MOLECULAR PHARMACOLOGY, 6, 357-382

On the Mechanism of Desensitization at Cholinergic Receptors

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(Received December 22, 1969)

SUMMARY

Evidence is presented that desensitization to cholinergic agonists in chick and leech muscle is a process involving receptor inactivation. Various possible mechanisms that could account for slow inactivation of receptors by agonists were analyzed mathematically, and expressions were obtained for the rate and extent of desensitization expected under various conditions.

With the majority of agonists tested there appeared to be the same relationship between the response and the amount of desensitization produced. Certain agonists, however, were relatively more effective in causing desensitization.

The kinetics of development of and recovery from desensitization were studied in chick and frog muscle. Reactivation of desensitized receptors occurred exponentially. In chick muscle the rate constant for recovery was the same (0.3 min^{-1}) regardless of what agonist had been used to produce the desensitization.

In chick muscle, tubocurarine decreased *pari passu* the response and the desensitization produced by carbachol or suxamethonium. Tubocurarine increased the desensitization produced by the partial agonist *n*-decyltrimethylammonium.

These results are compatible with the cyclic model for desensitization suggested by Katz and Thesleff [*J. Physiol.* (*London*) **138**, 63 (1957)], with the additional factor that certain drugs may have a preferential affinity for desensitized, compared with normal, receptors. The process of receptor activation may be very closely related to the transition from normal to desensitized receptors brought about by agonist drugs.

INTRODUCTION

In a study of an anomalous type of drug antagonism involving cholinergic receptors in chick slow muscle and in leech muscle (1) we found that the affinity of the receptors for certain antagonists was increased if the appropriate agonist was applied at the same time as, or shortly before, the tissue was exposed to the antagonist. The evidence suggested that this change in affinity was due to a conformational change in the receptors caused by the agonist and it was called the metaphilic effect. Because the effect was found to persist for several minutes after a conditioning dose of agonist was washed out of the tissue, we suggested that it might be related to the phenomenon of desensitization, which appears as a decrease in the sensitivity of a tissue to agonist drugs that occurs after a high concentration of agonist has been applied to the tissue, and from which recovery can take many minutes.

In this paper a number of possible mechanisms that could be responsible for desensitization are discussed, with the aim of

This work was supported by a grant from the Medical Research Council.

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determining whether the mechanism could also account for the metaphilic effect.

There have been several studies of drug desensitization in various tissues. Barsoum and Gaddum (2), Cantoni and Eastman (3), and Paton (4) have described specific and nonspecific desensitization in intestinal smooth muscle. Desensitization at the frog neuromuscular junction has been studied by Thesleff (5), Katz and Thesleff (6), Nastuk, Manthey, and Gissen (7), and Manthey (8). Khairallah, Page, Bumpus, and Türker (9) have studied the desensitization produced by angiotensin in arterial smooth muscle. The current status of the various theories put forward is discussed by Elmqvist and Thesleff (10), Taylor and Nedergaard (11), and Waud (12). It is safe to say that the mechanism is poorly understood. An important but not always very clear distinction has been made between specific desensitization, in which an agonist desensitizes the muscle only to agonists that act upon the same receptor (2, 13, 14), and nonspecific desensitization, in which an agonist desensitizes the tissue to agonists that act on different receptors (3, 4).

The first part of this paper is concerned with testing the specificity of the desensitization produced by agonists such as carbachol and suxamethonium in chick and leech muscle. If desensitization is related to the metaphilic effect, it would be expected to show the same pattern of specificity. The results show this to be true, and suggest that desensitization involves the inactivation of a fraction of the receptor pool, as proposed by Katz and Thesleff (6) and Nastuk (15). These authors suggested various theoretical models to account for desensitization, and we have analyzed the properties of these and other models and tried to test experimentally which one accounts most satisfactorily for desensitization to cholinergic agonists in chick and frog muscle. The results support the cyclic model proposed by Katz and Thesleff (6) and favored by them on kinetic grounds. This model postulates the production of a modified and insensitive conformation of the receptor, which could be the form for which metaphilic antagonists have a preferential affinity. The quantitative relationship between desensitization and the metaphilic effect is discussed in the accompanying paper (16). The results also suggest a relationship between the processes of receptor activation and desensitization which implies that desensitization is a necessary consequence of receptor activation and not simply a pharmacological curiosity.

Previous studies on desensitization (6–8) have expressed the effect as a fractional reduction in the response to a given concentration of agonist. In order to infer from such a measurement the fraction of the receptor pool that has been inactivated, some assumption about the relationship between the fractional receptor occupancy by the agonist drug and the measured effect is needed, and this can lead to considerable uncertainties in interpretation (see ref. 12). In the present studies, we have expressed desensitization in terms of agonist dose ratios. Though this involves more laborious experiments, being a null method it circumvents the need for these arbitrary assumptions, and has been used extensively in the analysis of drug-receptor interactions (4, 17, 18).

MECHANISM OF DESENSITIZATION—THEORETICAL ASPECTS

The main mechanisms that have been proposed to account for desensitization are the following.

Nonspecific mechanisms (3, 4, 12). When only one class of agonist is known, it is difficult to exclude nonspecific mechanisms. Results presented below show that mechanical fatigue or failure of excitation-contraction coupling is not appreciably involved in chick muscle, and there is abundant evidence that desensitization is associated with recovery of both the membrane potential and the conductance at the frog neuromuscular junction. Waud (12) has suggested that local ionic shifts brought about as a consequence of the conductance change could account for desensitization, but it is not clear to us how the entry of sodium ions or the loss of potassium ions could act to restore the membrane potential and nullify the increase in conductance.

Receptor mechanisms. Various suggestions have been made that have tried to account

for desensitization by the properties of the drug-receptor interaction itself. Thus Paton (4) put forward the rate theory of drug action, which suggests that the effect of an agonist is a function of its rate of association with the receptors (not of the fraction of receptors occupied, as the conventional theory holds). This theory predicts that the effect of an agonist will initially be large, when all the receptors are vacant and the association rate consequently high, but will fade away as the fraction of receptors occupied by agonist molecules increases, leaving fewer vacant for fresh associations. When the drug is washed out of the tissue, the rate of association will at once drop to zero, and so the effect will cease, but a fraction of the receptors will remain occupied, and this means that the tissue will be less sensitive for a time after an agonist is washed out of the tissue. The current status of this theory is discussed by Waud (12); though convincing evidence in its favor is difficult to obtain, it has not so far been refuted experimentally, and was considered as a possible mechanism for desensitization in the present study.

There are also various models that adhere to the basic occupation theory of drug action (i.e., response is a function of the fraction of receptors occupied by agonist molecules, and not of the rate of association), but add on extra reaction steps in order to explain desensitization. Four basic models of this type have been proposed and are shown in Fig. 1. The first three (Fig. 1a–c) were considered by Katz and Thesleff (6). In the first model, two types of agonist-receptor interaction are envisaged. The first type of complex, AR, is formed rapidly and is responsible for producing the drug effect. The second type of complex, AR', is formed only slowly, and is inactive. In the second type of model (Fig. 1b), the active complex, AR, slowly turns into an inactive form, AR'. In these two models, slow recovery from desensitization is ascribed to slow dissociation of AR' (in Fig. 1a) or to slow reconversion of AR' to AR (in Fig. 1b). The third model (Fig. 1c) also entails the conversion of the active complex, AR, to an inactive form, AR', but the status quo is restored by dissociation of AR', leaving free R', which then slowly reverts to R. As Fig. 1c is drawn, the desensitization and recovery steps are irreversible. As Katz and Thesleff discussed, these can be made reversible, but in the formulations that follow we have found that the irreversible model gives rather simpler equations, not differing in their general form from those of the reversible model, and we have confined our attention to this form.

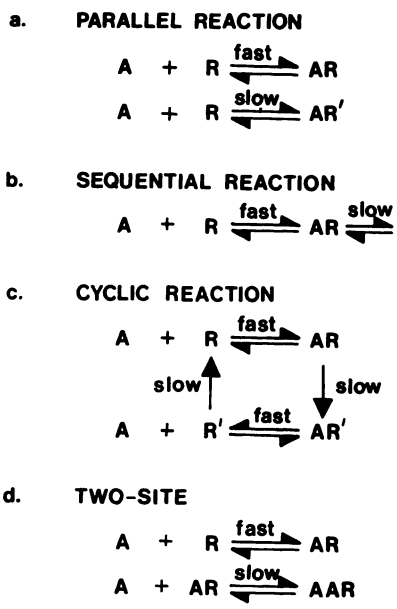


FIG. 1. *Hypothetical mechanisms for desensitization*

In each model the active complex AR is formed rapidly. Desensitization entails the slow formation of AR' (in models a, b, and c) or of AAR (model d). In the cyclic reaction model, the existence of unoccupied but desensitized receptors, R', is postulated.

Finally, Fig. 1d shows a model that derives from Nastuk and Gissen (19), who suggested that the receptor has more than one binding site, and that occupation of the first site activates the receptor while occupation of subsequent sites inactivates it. Thus desensitization is ascribed to the progressive occupation of the extra binding sites, which Nastuk and Gissen (19) suggested might be three phosphate groups. In our representation, only two sites are shown, because addition of extra sites merely com-

plicates the equations without basically altering their form.

Depolarizer entry (11). This theory suggests that depolarizing agents cross the postsynaptic membrane, and that their intracellular accumulation results in desensitization. Evidence in favor of this mechanism at present appears to be slender: indeed, del Castillo and Katz (20) found that intracellular injection of carbachol at frog motor end plates did not affect their sensitivity to externally applied carbachol, which is contrary to the prediction of the theory. This theory does not attempt to explain why intracellular accumulation of the agonist should result in desensitization, and therefore gives rise to little in the way of quantitative predictions. We shall not, therefore, consider it further, though it has clearly not been ruled out as a mechanism of desensitization.

Predicted Properties of Theoretical Models

We have derived equations, based on the law of mass action, to describe the extent and rate of desensitization as functions of the concentration of agonist, and the effect on desensitization of adding reversible competitive antagonists.

In all of the models the drug effect is greatest when the drug is first applied, and then declines to a steady-state value when the drug is left in contact with the tissue; it is useful to obtain equations describing the rate and extent of this decline predicted by the different models. When the agonist concentration is reduced to zero, a certain fraction of the receptors is left in the desensitized state, and this fraction can be measured by the dose ratio method, as described below. For each model, equations can be derived that describe the rate at which desensitization develops in the presence of the agonist, and the rate at which sensitivity is regained after the agonist is removed. Finally, we wish to know what effect a reversible antagonist would have on these systems. There are two ways of formulating this problem. First, we can find out how much desensitization is produced, and how rapidly, by a given concentration of agonist, alone and in the presence of the antagonist. A second approach is to compare the desensitization produced by a given concentration of agonist $[A]$ alone, with that produced by a concentration of agonist $[A]'$ giving the same initial response in the presence of the antagonist as was obtained with $[A]$ alone. In the following section the behavior of the theoretical models shown in Fig. 1 will be analyzed in this way.

Rate theory model.

$$A + R \underset{k_{2A}}{\overset{k_{1A}}{\rightleftharpoons}} AR \qquad (p_A)$$

$$B + R \underset{k_{2B}}{\overset{k_{1B}}{\rightleftharpoons}} BR \qquad (p_B)$$

Here k_{1A} and k_{2A} are the association and dissociation rate constants for the agonist, A; k_{1B} and k_{2B} are the corresponding rate constants for the antagonist, B. p_A and p_B are the fractional occupancies by A and B, respectively. According to rate theory the response of the tissue is a function of the rate of association of agonist molecules, $k_{1A} \cdot [A] \cdot (1 - p_A - p_B)$. If this model is to explain relatively slow desensitization, k_{2A} must be quite small. On the other hand, the antagonist, tubocurarine, is known to dissociate rather rapidly from end-plate receptors (21), and in the present studies we have found that the antagonism produced by tubocurarine in thin strips of chick biventer cervicis decreases with a half-time of about 30 sec (which is faster than recovery from desensitization). This is contrary to the rate theory as originally formulated (4), which requires that the dissociation rate constant, k_2, should always be greater for agonists than for antagonists. Thus, if this theory is to explain desensitization in the present case, we have to abandon Paton's proposition that the efficacy of a drug depends only on its k_2, and to introduce an efficacy term distinct from k_2. Though this robs the rate theory of its attractive formal simplicity, it remains a possible model for desensitization and needs to be considered further.

