuming competition, the combination should give a dose ratio $DR_1 + DR_2 - 1$. If the drugs do not compete at the same receptors, the combined dose ratio will be $DR_1 \cdot DR_2$. This test was applied using tubosurarine and gallamine. Tubocurarine, 7.5×10^{-7} M, by itself, gave a dose ratio of 3.2; gallamine, 7.5×10^{-6} M, gave a dose ratio of 16.0. Combining both drugs gave a dose ratio of 20, close to the predicted value of 18.2 for a competitive interaction.

These results show that the interactions of tubocurarine, gallamine, carbachol, and suxamethonium agree fairly well with the conventional model of competitive antagonism. This may be interpreted either as a real competitive equilibrium among agonist, antagonist, and receptors (1), or as an occlusive block by a relatively slowly dissociating antagonist, if only a small fraction of receptors is occupied by agonist (6, 13).

Measurements of the dissociation rate constants of the antagonists, and of the equilibrium constants of the agonists, which would be needed in order to distinguish between these possibilities, are not at present available. There is, however, some evidence that the rate of dissociation of

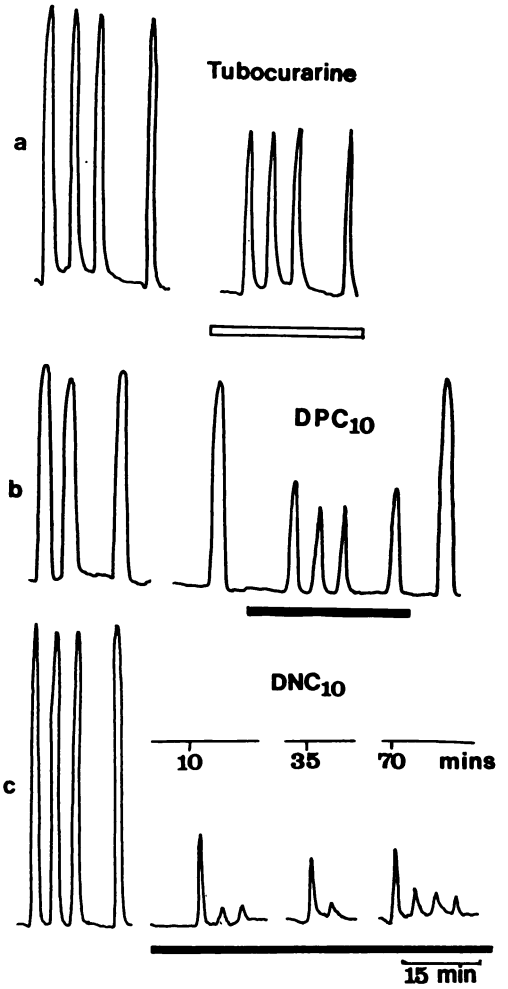

FIG. 3. *Tracings of isometric contractions of chick biventer muscle strips in response to carbachol*

a. Effect of tubocurarine, 5×10^{-7} M (for the time indicated by the horizontal bar), on contraction produced by 1.5×10^{-5} M carbachol. No desensitization is apparent.

b. Effect of DPC_{10}, 1.9×10^{-6} M (horizontal bar), on contraction produced by 3.7×10^{-5} M carbachol. There was little, if any, desensitization in the control period, but it was consistently found in the presence of DPC_{10}.

c. Effect of DNC_{10}, 7.4×10^{-7} M (horizontal bar), on contraction produced by 3.7×10^{-5} M carbachol. The tracing was interrupted, as shown by the breaks in the record, and no carbachol was applied during those periods. These desensitization-enhancing effect of DNC_{10} is clearly shown.

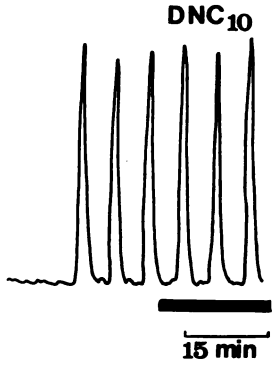

FIG. 4. *Tracing of contractions of chick biventer muscle strip in response to caffeine, 3.9 mM*

DNC_{10}, 7.4×10^{-7} M, was present during the period indicated by the bar, and had no effect on the contractions.

curare-like antagonists is much higher than that of atropine-like antagonists in smooth muscle. Thus, using iontophoretic application of tubocurarine, both del Castillo and Katz (14) and Waud (15) found rapid recovery from block at frog motor end plates, while in the present experiments the thickest strips of muscle were recovered from the action of tubocurarine within 2 min of washing the drug out of the tissue. In contrast to the situation in intestinal smooth muscle (13), it is thus possible that real competitive antagonism of the type envisaged by Gaddum (1) takes place at these receptors.

Effects of DPC_{10} and DNC_{10}. In contrast to tubocurarine and gallamine, DPC_{10} and DNC_{10} did not behave in an orthodox manner. Typical experiments are shown in Fig. 3. In the control period of each experiment, steady responses were produced by repeated doses of carbachol; altering the interval between doses did not affect the size of the contractions (i.e., no de- of 7.4×10^{-7} M DNC_{10}, the response to sensitization was apparent). In the presence carbachol was reduced, and marked desensitization to repeated doses of carbachol appeared. This is shown by the decreased

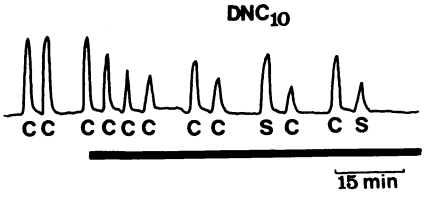

FIG. 5. *Tracing showing contractions of chick biventer muscle strip in response to carbachol (C), 1.5×10^{-5} M, and suxamethonium (S), 5.6×10^{-7} M*

During the period indicated by the bar, DNC_{10}, 1.9×10^{-7} M, was present. Note that equiactive doses of carbachol and suxamethonium had an equal "desensitizing" effect in the presence of DNC_{10}.

