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Modification of Dose-Response Curves by Effector Blockade and Uncompetitive Antagonism

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SUMMARY

Kinetic equations were derived for simple models of agonist-antagonist-receptor interaction and used to predict theoretical dose-response curves for the situation where there exists "receptor reserve" and a simple rectangular hyperbolic relation between tissue response and receptor occupancy by agonist, generated by an "effector chain" that links final response to receptor activation. Drug action within this chain, or an "uncompetitive" antagonism exerted by a reversible agonist that combines primarily with the active agonist-receptor complex, may give the appearance of classical noncompetitive or competitive antagonism, depending upon the amount of receptor reserve and upon the rate constants involved in both agonist-receptor and antagonist-receptor interaction. The apparent potency and nature of antagonism by such an uncompetitive antagonist may depend upon the particular agonist against which it is tested. The phenomenon whereby true competitive antagonists reduce the maximal response to a partial agonist can be explained if the partial agonist, in addition to activating receptors, also acts as an uncompetitive antagonist.

1. INTRODUCTION

The responses of tissues to agonist drugs and blockade of such responses by pharmacological antagonists can usually be explained in terms of conventional receptor kinetics. Agonists are considered to combine with a receptor recognition site to form a complex, the activated state of the receptor, which eventually leads to the final measured response. Antagonists, although they can combine with receptor, do not activate the effector system. By analogy with enzyme-substrate kinetics, competitive and noncompetitive inhibition are defined according to whether binding of antagonist to receptor is excluded by or is independent of attachment of agonist to receptor. The behavior predicted by such models, with various degrees of complexity, has been examined extensively with a view to explaining experimental observations (e.g., see refs. 1-3). In particular, it has long been recognized that noncompetitive antagonism or irreversible competitive inactivation of receptor binding sites for agonist may, in the experimental situation, mimic competitive antagonism. This occurs when the tissue is capable of maximal response with activation of only a small fraction of receptors ["spare receptors" (4); "receptor reserve" (5)]. It is our purpose in this communication to point out that an antagonism which is contingent upon prior activation of receptor by agonist, i.e., "uncompetitive" inhibition, resembles antagonism exerted at an intermediate stage in the effector system in that it may give the appearance of classical noncompetitive or competitive antagonism when there is receptor reserve. The phenomenon that true competitive antagonists can appear to act noncompetitively when tested against partial agonists (6-9) is compatible with an action by the partial agonists to block at a site distinct from that at which the agonist action is exerted. Moreover, the kinetics of uncompetitive antagonism is such that the potency of an antagonist may vary with the particular agonist against which it is tested.

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2. UNCOMPETITIVE INHIBITION AND EFFECTOR BLOCKADE

Basic equations. It is useful to begin with a restatement of some well-known results. In the simple situation where the combination of agonist and receptor always leads to an active complex, the form of antagonism by which the blocking drug acts only on this complex of the receptor is equivalent to uncompetitive antagonism. The formulae for equilibrium receptor occupancy in the absence and presence of antagonists are derived on the assumption (for simplicity) that only one molecule of agonist (A) is sufficient to activate receptor (R) by forming a complex AR. The dissociation constant of this complex is K_a. By definition, a "competitive" antagonist (I) binds (reversibly) only to the free receptor, to form an inactive complex (IR) with a dissociation constant K_I. Also, by definition, the binding of an "uncompetitive" antagonist (J) is contingent upon prior attachment of A

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to the receptor; i.e., the inactive complex AJR can exist, but JR cannot.

By mass action, and conservation of total receptor (R_t)

$$K_a AR = A \cdot R;\ K_I IR = I \cdot R;\ K_J AJR = J \cdot AR = J \cdot A \cdot R/K_a$$

$$R_t = R + AR + IR + AJR$$

$$AR = R_t/[1 + J/K_J + (1 + I/K_I)\,K_a/A] \quad (2.1)$$

where the italicized capital letters (except for K) represent concentrations.