When the agonist drug is added alone, the initial rate of association, a_0, is given by

$$a_0 = k_{1A}[A]$$
$$= k_{2A}c_A$$

where $c_A = k_{1A}/k_{2A} \cdot [A]$.

Desensitization, according to this model, corresponds with the build-up of p_A . Thus

$$\frac{dp_A}{dt} = k_{2A}c_A(1 - p_A) - p_A$$

$$p_A = \frac{c_A}{c_A + 1}\,[1 - \exp -k_{2A}(c_A + 1)t] \quad (1)$$

Thus, if desensitization is allowed to proceed to equilibrium, p_A reaches a value of $c_A/(c_A + 1)$. The rate of association, a_∞ , is then $(k_{2A}c_A)/(c_A + 1)$.

The associaton rate declines exponentially from a_0 to a_∞ with a rate constant $\tau_d^{-1} = -k_{2A}(c_A + 1)$. When the desensitizing agonist is removed, p_A decreases (i.e., sensitivity returns) with a rate constant $\tau_r^{-1} = -k_{2A}$.

If we now consider the effect of including an antagonist B, and assume for simplicity that it equilibrates instantaneously with the receptor pool available to it, we may write

$$p_B = \frac{c_B}{c_B + 1}\,(1 - p_A)$$

where $c_B = k_{1B}/k_{2B}\cdot[B]$ and

$$\frac{dp_A}{dt} = k_{2A}c_A(1 - p_A - p_B) - p_A$$

Integration gives the following result.

$$p_A = \frac{c_A}{c_A + c_B + 1} \cdot\left(1 - \exp\left[-k_{2A}\,\frac{c_A + c_B + 1}{c_B + 1}\cdot t\right]\right) \quad (2)$$

The initial rate of association, a_0 , is then given by $k_{2A}c_A(1 - p_B)$ or $k_{2A}c_A/(c_B + 1)$, and a_∞ is given by $k_{2A}c_A/(c_A + c_B + 1)$. The rate constant for this process, τ_d^{-1}, is $-k_{2A}(c_A + c_B + 1)/(c_B + 1)$, while the recovery rate constant, τ_r^{-1}, is, as before, $-k_{2A}$.

If we now imagine that the effect of the antagonist is countered by raising the agonist concentration, it can be seen that increasing c_A by a factor $(c_B + 1)$ will overcome the effect of the antagonist in reducing a_0 . If $c_A(c_B + 1)$ is substituted for c_A in Eq. 2, the result is identical with Eq. 1. In other words, the effect of concentration c_A of agonist in terms of the rate and extent of the desensitization that it produces, as well as the initial response, is exactly matched by concentration $c_A(c_B + 1)$ in the presence of antagonist concentration, c_B . These properties of the model are summarized in Table 1.

Parallel and sequential reaction models. These two models (Fig. 1a and b) can be considered together, as they give rise to identical equations. The assumptions made were that both A and B equilibrate instantaneously with the free receptor pool, the rate-limiting reactions being the desensitization and recovery reactions, governed by rate constants k_d and k_r , respectively. In this and all subsequent models we assume that the drug effect is a function of the agonist occupancy p_A and not, as above, of the association rate.

Sequential reaction model:

$$A + R \underset{k_{2A}}{\overset{k_{1A}}{\rightleftharpoons}} \underset{(p_A)}{AR} \quad \underset{k_r}{\overset{k_d}{\rightleftharpoons}} \quad \underset{(p'_A)}{AR'}$$

$$B + R \underset{k_{2B}}{\overset{k_{1B}}{\rightleftharpoons}} \underset{(p_B)}{BR}$$

Parallel reaction model:

$$A + R \underset{k_{2A}}{\overset{k_{1A}}{\rightleftharpoons}} AR \qquad (p_A)$$

$$A + R \underset{k_r}{\overset{k_d}{\rightleftharpoons}} AR' \qquad (p'_A)$$

$$B + R \underset{k_{2B}}{\overset{k_{1B}}{\rightleftharpoons}} BR \qquad (p_B)$$

In both these systems, the amount of desensitization existing at the moment the desensitizing agonist is washed out of the tissue is given by p'_A .

The properties of these two models are summarized in Table 2. The dimensionless concentration variables, c_A and c_B , have the same meaning as in the earlier section. As in Table 1, the first and third lines are the same, showing that addition of the antagonist B, with a compensating increase in the agonist concentration such that the initial response of the tissue is unchanged, leaves the extent and rate of desensitization unaltered.

TABLE 1

Rate theory model

	Initial Stimulus $e_A a_0$	Final Stimulus $e_A a_\infty$	Equilibrium Desensitization p_{A_∞}	Desensitization Rate Constant τ_d^{-1}	Recovery Rate Constant τ_r^{-1}
A alone	$e_A\, k_{2A}\, c_A$	$\dfrac{e_A\, k_{2A}\, c_A}{c_A + 1}$	$\dfrac{c_A}{c_A + 1}$	$-k_{2A}(c_A + 1)$	$-k_{2A}$
$A + B$	$\dfrac{e_A\, k_{2A}\, c_A}{c_B + 1}$	$\dfrac{e_A\, k_{2A}\, c_A}{c_A + c_B + 1}$	$\dfrac{c_A}{c_A + c_B + 1}$	$-k_{2A}\dfrac{(c_A + c_B + 1)}{c_B + 1}$	$-k_{2A}$
$A' + B$	$e_A\, k_{2A}\, c_A$	$\dfrac{e_A\, k_{2A}\, c_A}{c_A + 1}$	$\dfrac{c_A}{c_A + 1}$	$-k_{2A}(c_A + 1)$	$-k_{2A}$

e_A is the efficacy of the agonist (18). However, since rate theory is being considered, e_A relates stimulus to association rate, a, and not to occupancy as in the original definition. The expressions in the last line are obtained by substituting $c_A(c_B + 1)$ for c_A in the second line. The dose ratio, $c_B + 1$, is the ratio by which c_A has to be increased in order to overcome the effect of the agonist.

TABLE 2

Parallel and sequential reaction models

	Initial Stimulus $e_A p_{A_0}$	Final Stimulus $e_A p_{A_\infty}$	Equilibrium Desensitization $p_{A'}$	Desensitization Rate Constant τ_d^{-1}	Recovery Rate Constant τ_r^{-1}
A alone	$\dfrac{e_A c_A}{c_A + 1}$	$\dfrac{e_A c_A}{Kc_A + c_A + 1}$	$\dfrac{Kc_A}{Kc_A + c_A + 1}$	$-k_r \cdot \dfrac{Kc_A + c_A + 1}{c_A + 1}$	$-k_r$
$A + B$	$\dfrac{e_A c_A}{c_A + c_B + 1}$	$\dfrac{e_A c_A}{Kc_A + c_A + c_B + 1}$	$\dfrac{Kc_A}{Kc_A + c_A + c_B + 1}$	$-k_r \cdot \dfrac{Kc_A + c_A + c_B + 1}{c_A + c_B + 1}$	$-k_r$
$A' + B$	$\dfrac{e_A c_A}{c_A + 1}$	$\dfrac{e_A c_A}{Kc_A + c_A + 1}$	$\dfrac{Kc_A}{Kc_A + c_A + 1}$	$-k_r \cdot \dfrac{Kc_A + c_A + 1}{c_A + 1}$	$-k_r$

e_A, in this and subsequent tables, is the agonist efficacy, as defined by Stephenson (18). The expressions in the last line are obtained by substituting $c_A(c_B + 1)$ for c_A in the second line. For the parallel reaction model,

$$K = \frac{k_{2A}}{k_{1A}} \cdot \frac{k_d}{k_r}$$

For the sequential reaction model,

$$K = \frac{k_d}{k_r}$$

Cyclic reaction model.

$$\begin{array}{lll} B + R & \underset{k_{2B}}{\overset{k_{1B}}{\rightleftharpoons}} BR & (p_B) \\ A + R & \underset{k_2}{\overset{k_{1A}}{\rightleftharpoons}} AR & (p_A) \\ \uparrow k_r & \qquad \downarrow k_d & \qquad\quad B \\ A + R' & \underset{k'_{2A}}{\overset{k'_{1A}}{\rightleftharpoons}} AR' & (p'_A) \\ (p') & & \end{array}$$

$$B + R' \quad \underset{k'_{2B}}{\overset{k'_{1B}}{\rightleftharpoons}} \quad BR' \qquad (p'_B)$$

Again we assume that the only rate-limiting steps are the desensitization and recovery reactions, with rate constants k_d and k_r, respectively. It is convenient to define the ratio of the equilibrium constants of A for R' and for R, and similarly for B. Thus

$$\frac{k'_{1A} \cdot k_{2A}}{k'_{2A} \cdot k_{1A}} = X; \qquad \frac{k'_{1B} \cdot k_{2B}}{k'_{2B} \cdot k_{1B}} = Y$$

It is also convenient to define

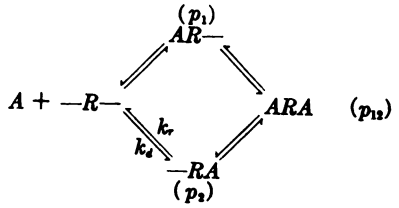
$$\frac{k_d}{k_r} = K$$

The amount of desensitization existing at any time, that could be detected if the desensitizing agonist were removed from the tissue, is given by $(p'_A + p'_B + p')$. The properties of this system are summarized in Table 3. An important property of this system is that the expressions in the third line of this table are not identical with those in the top line. Thus, if the effect of an antagonist is overcome by raising the agonist concentration by a factor $(c_B + 1)$, so that the initial occupancy, p_{A0}, is restored, the desensitization produced is greater than that produced by the concentration c_A in the absence of any antagonist, and the rate constants τ_d^{-1} and τ_r^{-1} are smaller.

It is obvious that an antagonist with a preferential affinity for R' $(Y > 1)$ will promote desensitization, because R' produced by the agonist will tend to be trapped by combination with B, instead of being free to revert to R. However, even if the antagonist has no affinity at all for R' $(Y = 0)$, it still affects desensitization, as is shown in the fourth line of Table 3. Desensitization is greater in extent, and slower in onset, than it is in the absence of the antagonist, but the time course of recovery is not affected.

Two-site model. This is formally more complicated than the models discussed so far, which have postulated only a single drug-binding site on the receptor. The complexity arises because, by analogy with allosteric proteins, there is a possibility of interaction between the two sites. If it is postulated that binding of a drug molecule at the "desensitizing site" renders ineffective the binding of a molecule at the "active" site, it cannot be reasonably assumed that the affinity of the drug for the active site is unaltered by occupation of the desensitizing site, and vice versa. Thus one has to take into account not only the affinity of the drug for the two sites, but also the interaction between the two sites.

The postulated reaction scheme is as follows.



When site 1 is occupied, the complex is represented by $AR—$; when site 2 is occupied, by $—RA$; and when both sites are occupied, by ARA; the fractions of the receptor in these three occupied states are written as p_1, p_2, and p_{12}, respectively. We assume that site 1 is the active site, so that response is a function of p_1, and that the forms $—RA$ and ARA are desensitized. To account for the slow kinetics of desensitization, it must be assumed that equilibration with site 2 is rather slow (i.e., k_d and k_r are small). The general kinetic and steady-state equations for this system are unwieldy, as they involve four separate equilibria, and the affinity for site 2 may depend on whether or not site 1 is occupied. We have therefore derived three special cases: (a) when there is no interaction between sites 1 and 2, (b) when site 2 can be occupied only when site 1 is occupied, and (c) when site 2 can be occupied only when site 1 is vacant. Analogous models have been described for enzymes with more than one binding site (see ref. 22). The behavior of the model is summarized in Table 4.