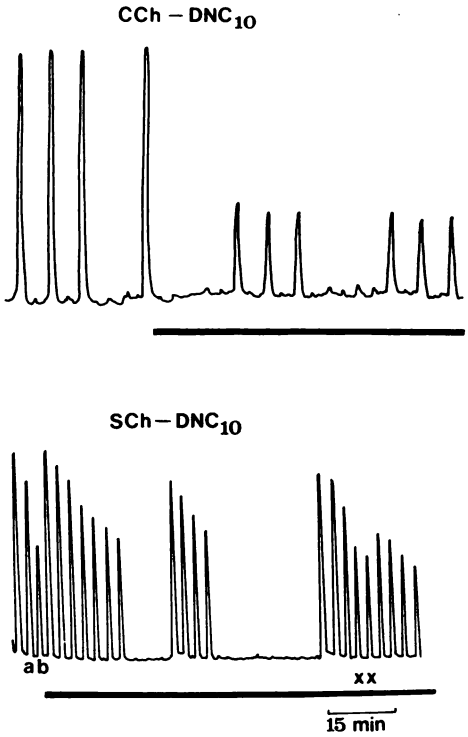

FIG. 6. *Tracings showing isometric contractions of leech muscle in response to carbachol (CCh) and to suxamethonium (SCh)*

In the upper trace, each contraction was produced by 4.8×10^{-7} M carbachol; during the period indicated by the bar, DNC_{10}, 1.2×10^{-6} M, was present, and the contractions were reduced. No effect on desensitization was apparent. In the lower trace, all unmarked contractions were produced by 1.9×10^{-5} M suxamethonium: *a*, 1.1×10^{-5} M; *b*, 7.4×10^{-6} M. The contractions marked *x* occurred when the automatic injector developed a fault. During the period indicated by the bar, DNC_{10}, 3.7×10^{-7} M, was present. The desensitization-enhancing effect of DNC_{10} was marked, while responses elicited after a period of quiescence were only slightly antagonized (cf. chick muscle, Fig. 4).

responses to doses of carbachol given at 4-min intervals, and by the fact that larger responses followed a period when carbachol was not applied. Increasing the dose interval to about 15 min gave progressively larger responses, but the responses never equaled those of the control period. The effect of DPC_{10} was similar but less marked.

Effects similar to these have been observed by Flacke and Yeoh (12), who found that antagonism of suxamethonium by tubocurarine in leech muscle was characterized by a "desensitization-enhancing" effect similar to that described above.

Several observations suggested that the actions of DPC_{10} and DNC_{10} were due specifically to combination with nicotinic receptors, rather than to some nonspecific effect such as rendering the tissue incapable of giving repeated contractions to any agonist. Caffeine is thought to cause contraction by a mechanism quite different from depolarizing drugs (16), and, as shown in Fig. 4, contractions produced by caffeine (3.9 mM) were unaffected by DNC_{10}, showing neither block nor enhanced desensitization. Similarly, contractions elicited by potassium ions were only slightly reduced by DNC_{10}, and there was no sign of desensitization when potassium chloride was applied repeatedly in the presence of DNC_{10}. The results with potassium were slightly less clear-cut than those with caffeine. This may have been due to a small cholinergic element in the response to potassium, revealed as a decrease by up to 20% of the potassium contraction in the presence of 3×10^{-6} M tubocurarine, and by slight enhancement of the potassium contraction in the presence of physostigmine, 7.4×10^{-7} M.

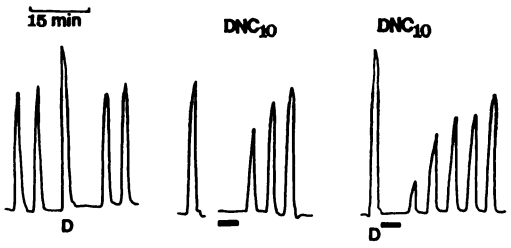

FIG. 7. *Tracing showing contractions of a chick biventer muscle strip in response to suxamethonium*

Unmarked responses were produced by 5.6×10^{-7} M suxamethonium. Responses marked *D* were produced by 2.2×10^{-5} M suxamethonium. DNC_{10}, 1.5×10^{-6} M, was present during the periods indicated by horizontal bars. The left-hand panel shows the small desensitization produced by 2.2×10^{-5} M suxamethonium on its own. The middle panel shows recovery from exposure to DNC_{10} without a preliminary desensitizing dose. The right-hand panel shows the slower recovery when the DNC_{10} was applied immediately after a desensitizing dose (D).

Carbachol and suxamethonium were both antagonized by DNC_{10}, and, in contrast to caffeine and potassium, both were effective in producing desensitization in the presence of DNC_{10} (Fig. 5).

Flacke and Yeoh (12) showed that tubocurarine antagonized the action of carbachol on leech muscle in the orthodox way, whereas it produced an effect very similar to the enhanced desensitization described above when suxamethonium was the agonist. In the present study, DNC_{10} was found to discriminate similarly between carbachol and suxamethonium in its blocking action on leech muscle. Figure 6 shows contractions of leech muscle produced by suxamethonium. During the control period, the preparation showed no sign of desensitization when 1.8×10^{-5} M suxamethonium was applied repeatedly at 3-min intervals. In the presence of DNC_{10}, 3.7×10^{-7} M, desensitization to suxamethonium was clearly seen, with the sensitivity recovering (though not to its control level) when the muscle was rested, just as in chick muscle (Fig. 3). In contrast, the effect of DNC_{10} on carbachol contractions was not unusual: responses to carbachol were antagonized by DNC_{10}, but there was only a very slight effect, if any, on desensitization. Thus, the interaction of DNC_{10} with suxamethonium in the leech closely resembled the effects seen in chick muscle with either carbachol or suxamethonium as agonist. Antagonism between DNC_{10} and carbachol in the leech muscle, on the other hand, showed no unorthodox features.