Classical "noncompetitive" antagonism, in which attachment of inhibitor is independent of attachment of A, is equivalent to a special case of combined competitive and uncompetitive antagonism, with $K_I = K_J$ and $I = J$. Here the equation can be written

$$AR = qR_t/(1 + K_a/A) \quad (2.2)$$

where $q = 1/(1 + I/K_I)$ and is the maximal fraction of total receptors that can be activated by agonist. The identical equation is obtained with irreversible competitive antagonism; here the antagonist reduces the maximal attainable AR from R_t to qR_t.

A general equation. In general, equations relating receptor occupancy to agonist concentration in the presence of antagonists (or after reduction of available receptor by an irreversible competitive blocker) can be written in the form

$$AR = R_t/[1 + u + (K_a/A)(1 + c)] \quad (2.3)$$

$$= \frac{R_t}{1 + u} \cdot \frac{1}{1 + (K_a/A)(1 + c)/(1 + u)} \quad (2.3a)$$

Equation 2.3a is, of course, the same as Eq. 2.2, with the substitution of $1/(1 + u)$ for q and $K_a(1 + c)/(1 + u)$ for K_a. Thus, the concentration of agonist at which AR is one-half its maximum is influenced by both u and c, whereas only u governs the extent by which the maximal possible AR is reduced. If more than one antagonist is present, u is simply the sum of normalized (relative to dissociation constants) concentrations of the uncompetitive antagonists, provided binding of these antagonists is mutually exclusive, and c is the sum of the normalized concentrations of competitive antagonists. Again there is the proviso that binding be mutually exclusive, which is likely to be the case for competitive antagonists since by definition each can be excluded by the presence of agonist.

Receptor reserve. The relationship between tissue response and receptor occupancy is generally obscured by a sequence of poorly understood transduction steps lying between the initial "stimulus" and the final effect. Often these steps serve to amplify the stimulus, and an essentially maximal response can occur when only a small fraction of the receptors is activated by the agonist. There is then said to be a "receptor reserve" (5). It is generally possible to analyze dose-response curves by employing the assumption that equal responses result from equal "stimuli," which in turn reflect equal occupancy of receptors by the one agonist that is present (4, 10–12).

From Eq. 2.3:

$$1/A_0 = (1 + c)/A + u/K_a \quad (2.4)$$

where A_0 and A are concentrations of agonist that give the same response in the absence and presence of antagonist. The dose ratio (DR) is

$$DR = A/A_0 = 1 + c + uA/K_a \quad (2.4a)$$

This can be rearranged to give

$$\mathrm{DR} = (1 + c)/(1 - uA_0/K_a) \quad (2.4b)$$

These expressions provide graphical methods for determining K_a from response-dose curves obtained before and after applying an irreversible competitive antagonist (which in effect gives $c = u$) sufficiently to reduce the maximal response to agonist; Eq. 2.4 then becomes the same as the equation derived by Furchgott (12) and Furchgott and Bursztyn (13), with $q = (1 + u)^{-1}$, i.e.,

$$1/A_0 = 1/(qA) + (1 - q)/(qK_a) \quad (2.4c)$$

Once K_a is known, dose-response curves obtained in the absence and presence of an antagonist provide estimates of u and c produced by the antagonist, i.e., its uncompetitive and competitive components. Equation 2.4b serves to make explicit the fact that DR is essentially independent of u when $uA_0/K_a \ll 1$. That is, when the testing values of A_0/K_a are very low because of very large receptor reserve, it is only when u becomes very high that the value of DR varies with A or A_0, and response-log dose curves are no longer parallel.

To visualize how dose-response curves are modified by receptor reserve, it is useful to consider the relatively simple case where response is related to amount of activated receptor by a rectangular hyperbolic function (12) the same as that relating receptor occupancy and agonist concentration. This will occur when the response is proportional to the amount of a second receptor (X) combined with a second messenger (B) which is produced in proportion to the amount of AR. It also occurs when the measured response is proportional to the change of membrane potential (v) of a cell and the agonist acts to alter membrane conductance to certain ions by amount g.