It was not worth considering the effect of antagonist with this model: no fewer than 12 separate equilibria are involved, with a corresponding number of disposable constants in the steady-state equations, and the predictive value of the equations is therefore very small. Moreover, this model attributes the slow kinetics of desensitization to the slowness of the reaction of agonist at site 2. If the reaction of the antagonist with site 2 is also allowed to be rate-limiting, complicated second-order kinetic equations result, which are again of little predictive value.

TABLE 3

Cyclic reaction model

	Initial Stimulus $e_A p_{A0}$	Final Stimulus $e_A p_{A\infty}$	Equilibrium Desensitization $(p' + p_A' + p_B')_\infty$	Desensitization Rate Constant τ_d^{-1}	Recovery Rate Constant τ_r^{-1}
A alone	$\frac{e_A c_A}{c_A + 1}$	$\frac{e_A c_A}{Kc_A(Xc_A + 1) + c_A + 1}$	$\frac{Kc_A(Xc_A + 1)}{Kc_A(Xc_A + 1) + c_A + 1}$	$-k_r \cdot \frac{Kc_A(Xc_A + 1) + c_A + 1}{(Xc_A + 1)(c_A + 1)}$	$-k_r$
$A + B$	$\frac{e_A c_A}{c_A + c_B + 1}$	$\frac{e_A c_A}{Kc_A(Xc_A + Yc_B + 1) + c_A + c_B + 1}$	$\frac{Kc_A(Xc_A + Yc_B + 1)}{Kc_A(Xc_A + Yc_B + 1) + c_A + c_B + 1}$	$-k_r \cdot \frac{Kc_A(Xc_A + Yc_B + 1) + c_A + c_B + 1}{(Xc_A + Yc_B + 1)(c_A + c_B + 1)}$	$\frac{-k_r}{Yc_B + 1}$
$A' + B$	$\frac{e_A c_A}{c_A + 1}$	$\frac{e_A c_A}{Kc_A[Xc_A(c_B + 1) + Yc_B + 1] + c_A + 1}$	$\frac{Kc_A[Xc_A(c_B + 1) + Yc_B + 1]}{Kc_A[Xc_A(c_B + 1) + Yc_B + 1] + c_A + 1}$	$-k_r \cdot \frac{Kc_A[Xc_A(c_B + 1) + Yc_B + 1] + c_A + 1}{[Xc_A(c_B + 1) + Yc_B + 1](c_A + 1)}$	$\frac{-k_r}{Yc_B + 1}$
$A' + B$ $(Y = 0)$	$\frac{e_A c_A}{c_A + 1}$	$\frac{e_A c_A}{Kc_A[Xc_A(c_B + 1) + 1] + c_A + 1}$	$\frac{Kc_A[Xc_A(c_B + 1) + 1]}{Kc_A[Xc_A(c_B + 1) + 1] + c_A + 1}$	$-k_r \cdot \frac{Kc_A[Xc_A(c_B + 1) + 1] + c_A + 1}{[Xc_A(c_B + 1) + 1](c_A + 1)}$	$-k_r$

The third and fourth lines are obtained by substituting $c_A(c_B + 1)$ for c_A in the second line.

$$K = \frac{k_d}{k_r}$$

$$X = \frac{k_{1A}'}{k_{2A}'} \cdot \frac{k_{2A}}{k_{1A}}$$

$$Y = \frac{k_{1B}'}{k_{2B}'} \cdot \frac{k_{2B}}{k_{1B}}$$

TABLE 4

Two-site model

	Initial Stimulus $e_A p_{10}$	Final Stimulus $e_A p_{1\infty}$	Equilibrium Desensitization $p_{2\infty} + p_{12\infty}$	Desensitization Rate Constant τ_d^{-1}	Recovery Rate Constant τ_r^{-1}
No interaction	$\frac{e_A c_A}{c_A + 1}$	$\frac{e_A c_A}{(Xc_A + 1)(c_A + 1)}$	$\frac{Xc_A}{Xc_A + 1}$	$-k_r(Xc_A + 1)$	$-k_r$
Co-operative interaction	$\frac{e_A c_A}{c_A + 1}$	$\frac{e_A c_A}{Xc_A^2 + c_A + 1}$	$\frac{Xc_A^2}{Xc_A^2 + c_A + 1}$	$-k_r \cdot \frac{Xc_A^2 + c_A + 1}{c_A + 1}$	$-k_r$
Negative interaction	$\frac{e_A c_A}{c_A + 1}$	$\frac{e_A c_A}{Xc_A + c_A + 1}$	$\frac{Xc_A}{Xc_A + c_A + 1}$	$-k_r \cdot \frac{Xc_A + c_A + 1}{c_A + 1}$	$-k_r$

In the first line it is assumed that occupation of site 1 has no effect on the affinity of the drug for site 2. In the second line it is assumed that site 2 can be occupied only when site 1 is occupied. In the third line it is assumed that site 2 can be occupied only when site 1 is vacant. X is the ratio of the affinity of the drug for site 1 and site 2.

Certain features of the analytical results presented in Tables 1–4 may be noticed.

1. In all cases except for the cyclic model, the rate constant for the development of desensitization is greater than that for recovery. Only with the cyclic model (Table 3) can desensitization develop more slowly than it disappears. It can be shown that the coefficient $[Kc_A(Xc_A + 1) + c_A + 1]/[(Xc_A + 1)(c_A + 1)]$, which is the ratio of the onset and recovery rate constants in Table 3, can become less than unity (i.e., onset is slower than recovery) at low values of c_A only if $X > K$. Drugs with a relatively high affinity for R' (X large) are thus more likely to show such kinetics than drugs with a low affinity for R'.

2. The effect of an antagonist does not fundamentally alter the properties of the rate, sequential, or parallel reaction mechanisms (Tables 1 and 2). If the agonist concentration is raised to overcome the effect of the antagonist, the desensitization produced is just the same as that produced by the lower concentration of agonist in the absence of any antagonist. With the cyclic model this is not true: an antagonist increases the amount of desensitization produced by the agonist if the agonist concentration is increased so as to overcome the blocking action in respect of the initial response. It can be seen from the last line of Table 3 that even an antagonist with no affinity for R' will affect desensitization considerably if X is large, but very little if X is small.

3. In all the models except for the cyclic model, the rate of recovery from desensitization would be likely to vary with different agonists. Thus, in the rate theory, parallel reaction, and two-site models, recovery from desensitization involves the dissociation of the agonist drug from the receptor, while in the sequential reaction model the process of recovery also involves the agonist, in the reaction $AR' \rightarrow AR$. In the cyclic model, on the other hand, the recovery process does not involve the agonist drug, and so long as the species R' is identical for all agonists, the rate of recovery from desensitization should be the same for all agonists.

METHODS

Strips of chick biventer cervicis muscle and leech dorsal muscle were prepared and set up as described by Rang and Ritter (1). The only difference was that the strips of chick muscle were connected to the isometric transducer via a phosphor-bronze spring of compliance 10 mm/100 mg. This was done because it was found that prolonged maximal isometric contraction tended to damage the muscle, sometimes even to break it, and this interfered with the study of desensitization. If the muscle could shorten without developing much tension, this difficulty was

avoided, and the muscle normally recovered its original sensitivity even after prolonged exposure to supramaximal agonist concentrations.

Experiments on frog muscle were done on extensor longus digiti IV of *Rana temporaria* during the period April–November 1969. The dissection and experiments were carried out in Ringer's solution of the following composition: NaCl, 116 mM; KCl, 2 mM; $CaCl_2$, 2 mM; and sodium phosphate (to give pH 7.0), 2 mM. A liquid/air interface was used as an extracellular electrode with which to record the depolarization at the end-plate region of the muscle (23). The muscle was mounted vertically on a Perspex assembly, which was drawn out of the bath at a constant rate (48 mm/min) by an electric motor operating a rack and pinion.

The volume of the bath was 50 ml, and the solution was bubbled with air, except for the 20-sec periods when recordings were being made; drugs were added in volumes of 0.5 ml or less. The organ bath was maintained at 20° by means of a thermostat, because the time course of desensitization was found to be very sensitive to temperature.

The end-plate depolarization was recorded by means of Ag-AgCl electrodes connected to the preparation by NaCl-agar bridges. Signals were fed into a solid-state differential amplifier with high-input impedance, and recorded on a potentiometric recorder. A microswitch on the rack-and-pinion device operated an event marker which enabled successive records to be accurately aligned. The end-plate depolarization was measured as the difference between the peak depolarization and the potential recorded from the same point on the muscle in the absence of any drug.

These methods were preferred to intracellular recording methods that might have given a more direct measure of end-plate conductance changes. This was because we wished to analyze desensitization by the dose ratio method, rather than simply to measure the fractional reduction of standard response, and this required a method that would give consistent drug effects for several hours. A number of experiments were done in which conventional microelectrode recording of end-plate depolarization in frog sartorius muscles was used, but it was rarely possible to obtain more than three or four consistent responses to depolarizing drugs. This made quantitative analysis very difficult, but many of the observations reported in this paper were qualitatively confirmed.

The following drugs were obtained commercially: carbamylcholine chloride (carbachol, Koch-Light), suxamethonium chloride (Allen & Hanbury), decamethonium iodide (Koch-Light), and (+)-tubocurarine chloride (Burroughs Wellcome). Other compounds used were diphenyldecamethonium iodide[2] (1), bistrimethylammonium compounds[3] ($Me_3N^+(CH_2)_nN^+Me_3 \cdot 2Br^-$), and bistriethylammonium compounds[3] ($Et_3N^+ \cdot (CH_2)_nN^+Et_3 \cdot 2Br^-$); other bisquaternaries[2] [$R_1R_2R_3N^+(CH_2)_{10}N^+R_1R_2R_3 \cdot 2Br^-$; R_1, R_2 = ethyl, R_3 = methyl; and R_1, R_2 = methyl, R_3 = 2-hydroxyethyl]; and monoquaternaries[2] (RN^+Me_3; R = *n*-decyl as bromide; R = phenyl as iodide).

RESULTS

Specificity of Desensitization

In work on the irreversible antagonist diphenyldecamethonium mustard, it was found (1) that in chick muscle cholinergic agonists produced the metaphilic effect but caffeine and potassium did not, whereas in leech muscle suxamethonium was effective, but not carbachol. We have investigated desensitization to see whether it shows the same pattern of specificity as the metaphilic effect in these two tissues.

Two kinds of experiment were done in chick biventer cervicis muscle. In the first (Fig. 2), we compared the shapes of the contraction produced by potassium and by cholinergic agonists. It was found (Fig. 2) that potassium caused a contraction that was well sustained for 10–15 min, whereas with carbachol and related agonists the muscle gradually relaxed despite the con-

[2] Supplied by Dr. E. W. Gill, Department of Pharmacology, Oxford University.

[3] Supplied by Dr. R. B. Barlow, Department of Pharmacology, Edinburgh University.

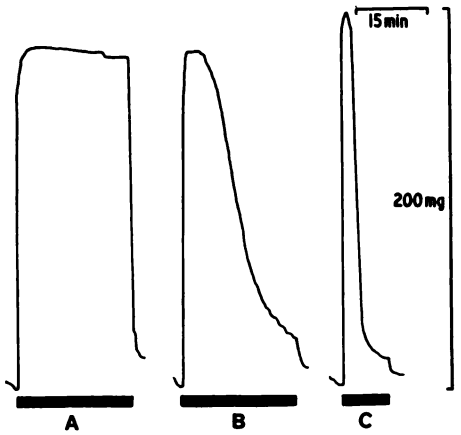


FIG. 2. *Responses of chick muscle to K^+ and carbachol.*

The records show the contractions of a strip of chick biventer produced by K^+, 28 mM, for 25 min (A); carbachol, 1.5×10^{-5} M, for 25 min (B); and carbachol, 7.4×10^{-5} M, for 25 min (C). The records show that the submaximal contraction produced by carbachol is much less well sustained than that produced by K^+. Increasing the carbachol concentration increases the rate of fade.