These observations argue strongly against a nonspecific action of DPC_{10} and DNC_{10}: both the block and the desensitization-enhancing action appear to affect only

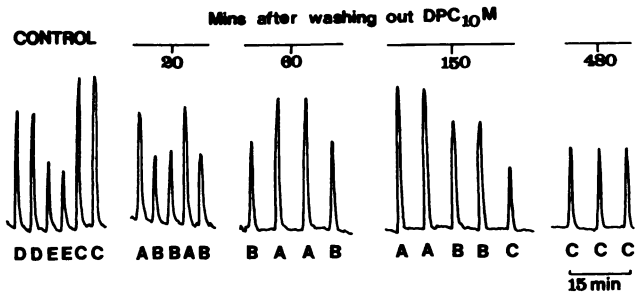

FIG. 8. *Tracing showing contractions of a chick biventer muscle strip in response to carbachol*

A, 3.7 × 10^{-5} M; *B*, 2.2 × 10^{-5} M; *C*, 1.6 × 10^{-5} M; *D*, 1.1 × 10^{-5} M; *E*, 7.4 × 10^{-6} M carbachol. Between the first and second panels, $DPC_{10}M$ was applied, giving a dose ratio of 2.5. During the 8-hr recovery period, the dose ratio fell to 1.7.

acetylcholine-like agonists in chick muscle, and both discriminate between carbachol and suxamethonium in leech muscle. This suggests that the mechanism of enhanced desensitization, as well as the antagonism, may involve an interaction with nicotinic receptors, and it was of interest to try to elucidate the mechanism further.

Two possible explanations of this phenomenon were considered: (a) that the DCP_{10} and DNC_{10} had a greater affinity for those receptors that had recently interacted with agonists, thus causing antagonist occupancy to increase after an agonist dose, and (b) that one effect of these antagonists was to increase the specific desensitization produced by a given dose of agonist, by a mechanism independent of their blocking action.

The experiment shown in Fig. 7 favored the former hypothesis: it showed that recovery from DNC_{10} block took longer if the DNC_{10} was applied immediately after a large dose of agonist had been washed out than if it was applied after a period of quiesence. This suggested that the large dose of agonist had produced some change in the receptors which gave them a higher affinity for the DNC_{10}, and consequently allowed the DNC_{10} to achieve a higher occupancy. It was difficult to reconcile this result with the second view, because the DNC_{10} was applied only after washing out the agonist, and it could not have altered the amount of desensitization produced.

It was not possible to study this type of block quantitatively in terms of occupancy, because every agonist dose changed the level of the block that it was intended to test. However, the irreversible antagonist $DPC_{10}M$ provided a useful analytical tool for a quantitative study, because the final occupancy achieved by a given dose applied for a given time could be studied after washing out the antagonist. Thus, the agonist doses used to test the level of block could not themselves alter that level, because they were applied when no free antagonist was present in the bath.

Action of $DPC_{10}M$. In contrast to the rapidly reversible blocking action of DPC_{10} and DNC_{10}, the blocking action of $DPC_{10}M$ persisted for many hours after the drug was washed out of the tissue.

Figure 8 shows stages in the recovery from $DPC_{10}M$ block of a strip of chick biventer muscle stimulated with carbachol.

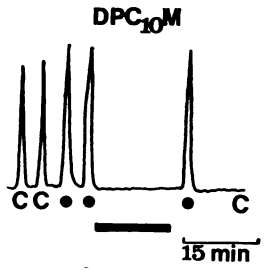

FIG. 9. *Tracing showing contractions of a chick biventer muscle strip*

C, Carbachol, 1.1 × 10^{-5} M; ●, caffeine, 3.9 mM. During the period indicated by the bar, $DPC_{10}M$, 8.8 × 10^{-5} M, was present in the bath. This abolished the responses to carbachol without affecting the responses to caffeine.

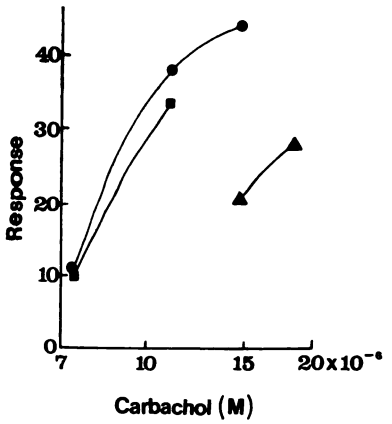

FIG. 10. *Log dose-response curves of chick biventer muscle strip to carbachol, showing protection by tubocurarine against the blocking action of $DPC_{10}M$*

●, Control curve; ■, after exposure to $DPC_{10}M$, 2.2×10^{-6} M, for 15 min, applied in the presence of tubocurarine, 2.2×10^{-5} M; ▲, after exposure to $DPC_{10}M$, 2.2×10^{-6} M, for 15 min without protection by tubocurarine.

After an initial phase of recovery (which varied markedly in different experiments and was complete in a few minutes), the occupancy was 0.6 (dose ratio, 2.5). After 8 hr the occupancy had declined only to 0.4. In this respect, the blocking action of $DPC_{10}M$ resembles that of benzilylcholine mustard on muscarinic receptors (11). In subsequent experiments, the degree of block was measured between ½ and 1 hr after washing out the $DPC_{10}M$. The rapid initial phase of recovery could have been due to the dissociation of a reversible ethyleniminium-receptor complex formed prior to alkylation (cf. ref. 11), or it could have been due to the presence of reversible antagonists in the mixture of substances formed as side products of the cyclization reaction. Only about 75% of the starting material gave rise to ethyleniminium ions, and it seems quite possible that the remaining 25% may have had appreciable blocking activity.