$$B = B' \cdot AR; \qquad B \cdot X = K_b BX$$

$$BX = X_t/(1 + K_b/B) = X_t/[1 + (K_b/B')/AR] \quad (2.5)$$

$$= X_t(B'R_t/K_b)/[B'R_t/K_b + R_t/AR]$$

In the second case, where G is the resting conductance of the cell (see ref. 14), one has

$$g = g'AR$$

$$v = v_{max}/(1 + G/g) = v_{max}/[1 + (G/g')/AR] \quad (2.5a)$$

$$= v_{max}(g'R_t/G)/[g'R_t/G + R_t/AR]$$

The equation becomes slightly more complicated if g is proportional to BX rather than to AR:

$$v = v_{max}/[1 + (G/g')/(BX)]$$

$$= v_{max}/[1 + (G/g')(1 + K_b/B)/X_t] \quad (2.5b)$$

$$= v_{max}\,\frac{(g'X_t/G)(B'R_t/K_b)}{(g'X_t/G)(B'R_t/K_b) + (B'R_t/K_b) + R_t/AR}$$

In all of these cases the expression may be written

$$E = e'/[e + R_t/AR] \quad (2.5c)$$

where E is effect and e and e' are independent of agonist concentration. In the absence of antagonists, $R_t/AR = 1 + K_a/A$. Equation 2.5c then takes a more familiar form (12):

$$E = \frac{Ae'/(1 + e)}{A + K_a/(1 + e)}$$

As pointed out by Furchgott (12), this expression arises whenever the final effect arises from the stimulus via a chain in which the "output" of each link is related to the "input" by an equation of the same form as that relating B or g to AR in the first two examples above (that is, the output is a rectangular hyperbolic function of the input). In general, e' and e are composed as follows:

$$e' = E'e_1e_2e_3 \ldots e_n \quad (2.5d)$$

$$e = e_1 + e_1e_2 + e_1e_2e_3 + \ldots + e_1e_2 \ldots e_n \quad (2.5e)$$

where there are n links in the chain, E' is the response to complete activation of the final link, and e_1, $e_2 \ldots e_n$ correspond to expressions such as $(B'R_t/K_b)$, $(g'X_t/G)$ above.

Combining Eq. 2.5c with Eq. 2.3a:

$$E = e'/[e + 1 + u + (1 + c)K_a/A] \quad (2.6)$$

Defining E_{max} as the maximal response that can be obtained by raising A, and E_m as E_{max} when $u = 0$,

$$E/E_m = 1/[1 + u/(1 + e) + (K_a/A)(1 + c)/(1 + e)] \quad (2.6a)$$

$$E/E_{max} = 1/(1 + K_a'/A) \quad (2.6b)$$

where

$$K_a' = \frac{K_a(1 + c)}{1 + e + u} = \frac{K_a}{1 + e} \cdot \frac{1 + c}{1 + u/(1 + e)} \quad (2.6c)$$

$$E_m = e'/(1 + e) \quad (2.6d)$$

$$E_{max} = e'/(1 + e + u) = E_m/[1 + u/(1 + e)] \quad (2.6e)$$

The effect of an irreversible competitive antagonist, which reduces R_t to qR_t, is given by substituting in the above equations qe' for e' and qe for e. In particular, at constant u the new maximal response to agonist becomes:

$$E''_{max} = E_{max} \cdot q(1 + e + u)/(1 + qe + u) \quad (2.6f)$$

Thus, when u is appreciable relative to e, i.e., E_{max} is noticeably less than E_m, any reduction of R_t will reduce E_{max}. The right-hand side of Eq. 2.6a is the same as that of Eq. 2.3 with the substitution of $u/(1 + e)$ for u and $K_a/(1 + e)$ for K_a. Thus, Eqs. 2.6–2.6e describe the same sigmoidal relationship between response and log A; the two differences imposed by receptor reserve are (a) the maximal response becomes reduced by u only when u is appreciable relative to e (Eq. 2.6e) and (b) the EC_{50} of the agonist, i.e., K_a', may be much less than K_a. The EC_{50} is proportional to $(1 + c)$ and is reduced by the presence of the u term by exactly the same factor as the maximal response is reduced (Eqs. 2.6c and 2.6e). As previously, a reduction in the number of receptors has the same effect as introducing the uncompetitive term "u" and an equal competitive term "c"; if the fraction of receptors remaining is q, the effect is given by Eqs. 2.6b–2.6e with $c = u = 1/q - 1$. When u and c are initially zero and the maximal response is reduced by a noncompetitive (or an irreversible competitive) antagonist, K_a' is changed from $K_a/(1 + e)$ to $K_a/(1 + qe)$. Thus, when e is initially much greater than 1, K_a' is reduced to a value very close to $K_a(1 - E_{max}/E_m)$.