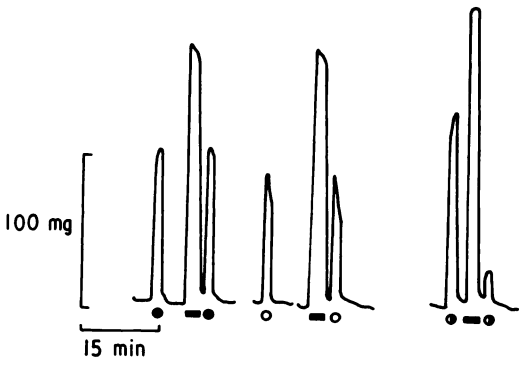


FIG. 3. *Specificity of desensitization in chick muscle.*

Conditioning doses of carbachol (7.4×10^{-5} M for 2 min) are indicated by the black bars. The test agonists were: ●, caffeine, 3.7×10^{-3} M; ○, K^+, 2.1×10^{-2} M; ◑, carbachol, 7.4×10^{-6} M.

tinued presence of the drug. After 15 min the relaxation was usually nearly complete, though muscles differed considerably in this respect. In the presence of potassium, the tension seldom decreased by more than about 20% in 15 min. This test revealed no difference between carbachol and suxamethonium.

This result shows that the muscle was capable of a well-sustained contraction when depolarized by raised potassium concentration, and implies that the fading contraction observed with cholinergic agonists reflected a fading depolarization. A number of studies at the motor end plate have shown that the depolarizing effect of cholinergic agonists is poorly sustained (5–7), and it seems likely that the same mechanism is responsible for the fading contraction in chick muscle. In Fig. 2 the contractions shown are nearly maximal; similar results were obtained when matching submaximal contractions produced by carbachol and potassium were compared.

In the second type of experiment, which was better suited to quantitative study, the sensitivity of the preparation to different substances was tested at various times after a conditioning dose of agonist had been washed out of the tissue. Figure 3 shows the effect of a large conditioning dose of carbachol on the sensitivity of a strip of chick muscle to carbachol, potassium, and caffeine, tested 1.5 min after the conditioning dose was washed out of the tissue. It can be seen that the desensitization affected only the sensitivity to carbachol. In six out of eight preparations on which this kind of experiment was done, the response to potassium was reduced by less than 10%, compared with a reduction of 50–80% in the response to carbachol. In one experiment, a conditioning dose of carbachol (7.4×10^{-5} M for 2 min) decreased a test response to potassium by 45%; in another experiment, 25% reduction of the response to potassium occurred. The desensitization to potassium seen in these two experiments may have been due to the cholinergic element in the action of potassium (1), which would presumably have been reduced by specific desensitization. In no case was the response to caffeine appreciably reduced.

The chick biventer muscle contains both singly innervated and multiply innervated fibers (24). Only the multiply innervated fibers would be expected to contract with cholinergic agonists, whereas both types might respond to potassium and caffeine.

Two experiments were done on strips of anterior latissimus dorsi, which contains only multiply innervated fibers (25). The same result was obtained, the contractions produced by caffeine and potassium being unaffected by desensitization with carbachol. Furthermore, the relative potencies of these three agonists were the same in biventer and anterior latissimi dorsi, which makes it unlikely that the twitch fibers in the biventer contributed appreciably to the response to caffeine and potassium.

In chick biventer cervicis muscle, carbachol and suxamethonium appear to have similar actions, except that suxamethonium is about 30 times as potent as carbachol. Both produce the metaphilic effect, and both are equally antagonized by a number of blocking agents (1). Their desensitizing action was compared in two experiments, in which the potency ratio of the two agonists was measured and equiactive conditioning doses were calculated accordingly. In one experiment the test drug was carbachol; in the other, suxamethonium. Equiactive conditioning doses of the two agonists caused the same amount of desensitization, whether the test agonist was carbachol or suxamethonium.

Leech muscle, unlike chick muscle, possesses separate receptors for carbachol and suxamethonium (26, 1), and we tested the specificity of the desensitization produced by these two agonists (Fig. 4). A conditioning dose of carbachol desensitized the muscle to carbachol much more than to suxamethonium, while desensitization with suxamethonium had the reverse effect. Thus desensitization in chick and leech muscle is specific, consistent with the idea that the mechanism involves inactivation of receptors. Furthermore, desensitization and the metaphilic effect have the same pattern of specificity, and the fact that in leech muscle both phenomena show the rather unexpected discrimination between carbachol and suxamethonium suggests a relationship between them.

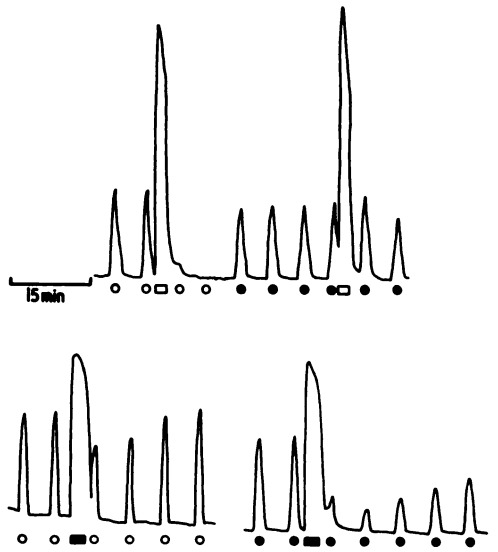


FIG. 4. *Specificity of desensitization in leech muscle.*

○, test doses of suxamethonium (2.2×10^{-5} M); ●, test doses of carbachol (3.7×10^{-7} M in upper record; 5.2×10^{-7} M in lower record); □, conditioning doses of suxamethonium (2.2×10^{-4} M); ■, conditioning doses of carbachol (7.4×10^{-6} M). The upper and lower tracings were obtained with different preparations from the same leech. The upper tracing shows that suxamethonium desensitized markedly to itself, but not at all to carbachol. The lower tracing shows a similar, but less marked, discrimination when the conditioning agonist was carbachol.

Quantitative Measurement of Desensitization

Chick muscle. If desensitization results from inactivation of a fraction of the receptors, it would be expected to affect the tissue in the same way as receptor blockade by specific antagonists. In chick muscle, alkylation of the receptors with $DPC_{10}M$[4] causes

[4] The abbreviations used are: DPC_{10}, decamethylene - 1,10 - bis[dimethylbenzylammonium bromide] (diphenyldecamethonium bromide); $DPC_{10}M$, decamethylene-1-(*N*-benzyl-2-chloroethylamino) -10-dimethylbenzylammonium chloride hydrochloride; $C_n$bis-TMA, bistrimethylammonium compounds, where n = 7, 14, 15, 16, 17, or 18; $C_n$bis-TEA, bistriethylammonium compounds, where n = 10, 11, or 12; C_{10}TMA, *n*-decyltrimethylammonium bromide; phenyl-TMA, phenyltrimethylammonium iodide; C_{10}bis-DEMA, $R_1R_2R_3N^+\cdot(CH_2)_{10}N^+R_1R_2R_3\cdot 2Br^-$, R_1, R_2 = ethyl, R_3 = methyl; C_{10}OH, R_1, R_2 = methyl, R_3 = 2-hydroxyethyl; DNC_{10}, decamethylene-1,10-bis[dimethyl-(1-naphthylmethylene)ammonium bromide]; $DNC_{10}M$, decamethylene-1-(2-chloroethyl-1-naphthylmethyl)amine-10-dimethyl-(1-naphthylmethyl)ammonium chloride hydrochloride.

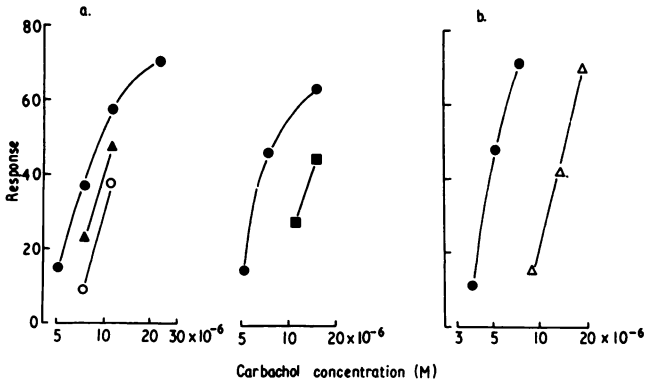


FIG. 5. *Desensitization in chick muscle with carbachol as test agonist*

a. Desensitization with carbachol. Results of two experiments are shown. ●, control dose-response curve; ▲, 2 min after washing out conditioning dose of carbachol (1.1×10^{-5} M applied for 15 min); ○, conditioning dose of 2.2×10^{-5} M for 15 min; ■, conditioning dose of 7.4×10^{-5} M for 15 min.

b. Desensitization by C_{10}TMA. ●, control; △, 1.5 min after washing out conditioning dose of C_{10}TMA (7.4×10^{-6} M for 10 min). Desensitization by either carbachol or C_{10}TMA causes a shift to the right of the log dose-response curve to carbachol, without any change in slope.

the log dose-response curve for carbachol to shift to the right without any change in slope, up to a carbachol dose ratio of about 3 (1). Figure 5 shows that desensitization by carbachol affects the tissue in exactly the same way. In this experiment, each point on the "desensitized" curves was obtained at a fixed time after washing out the conditioning dose of carbachol. Thus, each conditioning dose could be followed by only one test dose, after which the tissue was left for about 30 min to recover fully before the next test. The amount of information that could be obtained from a single preparation was thus limited, and Fig. 5 includes results from three separate experiments. The carbachol log dose-response curve is shifted to the right in the desensitized state without any change in slope up to a dose ratio of about 3. Figure 5 also shows the result of an experiment in which C_{10}TMA was used as the conditioning agonist. In this experiment also, the desensitization caused a parallel shift to the right of the carbachol log dose-response curve. This finding strengthens the interpretation of desensitization as a mechanism involving the inactivation of receptors, and also provides a convenient means of measuring the effect. It is clear from Fig. 5 that the percentage depression of a test response after desensitization will depend very much on the test dose used, whereas the dose ratio is independent of the test dose used. In order to be sure of obtaining a dose ratio measurement from a single test response in the desensitized state, it was necessary to obtain a number of points on the control (undesensitized) log dose-response curve, and to choose a test dose to give a response somewhere on this curve. The dose ratio for the single test response could then be determined by interpolation. Fortunately, the behavior of the chick biventer was extremely consistent, and the log dose-response curve was very steep (see Fig. 5), so that dose ratios as small as 1.2 could be measured quite reliably. The control dose-response curve after recovery was always compared with the initial control, and they usually agreed very closely. However, when large desensitizing doses of full agonists, such as carbachol or suxamethonium, were given repeatedly, the sensitivity of the preparation tended to decrease progressively, and often after three or four such desensitization tests the experiment had to be abandoned because further tests caused a marked permanent loss of sensitivity. Other problems were encountered in measuring the desensitization caused by carbachol or suxamethonium. After a large conditioning dose (more than about 2×10^{-4} M carbachol or 5×10^{-6} M suxamethonium), the muscle often failed to relax

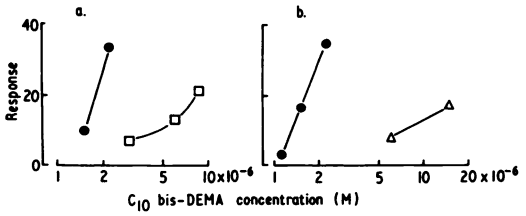


FIG. 6. *Desensitization in chick muscle with C_{10} bis-DEMA as test agonist*

a. Desensitization by carbachol. ●, control; □, 2 min after washing out conditioning dose of carbachol (1.1×10^{-4} M for 4 min).

b. Desensitization by C_{10}TMA. ●, control; △, 2 min after washing out conditioning dose of C_{10}TMA (2.2×10^{-5} M for 4 min). Desensitization by either carbachol or C_{10}TMA causes a marked decrease in the slope of the log dose-response curve when a partial agonist is tested.

fully, and indeed frequently went into a second contraction after the conditioning agonist was washed away. The test responses could not then be measured from the normal baseline, and no reliance could be put on the results. Thus, it was not feasible to measure dose ratios greater than about 3 produced by desensitization with carbachol or suxamethonium. Up to this limit the parallel shift of the log dose-response curve, as shown in Fig. 5, appeared to be obeyed.