At dose ratios greater than about 3, produced with $DPC_{10}M$, the log dose-response curve for carbachol or suxamethonium was flattened as well as shifted to the right, presumably because the antagonism was irreversible and therefore noncompetitive. In the remaining experiments the dose ratios were usually less than 2.5, and the log dose-response curves remained parallel, so that the dose ratio could be measured unambiguously.

If a solution of $DPC_{10}M$ was kept at 37° overnight, during which time all of the ethyleniminium ion was hydrolyzed, the long-lasting blocking action was lost, and the solution showed only a fully reversible blocking effect. It appeared, therefore, that, as with benzilylcholine mustard, the species responsible for the long-lasting block was the ethyleniminium ion.

The specificity of action of $DPC_{10}M$ was tested in the following ways.

1. Contractions produced by caffeine, 3.9 mM, were unaltered by $DPC_{10}M$. Figure 9 shows the effect of 8.8×10^{-5} M $DPC_{10}M$, applied for 15 min, which caused complete abolition of the response to carbachol without any effect on the caffeine contraction.

2. Application of $DPC_{10}M$ in the presence of tubocurarine failed to produce a persistent antagonism to carbachol (Fig. 10). This experiment showed that tubocurarine could protect the tissue against the action of $DPC_{10}M$, the most likely interpretation being that the receptors that were occluded by tubocurarine were rendered unavailable to the alkylating agent.

3. In leech muscle, carbachol and suxamethonium appear to act upon different receptors [see Flacke and Yeoh (12), and also results presented below]. The action of $DPC_{10}M$ on leech muscle was also selective, contractions produced by carbachol being unaffected by $DPC_{10}M$, while those produced by suxamethonium were antagonized as in chick muscle (e.g., Fig. 11).

In chick muscle there is evidence of only one receptor system, acted on by both suxamethonium and carbachol. It was surprising, therefore, to find that after application of $DPC_{10}M$ the dose ratio for suxamethonium was consistently slightly less than that for carbachol. For example, in one experiment, 4.5×10^{-6} M $DPC_{10}M$ applied for 15 min caused a dose ratio of 1.9 measured on carbachol responses, and 1.5 for suxamethonium; in another, 9.0×10^{-6} M $DPC_{10}M$ applied for 15 min produced a dose ratio of 2.1 for carbachol and only 1.7 for suxamethonium. Physostigmine, $7.9 \times$

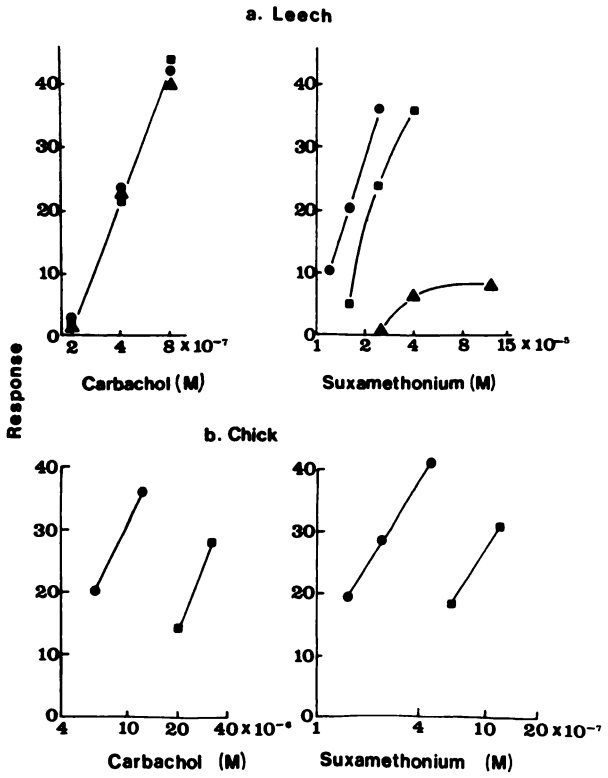

FIG. 11. *Log dose-response curves for chick and leech muscle, using carbachol and suxamethonium as agonists*

Each point represents the mean of two to four closely similar responses. The symbols ● in each panel show the control curve. The right-hand curves were obtained after exposure to $DPC_{10}M$. It can be seen that $DPC_{10}M$ discriminates between carbachol and suxamethonium in the leech, but not in the chick.

a. Leech muscle. ■, $DPC_{10}M$, 9.6×10^{-6} M, for 15 min; ▲, $DPC_{10}M$, 9.6×10^{-6} M, for 15 min with suxamethonium, 4.5×10^{-5} M, for 10 min concurrently.

b. Chick muscle. ■, $DPC_{10}M$, 7.9×10^{-6} M, for 15 min, in the presence of physostigmine, 7.9×10^{-8} M.

10^{-8} M, increased the responses of chick biventer muscle strips to suxamethonium, but not to carbachol, in contrast to its action on leech muscle (17). It therefore seemed possible that, in addition to alkylating the receptors, the $DPC_{10}M$ also irreversibly inactivated the tissue cholinesterase and that this caused the log dose-response curve of suxamethonium to shift less than that of carbachol. In accordance with this view, when experiments were performed in the presence of physostigmine (7.9×10^{-8} M), the dose ratios measured after $DPC_{10}M$ block were identical for carbachol and suxamethonium (Fig. 11b).

These findings confirmed that $DPC_{10}M$ produced its antagonism by a specific receptor-blocking action. It thus seemed a suitable compound with which to investigate the desensitization-enhancing effect seen with DNC_{10} and DPC_{10}.