Figure 1 shows theoretical response-log dose curves for competitive, uncompetitive, and noncompetitive antagonism, with $e = 200$. The existence of receptor reserve merely reduces the apparent potency of the uncompetitive antagonist. Shifts of dose-response curves similar to those of Fig. 1C, but with varying amounts of receptor reserve, are commonly seen with irreversible competitive blockade (e.g., Fig. 2E).

"Efficacy," and e as a measure of receptor reserve. By rewriting Eq. 2.5c as

$$E = (e'/e)/[1 + R_t/(e \cdot AR)]$$

it becomes evident that the term e in the above equations is the same as Stephenson's "efficacy" (4), since for agonists with different efficacies, responses that are 50% of e'/e (which is Stephenson's "true maximum," approached when e is large) are obtained when "stimulus" (i.e., $e\ AR/R_t$) is equal to 1. The value of e'/e must in general be identical for all agonists acting through the same receptor, since e' and e always share the common factor e_1, which corresponds to the first link in the effector chain. Moreover, e_2, e_3, etc. must be identical since one and the same effector chain is involved. However, the factor e_1 contains a proportionality factor (B' in the above example) that could conceivably vary from one agonist to another, giving different values of e for different agonists acting via the same receptor. Other models of "partial agonism" are considered in Section 4.

As emphasized by Furchgott (12), e is also directly proportional to R_t, the concentration of activatable receptors, and is a measure of receptor reserve. It may indeed be a generally convenient way in which to express receptor reserve, for which there seems to be at present no generally accepted mathematical definition. Values for e may readily be determined experimentally if an irreversible competitive antagonist is available, and expression of receptor reserve in terms of the fraction of receptors needed for a half-maximal response evades a number of problems that arise if one seeks to define reserve in terms of the fraction of receptors necessary for an "essentially maximal" response. From the above equations, one-half E_m is achieved when AR/R_t is $1/(2 + e)$ and, in the absence of inhibitors, this occurs at $A = K_a' = EC_{50} = K_a/(1 + e)$. Determination of K_a by the Furchgott method (see Eq. 2.4c) or, more simply, as the extrapolated maximal EC_{50} obtainable with an irreversible competitive antagonist, therefore gives initial e as $(K_a/K_a' - 1)$. Alternatively, assuming the hyperbolic model, e can be determined as $p - 2$, where p is the factor by which R_t must be divided to reduce E_m by 50%—p can of course be obtained from a plot of $1/q$ versus $1/E_m$ for various degrees of irreversible receptor blockade. In principle, the two methods are independent, and the same value for e is obtained if (and perhaps only if) the