If the test drug was a partial agonist, such as the ethylated decamethonium derivative C_{10}bis-DEMA, which has a log dose-response curve flatter than that of carbachol and reaches a maximum about 80% of that reached by carbachol, desensitization flattened the log dose-response curve as well as shifting it to the right (Fig. 6). It can be seen that desensitization with either carbachol or C_{10}TMA had the same effect. Stephenson (18) has shown in guinea pig ileum that partial agonists produce less than maximal responses even when they occupy all of the receptors. Thus any diminution in the number of receptors available will reduce the slope of the log dose-response curve for a partial agonist. Full agonists, on the other hand, produce maximal responses when occupying only a small fraction of the receptors, and inactivation of a considerable fraction can leave the slope of the log dose-response curve unaffected (27). This result

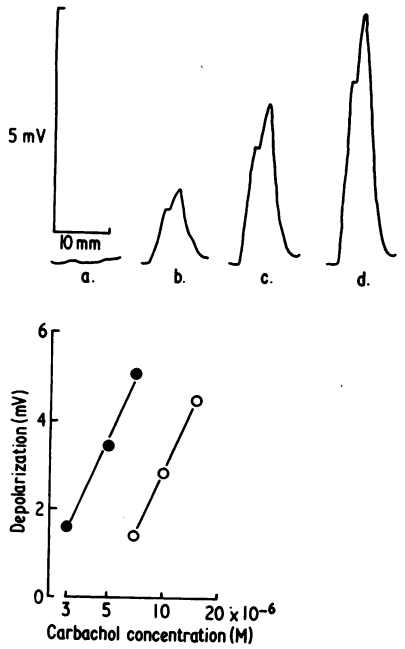


FIG. 7. *Desensitization in frog muscle*

The upper panel shows records obtained from frog toe muscle using a moving fluid electrode. The records were taken 1 min after adding carbachol to the bath. a. Control baseline. b. 3×10^{-6} M. c. 5×10^{-6} M. d. 7×10^{-6} M. The lower panel shows log dose-response curves. ●, control curve (each point is the mean of three to five measurements); ○, curve obtained when carbachol was added 1 min after washing out the conditioning dose of C_7TMA (5×10^{-4} M for 20 min). Each point represents a single measurement.

is thus consistent with the hypothesis that desensitization results from inactivation of the receptors and would not be expected of a nonspecific mechanism.

Frog muscle. Figure 7 shows the results of an experiment comparable to those shown in Fig. 5, but performed on frog toe muscle. The responses measured are the end-plate depolarization 1 min after the dose of carbachol was applied. The conditioning drug was C_7TMA, 5×10^{-4} M, applied for 20 min; 1 min after the conditioning drug was washed out, a dose of carbachol was applied and the response to this test dose was measured 1 min later. By repeating this operation using different test doses, a log dose-response curve for the muscle in the desensitized state was obtained, which was

parallel to the control curve. Thus, in frog as well as chick muscle, the desensitization dose ratio gives a quantitative measure of desensitization that is independent of the test dose of agonist used.

Comparison of Desensitizing Effects of Different Agonists

In all of the models discussed it is theoretically possible for different agonists to produce the same initial response of the tissue but to desensitize it by different amounts. Thus, in the rate theory model (Table 1), if two drugs have efficacies e and e' and dissociation rate constants k_2 and k_2', the concentrations (c and c') giving the same initial response will be governed by the relationship

$$ek_2c = e'k_2'c'$$

Thus

$$c' = \frac{ek_2c}{e'k_2'}$$

The steady-state stimulus, S_∞, produced by c is given by

$$S_\infty = \frac{ek_2c}{c + 1}$$

For c',

$$S'_\infty = \frac{e'k_2'c'}{c' + 1}$$

$$= \frac{ek_2c}{(ek_2c/e'k_2') + 1}$$

Thus, if $e'k_2'$ is smaller than ek_2, the second drug will produce responses that fade more rapidly than those produced by the first.

In the parallel and sequential reaction models (Table 2), increasing K, as well as reducing e_A, increases the amount of fade, while in the cyclic model (Table 3) or in the two-site model (Table 4), an increase in X has the same effect.

We have studied a number of agonists to see whether differences in the amount of fade produced by concentrations giving equal peak responses could be detected. The experimental results in Fig. 8 show that agonists vary markedly in this respect. In these experiments the agonists were left in contact with the tissue for 10 min and the contraction was recorded. Each test drug was compared with carbachol, the concentration being adjusted so that the peak tension was the same, usually about 50% of the maximum tension attainable with carbachol.

Figure 8 shows that full agonists such as decamethonium produce contractions indistinguishable in form from those produced by carbachol. Some partial agonists, such as C_{10}bis-DEMA and C_{10}bis-TEA, also produced contractions resembling those produced by carbachol, while others, such as C_{10}OH, C_{10}TMA, and the longer-chain bis-TMA compounds, C_{16}–C_{18}, produced contractions that faded much more rapidly than those produced by carbachol. This was studied quantitatively in 12 experiments. In each, a standard concentration of carbachol (between 5×10^{-6} and 7.5×10^{-6} M) was selected and the resulting contraction was compared with that produced by various other agonists at concentrations giving about the same peak response as that obtained with carbachol. The contraction height was measured when the drug had been in contact with the tissue for 5 min, and this was expressed as a percentage of the peak tension. With carbachol, in 12 separate muscles, the tension after 5 min was 71.4 ± 2.8% of the peak tension; after 10 min the value was 31.3 ± 4.8%. Since the fade produced by carbachol varied somewhat from one preparation to another, the percentage contraction at 5 min [i.e., (tension at 5 min × 100)/(peak tension)] for each test drug was expressed as a fraction of the percentage contraction at 5 min with carbachol in the same muscle. Thus a value less than unity means that the response faded more rapidly than with carbachol. The results of such experiments are shown in Table 5A. The compounds that produced significantly more fade than carbachol are footnoted. These compounds were all partial agonists. Not all partial agonists, however, caused more fade than carbachol; thus, C_7bis-TMA and C_{10}bis-DEMA were just the same as carbachol in this respect. The results with many of the other partial agonists were indeterminate, appearing to show rather more fade than carbachol, but

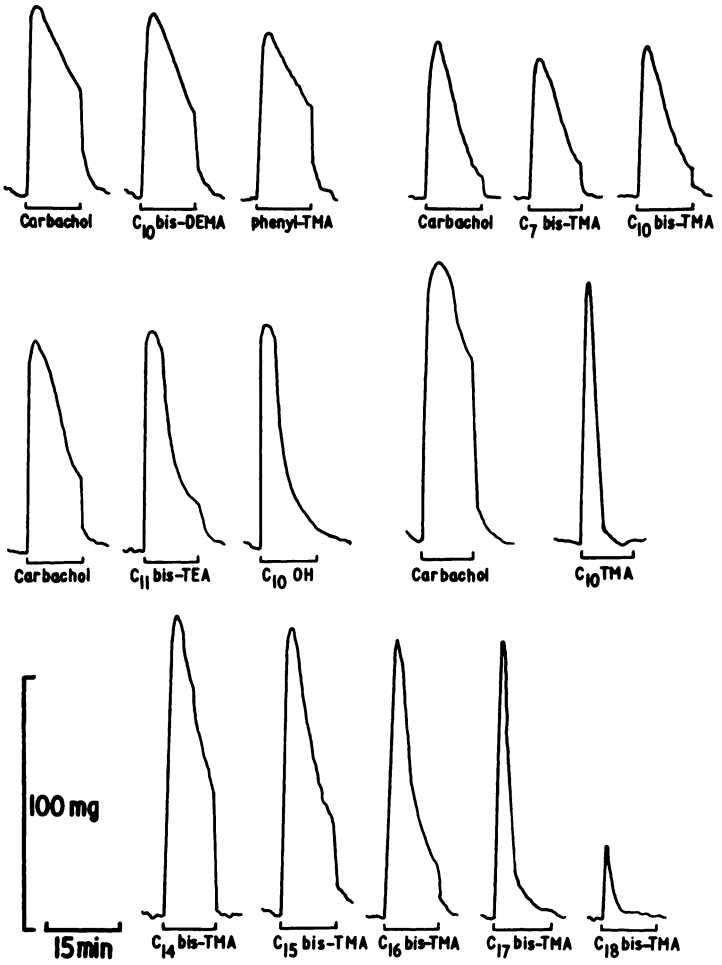


FIG. 8. *Fading responses of chick muscle to different agonists*

In each panel various drugs are compared on the same preparation. The contractions are about 50% maximal, and the drugs were present for the periods (10 min) indicated by the horizontal bars. Top left: carbachol, 7.4×10^{-6} M; C_{10}bis-DEMA, 3.7×10^{-6} M; Phenyl-TMA, 1.1×10^{-5} M. Top right: carbachol, 6×10^{-6} M; C_7bis-TMA, 2.2×10^{-5} M; C_{10}bis-TMA, 9×10^{-8} M. Middle left: carbachol, 6.3×10^{-6} M; C_{11}bis-TEA, 6.7×10^{-6} M; C_{10}OH, 2.2×10^{-5} M. Middle right: carbachol, 6×10^{-6} M; C_{10}TMA, 7.4×10^{-6} M. Bottom: C_{14}bis-TMA, 3.7×10^{-8} M; C_{15}bis-TMA, 3.7×10^{-8} M; C_{16}bis-TMA, 6.0×10^{-8} M; C_{17}bis-TMA, 1.5×10^{-7} M; C_{18}bis-TMA, 3.0×10^{-7} M.

failing to show a statistically significant difference at the 5% level of probability.

When the contraction produced by C_{10}TMA had disappeared, the tissue was still normally sensitive to potassium or caffeine, though quite insensitive to nicotinic agonists. As shown earlier (Fig. 5), exposing the tissue to C_{10}TMA reduces its sensitivity to carbachol in such a way that the log dose-response curve is shifted to the right without any change in slope. The same effect is seen after the tissue is desensitized with carbachol. There are thus many similarities between the desensitization produced by C_{10}TMA and by carbachol and we shall indicate further points of similarity in relation to the kinetics of desensitization, and to the metaphilic effect, in later sections. The evidence thus suggests that both carbachol and C_{10}TMA desensitize by essentially the same mechanism, but that C_{10}TMA is much more effective than carbachol in this respect.

TABLE 5

Fade produced by different agonists compared with that produced by carbachol

A. Chick muscle

Agonist	Full (F) or partial (P)	Fade ratio[a] ± SE	No. of trials
Suxamethonium	F	1.08 ± 0.05	5
C_7-bis-TMA	P	1.03 ± 0.03	3
C_{10}-bis-TMA	F	1.07 ± 0.07	5
C_{15}-bis-TMA	F	0.80 ± 0.09	3
C_{16}-bis-TMA	F	0.80 ± 0.10	3
C_{17}-bis-TMA	P	0.07 ± 0.06[b]	3
C_{18}-bis-TMA	P	0.0[b]	2
C_{10}-bis-TEA	P	0.89 ± 0.08	4
C_{11}-bis-TEA	P	0.72 ± 0.12	4
C_{12}-bis-TEA	P	0.20 ± 0.14[b]	4
C_{10}OH	P	0.67 ± 0.14	5
C_{10}TMA	P	0.07 ± 0.03[b]	3
C_{10}bis-DEMA	P	1.00 ± 0.07	6
Phenyl-TMA	F	0.94	2

B. Frog muscle

Agonist	Fade ratio[c] ± SE	No. of trials
Suxamethonium	1.00 ± 0.06	3
C_{10}bis-TMA	1.03 ± 0.11	3
Phenyl-TMA	0.07 ± 0.02[d]	4
C_7TMA	0.58 ± 0.12[d]	4
C_{13}bis-TMA	0.60 ± 0.04[d]	3

[a] Fade ratio = (percentage response at 5 min with test drug)/(percentage response at 5 min with carbachol).

[b] Fade significantly greater than with carbachol ($p < 0.01$). Other values do not differ significantly from carbachol ($p > 0.05$).