In a number of experiments, desensitization of chick muscle to carbachol was tested before and after the application of an amount of $DPC_{10}M$ sufficient to produce a dose ratio between 2 and 3. It was found that the desensitization produced by a given dose of carbachol was altered little, if at all, after the tissue had been exposed to the agonist. The effect seen in Fig. 3 with the reversible antagonists was not observed, showing that the blocking effect is not itself associated with an effect on desensitization. The next step was to test the

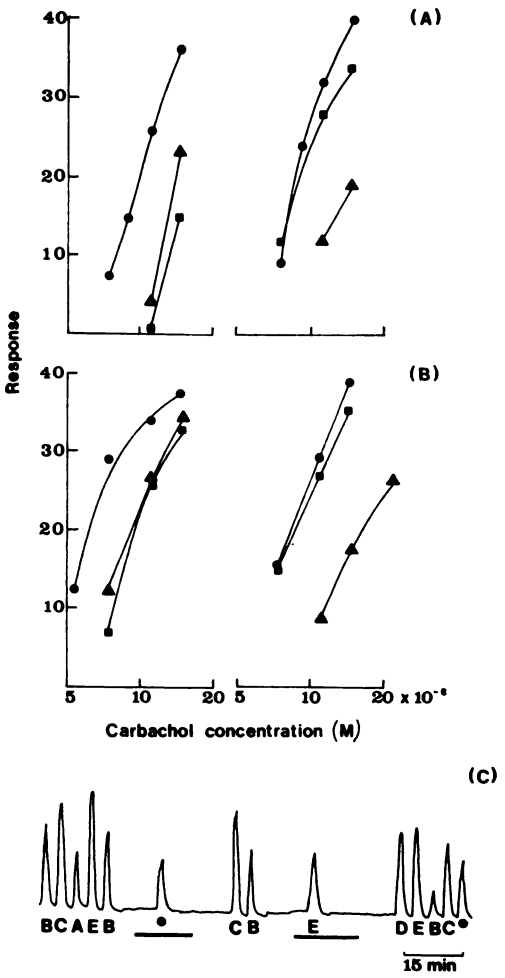

FIG. 12. *Enhancement of blocking action of $DPC_{10}M$ by concurrent application of agonists.*

A and B. Log dose-response curves for chick muscle. Each point represents the mean of two to four closely similar responses. ●, Control curves; ■, after exposure to 1.1×10^{-6} M $DPC_{10}M$ for 15 min with no concurrent application of agonist; ▲, after exposure to 1.1×10^{-6} M $DPC_{10}M$ for 15 min with simultaneous application of 1.5×10^{-5} M carbachol (two successive doses, each left in for 2 min (A) and 5.9×10^{-7} M suxamethonium (two doses for 2 min each) (B). In the left-hand panels, the agonist was applied during the first of the two exposures to $DPC_{10}M$; in the right-hand panels, the order was reversed. In both cases the antagonism produced was greater when agonist was applied simultaneously.

C. Tracing of responses of chick biventer muscle strip to carbachol (*A–E*) and caffeine (●), 4 mM. *A*, 5.9×10^{-6} M; *B*, 7.4×10^{-6} M; *C*, 1.1×10^{-5} M; *D*, 1.2×10^{-5} M; *E*, 1.5×10^{-5} M carbachol. During the periods indicated by horizontal bars, 2.2×10^{-6} M $DPC_{10}M$ was present. This experiment showed that the blocking action of $DPC_{10}M$ was increased by simultaneous application of carbachol, but not by an equiactive dose of caffeine.

alternative hypothesis, that the effectiveness of the antagonist was enhanced by the action of the agonist.

Effect of agonists on the blocking action of $DPC_{10}M$. Application of $DPC_{10}M$ for 15 min to a chick biventer preparation without simultaneous agonist applications caused a degree of irreversible block that varied with the concentration of $DPC_{10}M$ used (Fig. 13b). If, however, carbachol was added to the muscle during the application of $DPC_{10}M$, the block produced was greater. Thus Fig. 12A shows that 1.1×10^{-6} M $DPC_{10}M$ applied for 15 min to a quiescent preparation caused very little antagonism (dose ratio, about 1.1), but that substantially more antagonism was produced (dose ratio, about 1.6) when carbachol, 1.7×10^{-5} M, was applied for a total of 4 min in the presence of the antagonist.

This finding confirmed the earlier, tentative conclusion that the desensitization-enhancing action of DNC_{10} resulted from an increase in the affinity of the receptors for the antagonist, caused by application of the agonist, rather than from an effect of the antagonist on the desensitization process. Thus, with $DPC_{10}M$, the blocking effect lasted indefinitely and could be confidently ascribed to alkylation of receptors rather than to desensitization.

In describing the effects of DNC_{10} and DPC_{10}, evidence was presented that the desensitization-enhancing effect was specific, in that it was produced only by acetylcholine-like agonists in the chick, and much more by suxamethonium than by carbachol in leech muscle. We therefore investigated, in leech muscle, whether the effect of different agonists in promoting receptor alkylation by $DPC_{10}M$ showed the same specificity as was described for the desensitization-enhancing effect described earlier.

Figure 12B shows that suxamethonium, as well as carbachol, increased the blocking

TABLE 2

Selective action of $DPC_{10}M$ with respect to carbachol and suxamethonium in leech muscle

The table shows dose ratios for carbachol and suxamethonium produced by exposure of strips of leech muscle to $DPC_{10}M$. Control values represent the effect of $DPC_{10}M$ alone; experimental values were obtained after concurrent application of the conditioning agonist (concentrations and times indicated below). For each pair of measurements, two strips from a single muscle were used.