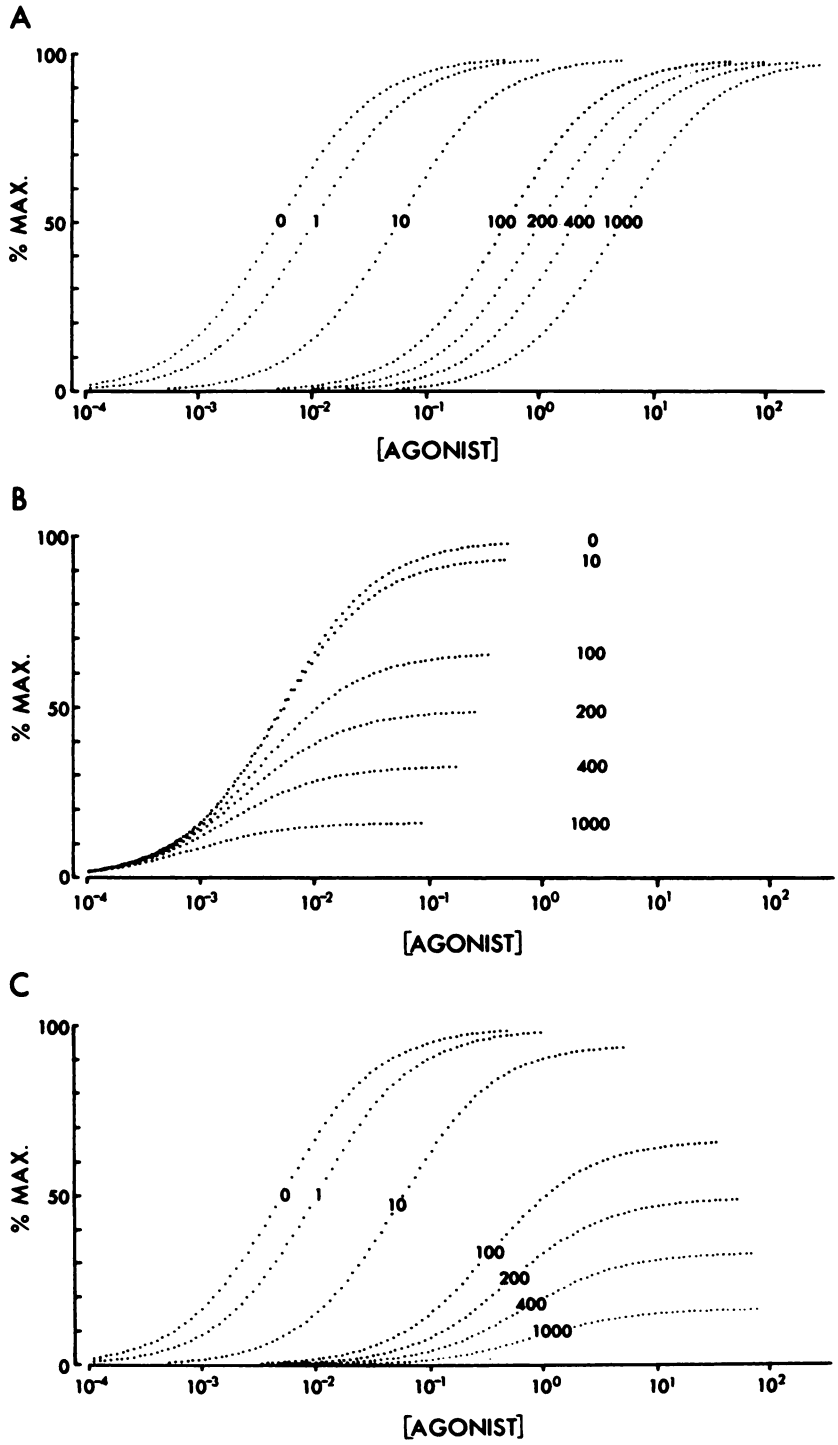


FIG. 1. *Theoretical response-log agonist concentration curves generated using Eq. 2.6, at varied concentrations (0–1000) of antagonist*

In all cases the receptor reserve term (e) was given an arbitrary value of 200. The concentrations of both agonist and antagonist are relative to their dissociation constants. A, Competitive inhibition: the antagonist binds only to free receptor; therefore, $u = 0$ and $c = I/K_I$. B, Uncompetitive inhibition: $u = I/K_I$ and $c = 0$; the antagonist binds only to receptors activated by the agonist. C, Noncompetitive inhibition: $u = c = I/K_I$; antagonist binding is the same whether or not agonist is associated with the receptor. Irreversible receptor inhibition gives identical curves for values of $q = 1/(1 + I/K_I)$ (and e held constant at 200). The ordinate indicates percentage of E_m, the maximal possible response to agonist when no antagonist is present.

hyperbolic model is applicable. The determination of e (and of q values) is complicated if there is cooperativity in agonist-receptor interaction (see Section 6).

The definition of "receptor reserve" as e conforms to the usual sense in which the term is employed in that e is zero when tissue response is directly proportional to the amount of receptor activated by the agonist; i.e., any reduction of R_t reduces maximal response in proportion. Provided the hyperbolic model can be applied, e indicates the fraction of receptors needed for any arbitrarily defined "essentially maximal" response.