[c] Fade ratio = (percentage response at 15 min with test drug)/(percentage response at 15 min with carbachol).

[d] Fade significantly greater than with carbachol ($p < 0.05$).

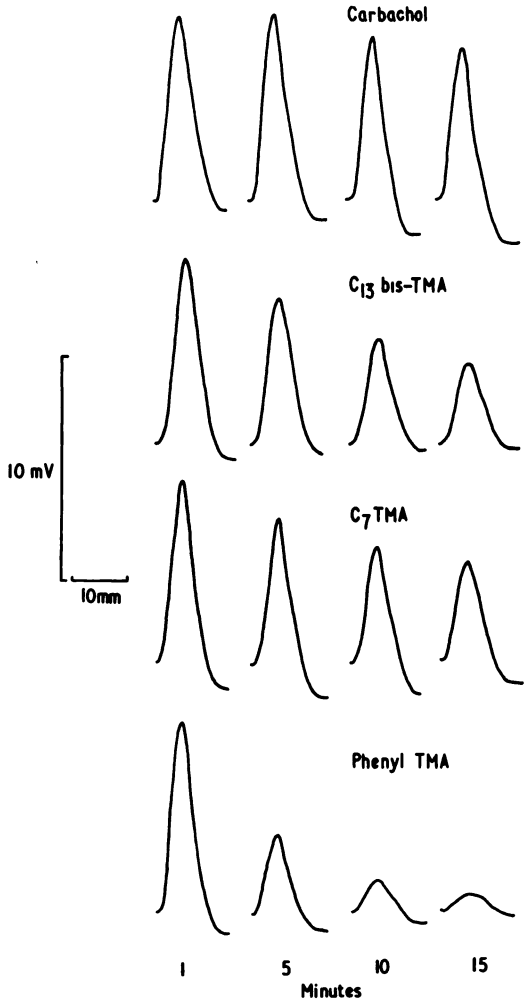


FIG. 9. *Fading depolarization in frog muscle*

Records were obtained from a single preparation when different agonists were left in contact with the muscle for 15 min. Records were taken 1, 5, 10, and 15 min after addition of the drug. The concentrations were selected to give equal effects at 1 min. The concentrations were: carbachol, 1.25×10^{-5} M; C_{13}bis-TMA, 10^{-5} M; C_7TMA, 10^{-4} M; phenyl-TMA, 3.5×10^{-4} M.

Similar results have been obtained on the frog motor end plate, though we have not investigated this in as much detail. Thus Fig. 9 shows that the end-plate depolarization produced by 10^{-5} M carbachol was quite well sustained when the drug was left in contact with the muscle for 15 min. Concentrations of C_{13}bis-TMA, C_7TMA, and phenyl-TMA that produced a depolarization after 1 min equal to that produced by carbachol had a much more poorly sustained action. Quantitative information on the relationship between the fade produced by various agonists relative to that produced by carbachol is summarized in Table 5B. In each of this series of experiments, a dose of carbachol between 7.5×10^{-6} and 1.8×10^{-5} M was selected, and the depolarization produced by this dose after 1 min and after 15 min in

contact with the muscle was measured; a dose of the agonist being compared with carbachol was then found that gave a response after 1 min within ±10% of the response to the standard dose of carbachol after 1 min. The responses at 15 min were expressed as a percentage of the response to the same agonist at 1 min, and these percentage responses were compared to see whether the agonist produced a significantly different fade from carbachol. Phenyl-TMA, C_7TMA, and C_{13}bis-TMA all faded significantly more than carbachol ($p < 0.05$), while C_{10}bis-TMA and suxamethonium did not differ from carbachol. Similar experiments in which the fades produced by these agonists were compared one with another (rather than with carbachol) showed that the order of fading tendency was: phenyl-TMA > C_{13}bis-TMA > C_7TMA > carbachol, suxamethonium, C_{10}bis-TMA. These findings are consistent with the observations of Gissen and Nastuk (28), using intracellular recording from frog sartorius fibers, that repolarization in the presence of phenyl-TMA was more rapid than in the presence of carbachol. The situation thus appears to be essentially similar to that described in chick muscle, though the compounds that produced fading depolarization in frog muscle did not cause fading contractions of chick muscle, while those that caused fading responses of chick muscle were without depolarizing activity at the frog end plate.

An interesting difference was found between the actions of the two partial agonists, C_{10}TMA and C_{10}bis-DEMA, on chick muscle. With C_{10}bis-DEMA, which produced no more fade than did carbachol, increasing the concentration beyond about 2×10^{-5} M had no effect on either the peak contraction or the degree of fade (Fig. 10). Thus contractions produced by 3.7×10^{-5} M and 7.4×10^{-5} M C_{10}bis-DEMA were superimposable. With C_{10}-TMA, on the other hand, increasing the concentration beyond 1.5×10^{-5} M increased the rate of fade even though it did not affect the peak tension (Fig. 10). The initial rate of contraction was faster, however, and the peak was reached earlier than with low concentrations. This produced the paradoxical result with C_{10}TMA that the tension measured at various times after the peak was greater at low than at high concentrations. The result with the partial agonist C_{10}bis-DEMA militates against the rate theory mechanism for desensitization. According to this theory, the initial stimulus is given by $e_A k_{2a} c_A$, and therefore increases indefinitely as the agonist concentration is increased. If the initial response does not increase indefinitely, this must be due, according to the rate theory, either to saturation of the effector system (as when the contractile mechanism is fully activated) or to the response being cut short by the rapidly developing fade (29). With C_{10}bis-DEMA, neither explanation can be upheld; the contraction produced is submaximal, and the fade is no more rapid than that seen with carbachol.

One feature of these results that merits consideration is that for chick muscle eight out of the 15 drugs studied, including two partial agonists, desensitized at the same

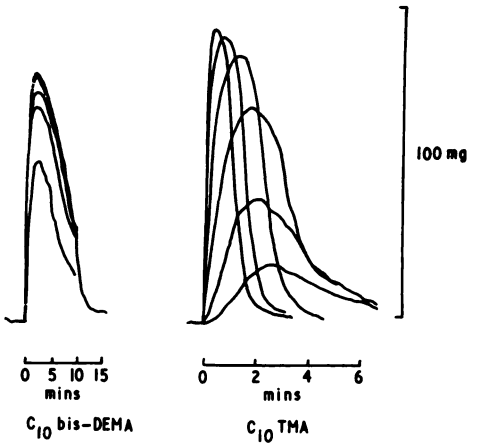


FIG. 10. *Responses of chick muscle to partial agonists*

Superimposed tracings of contractions of chick muscle to C_{10}bis-DEMA (left panel) and C_{10}TMA (right panel). The concentrations used were: C_{10}bis-DEMA, 3×10^{-6} M, 7.4×10^{-6} M, 1.9×10^{-5} M, 3.7×10^{-5} M, and 7.4×10^{-5} M; C_{10}TMA, 3×10^{-6} M, 4.4×10^{-6} M, 7.4×10^{-6} M, 1.5×10^{-5} M, and 3×10^{-5} M. With C_{10}bis-DEMA, increasing the concentration beyond maximal had no effect on the response. With C_{10}TMA, increasing the concentration accelerated the fade without affecting the peak tension.

rate (Table 5A), and no drugs were found that desensitized more slowly than this "standard" rate. Similarly, with frog muscle, three of the six drugs studied all desensitized at the same (minimum) rate (Table 5B). To explain this by the rate theory entails, as mentioned earlier, the assumption that all these drugs have the same efficacy and that no drug has a higher efficacy. This assumption is untenable for the partial agonists. On the parallel or sequential reaction models, it turns out that for drugs to differ in efficacy while producing the same degree of desensitization the assumption has to be made that $e_{A1}/e_{A2} = K_1/K_2$ (K being defined as in Table 2). On the other hand, to account for the fact that some partial agonists desensitize more than the "standard" drugs, it would have to be supposed that this relationship did not apply in these cases. Thus an interesting generalization is suggested by the existence of a "standard" rate of desensitization, but these models give no satisfactory account of deviations from this standard pattern.

The cyclic reaction model appears to offer a satisfactory explanation of these findings. For drugs with an affinity for R' much smaller than their affinity for R (i.e., $X \ll 1$), proportionality between efficacy and K would, as stated above, result in a standard rate of desensitization. But if a drug had an appreciable affinity for R', so that X were no longer negligible, it would be expected to desensitize more than the standard drugs. Thus we may tentatively suggest that agonists such as C_{10}TMA and the bis-TMA compounds C_{16}–C_{18} have relatively high affinities for R', while the other agonists have only a negligible affinity for R'. An exactly similar hypothesis is suggested as an explanation of the difference between conventional and metaphilic antagonists in the accompanying paper (16).

The two-site model readily explains why agonists should differ in their desensitizing action, in terms of differences in the relative affinity of the drugs for the active and the desensitizing sites. But it offers no satisfactory explanation why a large group of drugs should show the same, minimum, rate of desensitization.

Kinetics of Desensitization

The four theoretical models discussed above give rise to different predictions about the kinetics of desensitization. All predict that the fraction of desensitized receptors should rise and fall exponentially when the desensitizing drug is added or removed. However, on every model except for cyclic one, the rate constant for the onset of desensitization is always greater than the rate constant for recovery. Also, in the cyclic model, one would expect the recovery rate to be the same whatever drug had produced the desensitization, for in this model the recovery step is represented simply by $R' \rightarrow R$. In the other models, however, the agonist drug is involved in the recovery process, and the rate of recovery would not be expected to be the same for all agonists.

Chick muscle. The time course of recovery from desensitization was investigated by applying a conditioning dose of agonist for a standard length of time and allowing a variable period of time to elapse between washing away the conditioning drug and applying a test dose of carbachol. By measuring the test response and comparing it with the control log dose-response curve for carbachol, an estimate of the dose ratio, r_d, produced by desensitization was obtained. From this, the fraction of the receptors in the desensitized state, p_d, could be calculated using the equation

$$p_d = \frac{r_d - 1}{r_d}$$

This equation is valid so long as the log dose-response curves remain parallel. By repeating the experiment with different recovery times, the time course of the decline of p_d could be studied. This was done with several different agonists, and the results are shown in Fig. 11 and Table 6. The recovery was exponential, as predicted, and the rate constant was the same for all of the agonists tested, within the limits of error of the experiment. Lines were drawn by inspection through the semilogarithmic plots, and the mean recovery rate constant, r_r^{-1}, was found to be 0.30 min^{-1}, corresponding to a half-time of 2.3 min. The extrapolated semilogarithmic

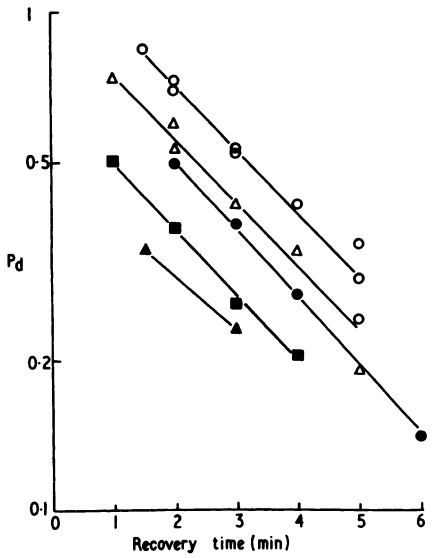


FIG. 11. *Kinetics of recovery from desensitization in chick muscle*

The fraction of receptors desensitized (p_d) is plotted semilogarithmically against the interval between washing out the conditioning agonist and applying the test dose of carbachol. The conditioning agonists were: ○, C_{10}TMA 2.2×10^{-5} M, for 10 min; △, C_{10}TMA, 7.4×10^{-6} M, for 10 min; ●, carbachol, 7.4×10^{-5} M for 4 min; ■, C_{16}bis-TMA, 2.0×10^{-7} M, for 5 min; ▲, C_{16}bis-TMA, 1.3×10^{-7} M, for 5 min.

plots sometimes met the ordinate at a value of p_d greater than unity. The explanation is probably that the early recovery was retarded by diffusion, and these results cannot, unfortunately, be used to estimate the amount of desensitization existing at the time when the conditioning drug was washed out. The timing is also somewhat ambiguous, because t_r in Fig. 11 refers to the interval between washing out the conditioning drug and adding the test drug. The test response actually took 60–90 sec to develop, and so the time taken to represent t_r is somewhat arbitrary. In spite of these uncertainties, the conclusion seems clear that the rate of recovery was the same for all of the drugs tested. As stated earlier, recovery after blockade with tubocurarine or gallamine was much more rapid, having a half-time no greater than about 30 sec. It is therefore very unlikely that the rate of recovery from desensitization was seriously limited by diffusion.