Conditions			Dose ratio	
Antagonist	Test agonist	Conditioning agonist	Control	Experimental
9.6×10^{-6} M, 15 min	Carbachol	Carbachol, 1.2×10^{-5} M, 5 min	No block	No block
	Carbachol	Suxamethonium, 4×10^{-5} M, 10 min	No block	No block
2.4×10^{-6} M, 20 min	Suxamethonium	Carbachol, 1.6×10^{-6} M, 4 min	1.24	1.25
	Suxamethonium	Suxamethonium, 4×10^{-5} M, 4 min	1.69	5.8[a]

[a] Log dose-response curves were markedly flattened; dose ratios were measured at roughly 50% of the maximal response.

effect of $DPC_{10}M$ in chick muscle; caffeine, however, did not (Fig. 12C). Suxamethonium was approximately 30 times as active as carbachol in producing contracture, and appeared to be similarly more active in enhancing block by $DPC_{10}M$. Caffeine in a concentration of 4 mM produced a contraction about equal to that produced by 1.5×10^{-5} M carbachol but was completely ineffective in enhancing the blocking action of $DPC_{10}M$.

In leech muscle, as Table 2 and Fig. 11 show, the sensitivity to carbachol was unaffected by $DPC_{10}M$, and carbachol was correspondingly ineffective in enhancing the blocking action of $DPC_{10}M$. Suxamethonium, on the other hand, was antagonized by $DPC_{10}M$, and was also very effective in increasing the blocking effect of $DPC_{10}M$.

These relationships, in chick and leech muscle, are summarized in Table 3. Thus, the specificity of action of each agonist in enhancing the blocking effect of $DPC_{10}M$ corresponds exactly to its receptor specificity as an agonist. The phenomenon described can therefore be regarded as an effect of agonists upon their receptors, the effect being to increase the affinity of the receptors for antagonists of the particular type represented by DPC_{10}, DNC_{10}, and $DPC_{10}M$. We have chosen to call this a metaphilic effect of agonists, denoting their ability to alter the affinity of receptors for other compounds.

Time course of the metaphilic effect. It was of interest to determine whether the metaphilic effect vanished as soon as the agonist was washed out of the tissue, or

TABLE 3

Enhancement of block by $DPC_{10}M$ by different agonists

Species and test agonist	Effect[a] of conditioning agonist		
	Carbachol	Suxamethonium	Caffeine
Chick			
Carbachol	+	+	0
Suxamethonium	+	+	0
Caffeine	0	0	0
Leech			
Carbachol	0	0	—
Suxamethonium	0	+	—

[a] + indicates enchancement of block by conditioning agonist; 0 indicates little or no effect; — indicates not tested.

whether it remained after removal of the agonist. The experiment in which block by DNC_{10} was enhanced if the drug was immediately preceded by a large dose of agonist (Fig. 7) suggested the latter, but the point could be tested more satisfactorily by using $DPC_{10}M$. The design of the experiments was as follows. After establishing the control dose-response curve to carbachol, the preparation was rested for at least 15 min and was then exposed to 2.4×10^{-5} M $DPC_{10}M$ for 15 min, without concurrent application of agonist. A second dose-response curve to carbachol was then determined in order to measure the occupancy produced. $DPC_{10}M$ was then applied again at the same concentration, preceded, at intervals varying from 0 to 2½ hr, by a large dose of carbachol (1.5×10^{-4} M for 1½ min). In control experiments, the conditioning dose of carbachol was omitted. After washing out the antagonist, the carbachol dose-response curve was again measured, and the dose ratio produced by the second application of $DPC_{10}M$ was compared with that produced by the first. Figure 13a shows the results of these experiments. The metaphilic effect clearly outlasted the presence of carbachol, being still detectable 15 min after washing out the agonist. The effect of $DPC_{10}M$ applied 2½ hr after the conditioning dose of carbachol did not differ significantly from the control, in which no conditioning dose was given.

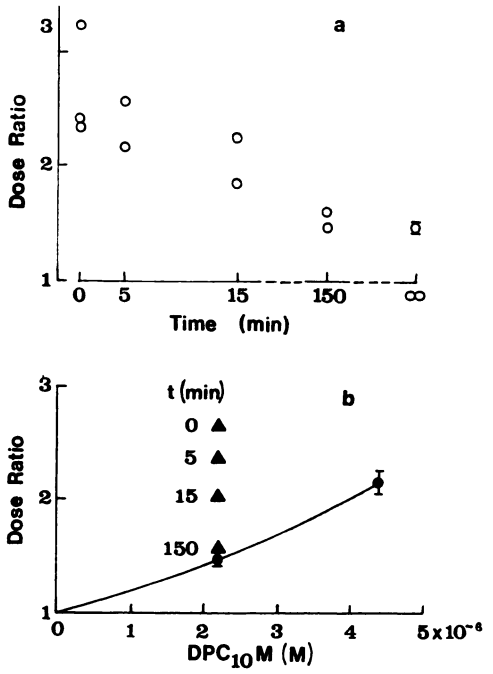

FIG. 13. *Time course of block-enhancing (metaphilic) effect of carbachol in chick muscle*

a. Dose ratios produced by applications of $DPC_{10}M$, 2.2×10^{-6} M, for 15 min at various times after washing out a dose of carbachol (1.5×10^{-4} M, for 1½ min). The point shown at time infinity is the mean and standard error of 11 measurements of dose ratio after application of $DPC_{10}M$ as above, but with *no* previous large dose of carbachol. There is clearly a regression with time, and some effect is apparent even 15 min after washing the carbachol out of the tissue.

b. Dose ratio plotted against concentration of $DPC_{10}M$ (applied for 15 min). The point at 2.2×10^{-6} M is the mean of 11 observations (and is the same as the point at time infinity in Fig. 13a). The point at 4.4×10^{-6} M is the mean (with standard error) of five observations. The filled triangles are the means of the single observations plotted in Fig. 13a, the appended numbers being the intervals (in minutes) between washing out the large dose of carbachol and applying $DPC_{10}M$. It is apparent that a dose of 1.5×10^{-4} M carbachol for 1½ min may at least double the blocking potency of $DPC_{10}M$ applied shortly afterward.