Effector blockade. Using the above equations, it is

possible to examine the consequences of a "functional" or "physiological" antagonism exerted somewhere in the effector system, i.e., an antagonism such that equal responses do not represent equal AR. Expressing e' and e in terms of $e_1, e_2 \ldots e_n$, Eq. 2.6 becomes

$$E = E'e_1e_2 \ldots e_n/[1 + u + e_1 + e_1e_2 + \ldots e_1e_2 \ldots e_n + (1 + c)(K_a/A)] \quad (2.7)$$

where E' is the response that would occur with full activation at the final step in the chain (e.g., v_{max} in the example given above) and e_1, e_2, etc. represent expressions such as $(B'R_t/K_b)$ and $(g'X_t/G)$. In general, a competitive antagonism exerted anywhere within the chain is equivalent to a change of an affinity (e.g., K_b), whereas a noncompetitive (or irreversible competitive) antagonism is equivalent to change of an amount of an intermediate receptor (e.g., X_t). Thus, either kind of antagonism will be equivalent in effect to a mixture of uncompetitive and competitive action at the level of the receptor where A acts. For example, let us suppose that $n = 2$ and e_2 is reduced to e_2/p (e.g., by reduction of g' or X_t or increase of G in Eq. 2.5b). Then,

$$E = E'e_1e_2/[e_1e_2 + p(e_1 + 1) + pu + p(1 + c)K_a/A] \quad (2.8)$$

This is just the same as increasing u by $(p - 1)(1 + e_1 + u)$ and replacing $(1 + c)$ by $p(1 + c)$. Since by definition $e_1 > 0$, the net effect is the same as would be produced by irreversible competitive or noncompetitive blockade at the receptor level, in the presence of a receptor reserve smaller than actually exists. It may easily be verified that in general any mode of reduction of the e_1 term is equivalent to noncompetitive (or irreversible competitive) antagonism with full receptor reserve (since R_t is a factor of e_1) and that the apparent receptor reserve becomes less the more remote the site of antagonism from the receptor itself. In particular, if $p_1, p_2, p_3 \ldots p_n$ are the factors by which $e_1, e_2 \ldots e_n$, respectively, must be divided in order to reduce maximal response to agonist by 50% (i.e., the apparent receptor reserves are $p_1 - 2$, $p_2 - 2$, etc.), then:

$$p_1 - 2 = e_1 + e_1e_2 + e_1e_2e_3 + \ldots e_1e_2 \ldots e_n = e$$

$$p_2 - 2 = (e_1e_2 + e_1e_2e_3 + e_1e_2 \ldots e_n)/(1 + e_1)$$

$$p_3 - 2 = (e_1e_2e_3 + \ldots e_1e_2 \ldots e_n)/(1 + e_1 + e_1e_2)$$

etc.

Of course, at the final step (corresponding to E' in the above equation), there is no apparent receptor reserve; any reduction in E' will appear "noncompetitive," in the sense that responses are depressed by the same factor at all agonist concentrations. Whenever apparent receptor reserve is less than full receptor reserve (i.e., $p < e + 2$), the remaining reserve may be revealed by an irreversible competitive antagonist acting on the receptor.

From Eq. 2.3a it is evident that uncompetitive antagonism is equivalent to reduction in the amount of a receptor together with an increase by the same factor of that receptor's affinity for its agonist. Any such action exerted within the effector chain will be equivalent to equal and opposite effects on two adjacent terms in the series $e_1, e_2 \ldots e_n$; the net effect is to alter only one term in the series $e_1 + e_1e_2 + e_1e_2e_3$, etc. For example, an uncompetitive action at the level of receptor X in the scheme corresponding to Eq. 2.5b will reduce X_t and K_b equally. If the factor is p, then the expression corresponding to Eq. 2.8 is:

$$E = E'e_1e_2/[e_1e_2 + pe_1 + 1 + K_a/A]$$

A similar result appears whatever the point in the chain the uncompetitive antagonism is exerted. The net effect is equivalent to introducing only an uncompetitive term, u; this is the same as saying that uncompetitive antagonism at any stage of the effector chain should change response-dose curves in the same way as uncompetitive antagonism at the receptor level.