TABLE 6

Kinetics of desensitization in chick muscle

A. Recovery kinetics

Conditioning drug	Concentration	Duration	Recovery half-time	Recovery rate constant, r_r^{-1}
	M	*min*	*min*	*min*$^{-1}$
Carbachol	7.4×10^{-5}	4	2.2	0.31
	7.4×10^{-5}	5	2.5	0.28
C_{10}TMA	7.4×10^{-6}	10	2.0	0.35
	2.2×10^{-5}	10	2.5	0.28
	2.2×10^{-5}	10	2.4	0.29
C_{16}bis-TMA	1.3×10^{-7}	5	2.7	0.26
	2.0×10^{-7}	5	2.3	0.30
C_{17}bis-TMA	1.5×10^{-7}	5	1.8	0.38
C_{18}bis-TMA	7.4×10^{-8}	5	2.8	0.25
	7.4×10^{-8}	6	2.3	0.30
Mean ± SE				0.30 ± 0.01

B. Kinetics of onset

Conditioning drug	Concentration	Recovery time	p	Onset half-time	Onset rate constant r_d^{-1}
	M	*min*		*min*	*min*$^{-1}$
Carbachol	3.7×10^{-5}	1.5	0.55	0.5	1.4
	7.4×10^{-5}	1.0	0.52	0.5	1.4
	7.4×10^{-5}	1.5	0.57	~0.6	~1.2
Suxamethonium	3.7×10^{-7}	1.0	0.30	~0.7	~1.0
	7.4×10^{-7}	1.0	0.50	~1.0	~0.7
C_{10}TMA	5.1×10^{-6}	1.5	0.45	2.4	0.29
	7.4×10^{-6}	2.0	0.35	2.5	0.28
	1.1×10^{-5}	1.5	0.55	1.8	0.38
	1.1×10^{-5}	2.0	0.42	1.2	0.58
	2.2×10^{-5}	2.0	0.67	~0.6	~1.2
	2.2×10^{-5}	2.0	0.53	~0.4	~1.7
C_{16}bis-TMA	1.3×10^{-7}	1.5	0.40	2.0	0.35
C_{17}bis-TMA	1.5×10^{-7}	1.0	0.55	~0.8	~0.9

The finding that the recovery rate is independent of the desensitizing agonist and of the depth of desensitization produced suggests that the same mechanism underlies the process of desensitization for the different drugs tested, whether they were highly effective at desensitizing, like C_{10}TMA, or produced only the "standard" amount of desensitization, like carbachol.

The kinetics of onset of desensitization was rather less easy to study than the recovery. A

qualitative idea could be obtained by observing the shape of the contraction when the agonist was left in contact with the tissue, but this was not satisfactory for quantitative purposes, because even with presumably "nondesensitizing" substances, such as potassium, an appreciable amount of fade was seen. Since the recovery rate constant was the same for different agonists, the fraction of desensitized receptors measured after a fixed recovery period (usually 1–2 min) was assumed to be directly proportional to the fraction desensitized at the time when the conditioning agonist was washed out. The nonexponential start to the recovery time course mentioned earlier introduces some uncertainty into this measurement, but fairly consistent results could nevertheless be obtained.

The kinetics of onset appeared to be satisfactorily fitted by exponentials, though with carbachol and suxamethonium the onset was too rapid for the time course to be established with any certainty. The results obtained on 10 preparations are shown in Table 6. The value p_∞ given in Table 6 refers to the value of p_d measured after prolonged exposure to the conditioning drug, with a recovery period t_r as shown in the table. With C_{10}TMA as conditioning agonist, the rate constant for the onset of desensitization, τ_d^{-1}, appeared to increase with the concentration of C_{10}TMA, though there was a good deal of variation among preparations. At the lowest concentration of C_{10}TMA (5.1×10^{-6} M), the rate constant τ_d^{-1} was 0.29 min^{-1}, which is not appreciably different from the recovery rate constant, 0.30 min^{-1}.

When concentrations of C_{10}TMA and carbachol were selected that gave the same equilibrium level of desensitization, and the rates of onset of desensitization by the two drugs were compared in the same muscle, carbachol was found to desensitize much more rapidly than C_{10}TMA (Fig. 12).

Using chick muscle, we have not succeeded in showing unequivocally that the onset rate is slower than the offset rate, as Katz and Thesleff (6) did in frog muscle. We have, however, shown that at low concentrations of partial agonists the two rates may be about the same. In terms of the

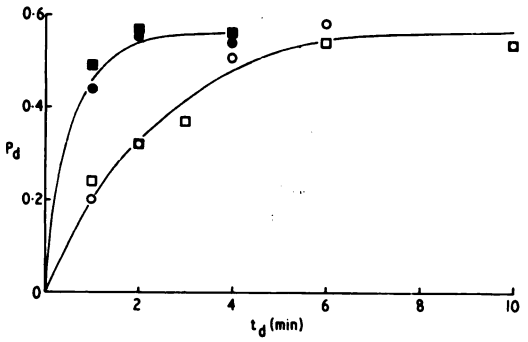


FIG. 12. *Kinetics of onset of desensitization in chick muscle*

Relation between the duration of application of the conditioning agonist (t_d) and the desensitization to carbachol, measured 1.5 min after washing out the conditioning agonist. The fraction of receptors in the desensitized state (p_d) is shown on the ordinate. The square and round symbols show the results obtained in two different preparations. ○ and □, conditioning agonist, C_{10}TMA, 1.1×10^{-6} M; ● and ■, conditioning agonist, carbachol, 3.7×10^{-6} M.

models discussed earlier, the only mechanism that allows this is the cyclic model. In all the other models τ_d^{-1} is greater than τ_r^{-1}. Examination of the expressions for τ_d^{-1} in Tables 1, 2, and 4 reveals that the ratio τ_d^{-1}/τ_r^{-1} is always equal to the dose ratio corresponding to the amount of desensitization at equilibrium. Thus, in the sequential or parallel reaction model, the fraction of receptors desensitized at equilibrium is

$$p'_{A\infty} = \frac{Kc_A}{Kc_A + c_A + 1}$$

The dose ratio corresponding to this is given by

$$r_{d\infty} = \frac{1}{1 - p'_{A\infty}} = \frac{Kc_A + c_A + 1}{c_A + 1}$$

From Table 2 is can be seen that this quantity is the same as the ratio of the rate constants for onset and recovery, so that $\tau_d^{-1}/\tau_r^{-1} = r_{d\infty}$. A similar analysis shows the same to be true of the rate theory and two-site models, but not of the cyclic model, where $\tau_d^{-1}/\tau_r^{-1} = r_{d\infty}/(Xc_A + 1)$. As already explained, the equilibrium desensitization is experimentally inaccessible, but it is safe to

say that it must be greater than the desensitization actually measured 1–2 min after washing out the conditioning drug. Thus, in the experiment on C_{10}TMA (Table 6), a maximum p_d value of 0.44 was measured when the conditioning drug was left in contact with the tissue for 12 min. This corresponds to a dose ratio of 1.8, and we should expect from the preceding analysis that the onset rate constant should be more than 0.54 min^{-1} (= 1.8 × τ_r^{-1}). In fact, it was only 0.29 min^{-1}. This finding provides evidence against the mechanisms analyzed in Tables 1, 2, and 4, but is quite compatible with the cyclic model (Table 3).

In the theoretical section it was pointed out that the cyclic model does not differ formally from the sequential reaction model if the agonist has no affinity for R'. Thus, we would expect the slow onset of desensitization to be confined to those drugs with an appreciable affinity for R'. In the preceding section it was suggested that the difference between agonists such as carbachol, on the one hand, and C_{10}TMA, on the other, might consist in the latter group having a relatively high affinity for R'. The kinetic results support this hypothesis, for carbachol was found to desensitize rapidly, the rate constant being greater than 1 min^{-1} (Fig. 12), in contrast to C_{10}TMA and C_{16}bis-TMA. This estimate of the onset rate with carbachol is only very rough, for the measurements showed a good deal of scatter, and the kinetics may well have been distorted by diffusion delays. Nevertheless it appeared that carbachol desensitized much more rapidly than C_{10}TMA when the concentrations of the two drugs were such as to give the same final level of desensitization.

Frog muscle. Experiments similar to those described above were performed on frog muscle. Both the rate and extent of desensitization were substantially increased by small increases in temperature, and so the experiments were performed at 20°. To determine the kinetics of development of desensitization, we applied the conditioning agonist for varying lengths of time and then added a test dose of carbachol 1 min after washing out the conditioning agonist. The depolarization produced by this test dose was measured 1 min after it had been applied,

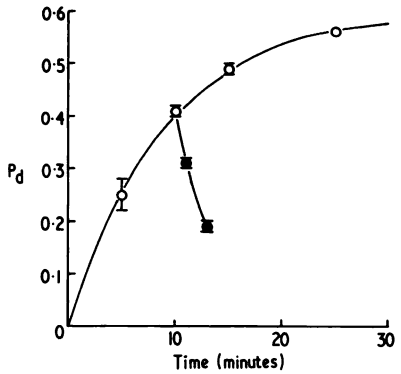


FIG. 13. *Kinetics of desensitization and recovery in frog muscle*

○, fraction of receptors (mean ± standard error) in the desensitized state (p_d), measured by adding a test dose of carbachol 1 min after washing out the conditioning agonist (C_7TMA, 5 × 10^{-4} M). The duration of exposure to the conditioning agonist is shown on the abscissa. ●, time course of recovery from desensitization when the conditioning agonist was left in the bath for 10 min. The curves are exponential, with time constants of 8.9 min for the development of desensitization, and 3.9 min for recovery.

and the desensitization dose ratio (r_d) was determined by reference to a control log dose-response curve obtained for carbachol. In order to test whether the development of desensitization could be slower than recovery, C_7TMA was used as the conditioning agonist, since it had been found to produce more rapidly fading responses than carbachol (Fig. 8 and Table 5B) and so might have a selective affinity for R' (i.e., $X > 1$). Figure 13 shows the values of p_d obtained in this way plotted against the time during which C_7TMA, 5 × 10^{-4} M, was applied to the tissue. The points were fitted by an exponential curve (as the model demands) with time constant τ_d = 8.95 min and going toward an equilibrium value of p_∞ = 0.60. Recovery from desensitization was studied by applying the same conditioning dose for 10 min, and then applying the test dose of carbachol 1, 2, or 4 min after washing out the conditioning dose (Fig. 13). Again, the results were fitted by an exponential, but with a time constant τ_r = 3.9 min. Though the points on the recovery curve do not show whether or not it is truly exponential, it is clear from Fig. 13 that the half-time for recovery was con-

siderably shorter than for the onset of desensitization.

Effect of Tubocurarine on Desensitization

The results presented so far have been consistent with the cyclic model for desensitization, and inconsistent in various respects with the other hypothetical models discussed. The effect on desensitization of a rapidly reversible antagonist provides another means of distinguishing between these models. Thus the rate theory, sequential, and parallel reaction models have the property that the antagonist should inhibit both the response and the desensitization produced by the agonist *pari passu* (see Tables 1 and 2). If agonist concentration $[A]$ produces a given response and a given amount of desensitization when tested in the absence of the antagonist, and concentration $[A]'$ produces the same peak response in the presence of the antagonist, then it is predicted that $[A]'$ will also produce the same amount of desensitization as $[A]$.

The cyclic mode predicts, however that $[A]'$ should produce more desensitization than $[A]$, and that the rate constant for the onset of desensitization should be smaller. The size of this effect on desensitization depends on (a) the value of Y for the antagonist and (b) the value of X for the agonist. We have argued that for carbachol, suxamethonium, etc., X is small, while for C_{10}TMA it is large, and so we should expect the desensitizing effect of suxamethonium to be less affected by the antagonist than that of C_{10}TMA.