The time course of the metaphilic effect produced by a 1½-min application of 1.5×10^{-4} M carbachol is shown in Fig. 13a: when $DPC_{10}M$ was applied immediately after the conditioning dose of carbachol, it produced a dose ratio of 2.5–3, whereas when no conditioning dose preceded the $DPC_{10}M$ application, the dose ratio was only 1.4. Even when 15 min elapsed between the conditioning dose and exposure to the antagonist, the dose ratio was about 2, showing that the effect of the conditioning dose persists for at least 15 min after it has been washed out of the tissue.

The magnitude of the metaphilic effect may be expressed as an increase in potency of $DPC_{10}M$ produced by the conditioning dose of agonist. Figure 13b shows the relationship between $DPC_{10}M$ concentration and the occupancy produced in the absence of any conditioning dose, and also the increase in the effectiveness of $DPC_{10}M$ when it was preceded by the conditioning dose of carbachol. It can be seen that the potency

of $DPC_{10}M$ was roughly doubled by the conditioning dose of carbachol given 5 min before.

Effect of tubocurarine applied before $DPC_{10}M$. It was important to establish whether the antagonist, tubocurarine, when applied before $DPC_{10}M$, would enhance $DPC_{10}M$ block in the same way that carbachol did, or whether a drug acting on the same receptor as $DPC_{10}M$ had to be an agonist to show the metaphilic effect. The same experimental design was used as with the experiments on the time course of the metaphilic effect of carbachol, but instead of a large dose of carbachol before the second $DPC_{10}M$ application, a dose of tubocurarine (1.5×10^{-6} M) was applied for 4 min and washed out 5 min before the second dose of $DPC_{10}M$. This concentration of tubocurarine produced an occupancy of approximately 0.8, and on washing out of the tubocurarine the antagonism declined rapidly, so that the occupancy was negligible at the time when the $DPC_{10}M$ was applied. If any metaphilic effect had been produced by the tubocurarine, it would have caused a larger dose ratio after the second dose of $DPC_{10}M$ than after the control.

This was not found to be true, however, the effect of $DPC_{10}M$ applied after exposure to tubosurarine being identical with the effect produced without any conditioning dose of tubocurarine. This result was consistent with the idea that *activation*, and not merely *occupation*, of the receptors is necessary for the metaphilic effffect.

DISCUSSION

The results presented in this paper show that the antagonists DPC_{10}, DNC_{10}, and $DPC_{10}M$ produce their effects by blocking receptors in both chick and leech muscle, and that this action is specifically favored by concurrent application of the agonists that act upon these receptors. With the reversible antagonists, DPC_{10} and DNC_{10}, the effect takes the form of "enhanced desensitization." There are, however, reasons for ascribing this phenomenon to the enhancement, by the agonist, of the action of the antagonist, rather than to the enhancement of desensitization by the antagonist. Thus, DNC_{10} applied in the aftermath of a large dose of agonist produced a greater block than when it was applied after a rest from agonist (Fig. 7). This finding shows that DNC_{10} cannot act by accentuating the development of desensitization; it could, however, retard the recovery process. The experiments with the alkylating agent, $DPC_{10}M$, suggest that this is not so, for in a preparation blocked by $DPC_{10}M$, desensitization was not enhanced; thus, the irreversible block was not associated with an irreversible effect on desensitization. The interpretation that is consistent with all of these findings is that the antagonists have no direct effect on desensitization, but that their affinity for receptors is increased by prior or concurrent application of agonist to the tissue.

Another interpretation that has to be considered is that the agonists facilitate the access of metaphilic antagonists to the receptors. Thus, for instance, contraction of the tissue might increase the rate at which these antagonists equilibrate throughout the extracellular space of the tissue; however, this cannot be the case, because caffeine contractions in the presence of $DPC_{10}M$ did *not* affect the final level of block (Table 3 and Fig. 12C). Again, if the site of action of metaphilic antagonists were intracellular, the block-enhancing action of agonists might result from their causing an increase of membrane permeability, thereby facilitating the entry of the antagonist into the cell; but DPC_{10}, DNC_{10}, and $DPC_{10}M$ act specifically on acetylcholine receptors of chick muscle (Figs. 4, 9, and 10), not on the contractile mechanism, and there is good evidence that acetylcholine receptors are accessible only from *outside* the cell (18, 14). Furthermore, if DNC_{10} were let into the cell by the action of carbachol, the finding that a period of rest partly relieved the block (Figs. 3, 5, and 6) means that some mechanism for the *removal* of DNC_{10} in the absence of carbachol has to be postulated, for the electrochemical gradient discouraging the escape of a cationic drug, such as DNC_{10}, from the cell would be greatest when the membrane potential is large (i.e., in the *absence* of carbachol). Thus, not

only is there strong circumstantial evidence against an intracellular site of action of metaphilic antagonists, but also, if facilitated access to such a site were to be the basis of the metaphilic effect, a special mechanism for the removal of metaphilic antagonists—such as active pumping out of the cell, or metabolic breakdown—would have to be proposed. Such a mechanism appears highly improbable.

It appears, therefore, that agonists must alter the structure of the receptor site in some way, increasing its affinity for this particular type of antagonist, but not for conventional antagonists, such as gallamine. It is this postulated molecular alteration of the receptor by agonists that we have called the metaphilic effect.

Although we have shown the metaphilic effect only in relation to the affinity of receptors for antagonists, an obvious possibility is that the affinity of receptors for agonists is similarly altered. Such an effect, if present, could be involved in the mechanism of desensitization.