As emphasized by Stephenson (4) and by Furchgott (12), response-dose curves do *not* generally follow a rectangular hyperbola; equations such as those above therefore cannot be employed directly. Nevertheless, it is evident that if the measured response is any arbitrary function of E, as defined in the above equations, rather than E itself, the equations will still hold true in the sense that antagonism exerted at any level in the effector chain will be equivalent to a mixture of competitive and uncompetitive action at the receptor level. Thus, an analysis using the Furchgott equal-response method (12, 13), should give linear plots of $1/A_0$ versus $1/A$ (Eq. 2.4) and will define the antagonist action in terms of apparent u, c, and K_a, and a value for apparent receptor reserve will be obtained if the action is construed as equivalent to irreversible competitive blockade. Moreover, " . . . the set of curves constructed with the use of a single K_a value, the respective determined q values for each level of blockade, and the original dose-response curve, should fit the sets of dose-response points obtained at each level of blockade" (12). For an antagonism that is exerted at a stage beyond that which causes the response-dose curve to deviate from a rectangular hyperbola, i.e., an antagonism that alters the functional relationship between response and E, the last statement may of course not be true, but this does not preclude the superficial resemblance of such an antagonism to a mixed antagonism at the level of the receptor.

Examples of "noncompetitive" receptor antagonism, exerted on nicotinic receptors, are shown in Fig. 2. With atropine (15) (Fig. 2A) or tetracaine (16) (Fig. 2B), the picture resembles that found with irreversible competitive blockade (19) (cobra α-toxin; Fig. 2E) except that the apparent receptor reserve is much less. It should be noted that with cobra α-toxin on frog rectus the apparent receptor reserve for acetylcholine is reported to be unaltered by poisoning acetylcholinesterase (although the response-log dose curve becomes steeper, as in Fig. 2A) and is much the same for carbachol (19). In the case of blockade of responses to butyltrimethylammonium by decyltrimethylammonium, as reported by Ariens and van Rossum (18), there would appear to be no receptor reserve at all (Fig. 2D), although butyltrimethylammonium is a full agonist in frog rectus (18) and is known to have an efficacy about one-half that of acetylcholine or carbachol in terms of opening ionic channels at the frog neuromuscular junction (20). Tetracaine clearly acts on the nicotinic receptor-ionophore in *Torpedo* electroplax