Results with suxamethonium as the conditioning agonist are presented in Table 7. It can be seen that tubocurarine in concentrations up to 3.7×10^{-6} M had no consistent effect on desensitization. In one experiment the desensitization appeared to be rather greater in the presence of tubocurarine, but in other experiments the reverse was seen. This result is expected of the cyclic model only if neither agonist nor antagonist has a high affinity for the desensitized receptors. If the antagonist was DPC_{10}, the desensitization was enhanced, even when the agonist concentration was not increased in the presence of the antagonist (last line of Table 6), confirming what had been shown qualitatively by Rang and Ritter (1). In terms of the cyclic model, DPC_{10} is an antagonist with a high affinity for R' ($Y > 0$).

TABLE 7

Effect of antagonists on desensitization produced by suxamethonium in chick muscle

Expt.	Antagonist	Dose ratio	Conditioning concentration of suxamethonium	t_d[a]	t_r[b]	p_d[c]
			M	min	min	
1	None		3×10^{-6}	5	3	0.55
	Tubocurarine, 3×10^{-6} M	8.0	2.4×10^{-5}	5	3	0.58
2	None		3×10^{-6}	5	3	0.50
	Tubocurarine, 3×10^{-6} M	9.0	2.7×10^{-6}	5	3	0.43
3	None		7.4×10^{-7}	4	2	0.38
	Tubocurarine, 3.7×10^{-7} M	2.2	1.5×10^{-6}	4	2	0.41
4	None		3×10^{-6}	5	3	0.43
	Gallamine, 3.7×10^{-6} M	10.0	3×10^{-5}	5	3	0.55
5	None		7.4×10^{-7}	2	2	0.22
	DPC_{10}, 1.9×10^{-6} M	2.2	7.4×10^{-7}	2	2	0.35

[a] t_d = duration of application of conditioning dose.

[b] t_r = interval between washing out conditioning dose and applying test dose.

[c] p_d = fraction of receptors desensitized.

When the conditioning agonist was C_{10}TMA, desensitization was consistently increased in the presence of tubocurarine, as shown in Fig. 14. In these experiments the dose ratio produced by tubocurarine was measured from the decrease in sensitivity to carbachol, and the conditioning concentration of C_{10}TMA was increased accordingly. It was found that the contraction produced by the conditioning concentration of C_{10}TMA was much smaller in the presence of tubocurarine than in the absence of any antagonist (in spite of increasing the concentration of C_{10}TMA in proportion to the carbachol dose ratio). This was presumably because the dissociation of tubocurarine from the receptors was not instantaneous, so that competitive equilibrium could not be estab-

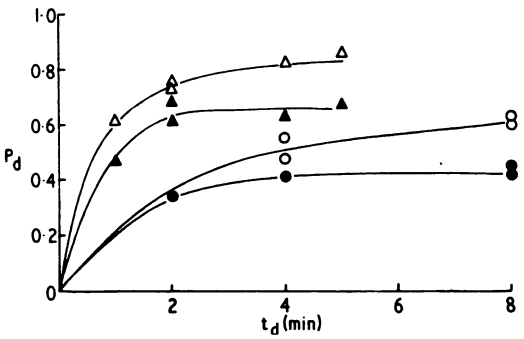


FIG. 14. *Effect of tubocurarine on desensitization by $C_{10}TMA$ in chick muscle*

Curves show the kinetics of onset of desensitization in chick muscle, plotted as in Fig. 11. ▲, conditioning agonist, $C_{10}TMA$, 2.2×10^{-5} M; △, conditioning agonist, $C_{10}TMA$, 1.1×10^{-4} M; experiment carried out in the presence of tubocurarine, 1.34×10^{-6} M; ●, conditioning agonist, $C_{10}TMA$, 1.1×10^{-5} M; ○, conditioning agonist, $C_{10}TMA$, 5.5×10^{-5} M; experiment carried out in the presence of tubocurarine, 1.34×10^{-6} M. The concentration of tubocurarine used gave a dose ratio for carbachol of 5.0. The conditioning dose of $C_{10}TMA$ in each experiment was increased by this factor in the presence of tubocurarine.

lished before the contraction was cut short by desensitization. It can be seen from Fig. 14 that the desensitization by $C_{10}TMA$ appeared to be slower in developing in the presence of tubocurarine, in accordance with the predicted behavior of the cyclic model.

DISCUSSION

The results presented in the first part of this paper show that the phenomenon of desensitization produced by nicotinic agonists in chick and leech muscle is quite specific. Thus desensitization to carbachol or related drugs does not affect the sensitivity of chick muscle to potassium or to caffeine, and in leech muscle desensitization with carbachol affects the sensitivity of the muscle to carbachol much more than to suxamethonium, while desensitization with suxamethonium has the reverse effect. Since studies with antagonists in leech muscle (26, 1) indicate that carbachol and suxamethonium act at different receptors in this tissue, the finding with desensitization implies that the mechanism entails the inactivation of specific receptors rather than any nonspecific depression of the tissue.

Another finding that suggests that receptor inactivation is the mechanism underlying desensitization in chick muscle is that the log dose-response curve to carbachol is displaced to the right in the desensitized state, without any change in slope, whereas the log dose-response curve to a partial agonist is flattened. This is to be expected of a mechanism involving receptor inactivation, but would be entirely unexpected if the receptors were not directly involved.

The theoretical models discussed in this paper represent various possible mechanisms that could account for specific desensitization. Many different models are conceivable; the ones selected for analysis were the simplest that could be devised, and it could not necessarily be expected that any of them could give a complete account of the phenomena. Nevertheless, the experimental evidence all appears to be consistent with the cyclic model, while being inconsistent in various ways with the other models. The observation that the onset of desensitization could be slower than the recovery was made by Katz and Thesleff (6) in a study of the desensitization produced by iontophoretic application of acetylcholine at the frog motor end plate, and led them to postulate the cyclic model. There is a large discrepancy between the rapid desensitization and recovery observed by Katz and Thesleff (6), using iontophoretic application, and the slow changes observed by Thesleff (5), Nastuk and Gissen (19), Harrington (30), and ourselves, using bath-applied drugs. At present the explanation of this discrepancy is not at all clear. It is possible that two separate mechanisms exist, and that the rapid mechanism studied by Katz and Thesleff (6) was obscured in these other experiments by the relatively slow diffusion of the drug to its site of action. Katz and Thesleff (6) themselves noted that a slower mechanism appeared when prolonged conditioning doses were used. Further study is clearly needed to resolve the relationship between these processes.

The cyclic mechanism for desensitization favored by our experimental results suggests a possible interpretation of the concept of agonist efficacy. This term was coined by Stephenson (18) to describe the property of a drug that determines the amount of "stimulus" associated with a given fractional oc-

cupancy of the receptors by the drug. Stephenson (18) showed that some agonists produced a large effect when occupying only a very small fraction of the receptors, while others (partial agonists) produced only a small effect even when the receptors were fully saturated. This interpretation has been substantiated for a number of drug-receptor systems (27, 31, 12). We have found that a number of full and partial agonists cause the same amount of desensitization when tested in concentrations that produce equal peak responses. According to the cyclic model these are drugs with little or no affinity for R', and the standard rate of desensitization implies that for any drug efficacy is proportional to K, the ratio of the desensitization and recovery rate constants, k_d and k_r. Since k_r was found to be the same for all the agonists tested, the implication is that efficacy is proportional to k_d. The theory then becomes very like the original rate theory (4), in that efficacy is equated with a reaction rate constant, but the reaction whose rate determines the stimulus delivered to the tissue is the reaction $AR \rightarrow AR'$ rather than $A + R \rightarrow AR$, as in the original rate theory. The efficacy of a drug would thus be a measure of the tendency of the complex AR to alter its conformation to AR', and a mechanistic interpretation would be that the stimulus is a quantal event associated with a transient intermediate conformation between AR and AR'. The over-all effect of a drug would thus be a function of the frequency with which receptors were caused to undergo this conformational change, rather than of the proportion of receptors in the form AR. The theory is thus, in a general sense, a rate theory rather than an occupation theory, even though it differs substantially from Paton's original formulation (4). The type of mechanism discussed by Paton (4), which entailed the stoicheiometric transfer of ions across the membrane, coupled with the association and dissociation of agonist molecules, does not seem appropriate at the neuromuscular junction, because the effect of the transmitter has been shown to be a conductance change (32, 33), the rate and direction of the transfer of ions depending on the electrochemical gradients. It seems preferable, therefore, to think in terms of an intermediate conformation conferring the property of raised conductance, rather than of a stoicheiometric ion transfer.

Of the theoretical models that we have discussed, the two-site model is the most difficult to formulate precisely and to test experimentally. There are, however, two pieces of evidence that appear to argue against the two-site model. They are (a) the fact that the rate of recovery from desensitization is the same for all agonists, and (b) the existence of a standard rate of desensitization. Since the two-site model must account for the slowness of desensitization by the slowness of combination of the agonist with the desensitizing site, it would be an extraordinary coincidence if recovery occurred at the same rate with different drugs. Similarly, the standard rate of desensitization has to be ascribed to coincidence according to the two-site model, whereas the cyclic model offers a more satisfactory explanation. These results are, however, far from eliminating the two-site model conclusively, and much more direct evidence will be needed to settle the question.

The results obtained on chick muscle with agonists such as C_{10}TMA and C_{16}bis-TMA, and with antagonists such as diphenyldecamethonium (DPC_{10}) and dinaphthyldecamethonium (DNC_{10}) and their alkylating derivatives $DPC_{10}M$ and $DNC_{10}M$ (1, 16), suggest that the conformational difference between R and R' is characterized by the fact that these drugs have a relatively high affinity for R', whereas drugs such as carbachol (and all of the agonists that produce the standard rate of desensitization), as well as the conventional, nonmetaphilic antagonists, tubocurarine and gallamine (1), have only a relatively small affinity for R'. We suggest that drugs that behave like C_{10}TMA and C_{16}bis-TMA might therefore be termed metaphilic agonists. In frog muscle C_7TMA, C_{13}bis-TMA, and phenyl-TMA belong to this class.

According to this nomenclature, metaphilic agonists are those that produce marked desensitization of the receptors without necessarily causing very marked depolarization. It seems possible that some of the puzzling behavior of depolarizing blocking agents at the neuromuscular junction might be explained in this way. It is still not at all clear to what extent depolarization

per se and desensitization contribute to the transmission block produced by decamethonium and suxamethonium at the neuromuscular junction. There appear to be differences between species, between experiments *in vivo* and *in vitro*, and effects of temperature (34). We have found marked differences between chick and frog muscle: phenyl-TMA, for example, behaves as a metaphilic agonist in frog muscle, but as a normal agonist in chick muscle. Also, temperature has been found to affect very markedly the rate and extent of desensitization in frog muscle.[5] The theory might, therefore, provide an explanation of some of the phenomena relating to depolarization block. Another result concerning neuromuscular blocking agents that might be explained by our hypothesis is the interaction between suxamethonium and hexafluorenium at the frog neuromuscular junction studied by Nastuk and Karis (35). They found that although hexafluorenium produced neuromuscular blockade without causing any depolarization, and its blocking action summed with tubocurarine, its effectiveness was *increased* rather than reduced by concurrent application of suxamethonium. Hexafluorenium is a symmetrical bisquaternary ammonium compound with aromatic substituents on the nitrogen atoms, similar to the metaphilic antagonists DPC_{10} and DNC_{10} described by Rang and Ritter (1). It seems likely that hexafluorenium is also a metaphilic antagonist; this would explain its otherwise unexpected synergism with suxamethonium, for the fraction of the receptors that it occludes (and thus the amount of block it produces) will be *increased* by simultaneous application of a depolarizing drug.

ACKNOWLEDGMENTS

We are grateful to Dr. E. W. Gill and Dr. R. B. Barlow for their generosity in supplying many of the compounds used.

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[5] Unpublished results.