The metaphilic effect in some ways resembles the well-known allosteric effect (19–21), in which an allosteric effector molecule is thought to bring about a conformational change in a macromolecule, thereby altering the affinity of a spatially separate active site for its substrate. Models of drug-receptor interactions based on allosteric mechanisms have recently been proposed by Karlin (22) and by Changeux, Thiéry, Tung, and Kittel (23). In our experiments, the chemical resemblance between the agonists and the antagonists makes it rather unlikely that they were acting at spatially separate sites. Moreover, tubocurarine prevented receptor blockade by $DPC_{10}M$; thus it is likely that these substances compete at the same site, and it is generally accepted that tubocurarine combines with the same site as agonists (3, 5). It therefore seems unlikely that $DPC_{10}M$ can combine with a receptor actually occupied by agonist, and we must assume either that the change induced in the receptor by the agonist outlasts the occupation of the receptor, or that occupation of a receptor by the agonist can induce changes in adjacent, unoccupied receptors. The latter possibility was elaborated by Changeux *et al.* (23) into a rather general theory of drug action based on "cooperativity" between adjacent receptors. Our finding that the metaphilic effect persists for at least 15 min after washing out a large dose of agonist supports the former hypothesis, and it seems unnecessary to invoke a mechanism depending on interaction between adjacent receptors.

The metaphilic effect appears to represent a change in the molecular structure of the receptor brought about by the action of agonists, but not of antagonists. An interesting possibility is that it may be an indirect manifestation of the molecular change in the receptor involved in the process of stimulation.

A possible example of the metaphilic phenomenon is provided by the study of Flacke and Yeoh (12), using leech muscle. They found that tubocurarine produced a conventional block when measured against carbachol or acetylcholine, but produced only enhanced desensitization when tested against suxamethonium. The response to infrequent doses of suxamethonium was quite unaltered in the presence of tubocurarine. In this case one would have to propose that tubocurarine had no appreciable affinity for suxamethonium receptors until they had been acted upon by suxamethonium. The enhancing effect of suxamethonium on the blocking action of tubocurarine could be detected when the tubocurarine was added shortly after washing out a dose of suxamethonium (as in the present study, with carbachol and $DPC_{10}M$). Thus, in the leech it would appear that the two types of antagonism—conventional and metaphilic—are demonstrable with a single antagonist acting on different receptors; in contrast, in chick muscle suxamethonium and carbachol act at the same receptor, and the type of antagonism produced is a function only of the antagonist used. These somewhat involved relationships are summarized in Table 4.

A series of *N*-substituted decamethonium derivatives was studied for blocking action against butyltrimethylammonium in the frog rectus abdominis preparation by van Rossum (24). The cumulative method for

TABLE 4
Summary of actions of compounds studied on chick and leech muscle

Compound	Chick muscle	Leech muscle
Carbachol and suxamethonium	Agonists acting on same receptor	Agonists acting on different receptors[a]
Gallamine	Conventional antagonist	Conventional antagonist on both receptors[a]
Tubocurarine	Conventional antagonist	Conventional antagonist of carbachol;[a] metaphilic antagonist of suxamethonium[a]
DPC_{10} and DNC_{10}	Reversible metaphilic antagonists	Conventional antagonists of carbachol; metaphilic antagonists of suxamethonium
$DPC_{10}M$	Irreversible metaphilic antagonist	Not irreversible antagonist of of carbachol; irreversible metaphilic antagonist of suxamethonium

[a] From results of Flacke and Yeoh (12).

measuring agonist dose-response curves was used, and several of the more heavily substituted compounds caused flattening as well as a shift to the right of the log dose-response curve. This was attributed to "noncompetitive" antagonism, but it seems possible that the metaphilic phenomenon could have accounted for at least part of the flattening that was observed. Thus, the progressively increasing agonist concentration might, by a metaphilic action, have progressively increased the antagonist occupancy during the determination of the dose-response curve.

Another recent study concerning an alteration of receptor specificity is that of Karlin and Winnik (25); here, however, the agent used to change the receptors was not an agonist but a reducing agent, dithiothreitol. *N*-Ethylmaleimide appeared to react with the acetylcholine receptor of the electroplaques of *Electrophorus electricus* only after the tissue had been treated with dithiothreitol. Karlin and Winnik suggested that the dithiothreitol reduced a disulfide bond in the receptor, rendering it susceptible to the action of *N*-ethylmaleimide.

Waud (26) has criticized the use of "cross-protection" experiments, in which large doses of agonist are applied to tissues that are then exposed to an irreversible antagonist. If the blocking effect of the antagonist is reduced when agonist is applied simultaneously, this is taken as evidence of a common receptor site. The present studies suggest another possible pitfall in the interpretation of cross-protection experiments, for here application of agonist has been shown actually to *promote* the occlusion of receptors by an irreversible antagonist.

The long-lasting nature of the metaphilic effect suggests that the altered form of the receptor for which the antagonist has high affinity persists for many minutes after removal of the agonist, reverting only slowly to the "natural" form. An interesting example of a change in protein conformation that takes several minutes to occur was described by Massey and Curti (27). They found that when the apoenzyme D-amino acid oxidase was activated by adding flavin adenine dinucleotide at 15–16°, the resulting conformational change occurred exponentially with a time constant of about 5 min, even though the complex was formed almost instantaneously. Thus the slow time course of the metaphilic effect would appear to be compatible with a slow conformational rearrangement of a macromolecule. One effect of agonists which may also persist for many minutes is desensitization, and this raises the possibility that the receptors for which $DPC_{10}M$ has high affinity may be "desensitized" receptors.

The mechanisms involved in drug de-

sensitization are at present quite uncertain. It seems clear that at least one mechanism is nonspecific (28, 6) and therefore not to be construed in terms of specific receptors. On the other hand, agonist-specific desensitization also exists (29, 30), and Katz and Thesleff (31) suggested a model for desensitization at the neuromuscular junction involving the gradual formation of desensitized receptors, which revert spontaneously to the natural form when the agonist is removed. We are currently studying desensitization in chick and leech muscle to try to determine whether there is a relationship between this and the metaphilic phenomenon. The possibility that the same molecular process underlies stimulation, desensitization, and the metaphilic phenomenon also remains to be explored.

ACKNOWLEDGMENT

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