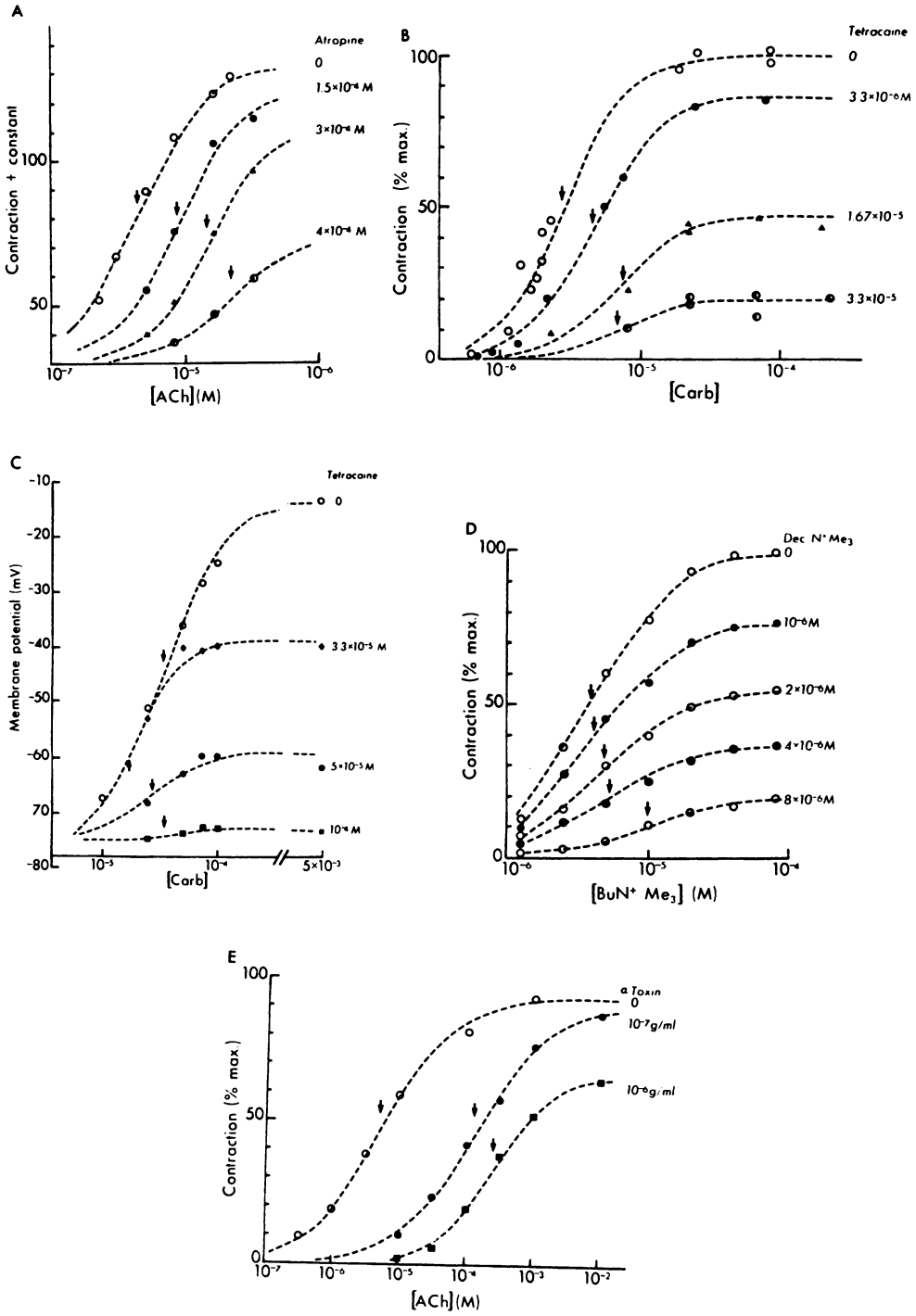


FIG. 2. *Comparison of "noncompetitive" shifts of response-log dose curves with shifts produced by an irreversible competitive antagonist*

A, atropine on contraction of frog rectus produced by acetylcholine (*ACh*) after irreversible blockade of acetylcholinesterase (15). B, Tetracaine on contraction of frog rectus by carbachol (*Carb*) (16). C, Tetracaine on the depolarization of *Electrophorus* electroplax by carbachol (*Carb*) (17). D, Decyltrimethylammonium on contraction of frog rectus by butyltrimethylammonium (BuN^+Me_3) (18). E, Cobra α-toxin on contraction of frog rectus produced by acetylcholine (*ACh*) (19), acetylcholinesterase not inhibited. Note change of scale on the abscissa in E. The receptor reserve in frog rectus revealed by the irreversible antagonist is much larger than the apparent receptor reserve in A, B, or D. In each case original data have been replotted, and the *arrows* indicate K_a' for each curve, obtained by fitting points to

$$\text{Response/max response} = [1 + (K_a'/A)^n]^{-1}$$

In all except E, chosen values for n were the same for each curve in the series: 1.8 in A and B, 1.6 in C, and 1.4 in D. In E, n was 0.8 for the control and 10^{-7} g/ml of toxin, and 1.0 for 10^{-6} g/ml of toxin. The relatively low n in E may be related to failure of agonist concentrations to achieve bath concentration, expecially deep in the tissue. An increase of n with toxin is then to be expected if receptors near the tissue surface are blocked more than receptors deep in the tissue.

in an uncompetitive manner (17, Fig. 2C), but it is unclear whether the blockade of frog rectus contraction at less than 3.3×10^{-5} M (16) (Fig. 2B) can be attributed to this mechanism. With atropine, on the other hand, the depression of contracture occurs at doses that markedly depress depolarization at frog end-plates by acetylcholine (see ref. 21); there is no need to postulate any effect on the effector chain (i.e., contractile mechanism). It is now known that at these high concentrations atropine acts on nicotinic receptors (22, 23) in the same way as local anesthetics, to shut or block ionic channels opened by acetylcholine. Since the target site is the active agonist-receptor-channel complex, it is to be classified as an uncompetitive antagonist; the shift to the right of the response log-dose curve indicates the existence of a competitive component to the blockade.

In all of these examples the "noncompetitive" blockade is best described as a mixed blockade, with (since receptor reserve in fact exists) uncompetitive terms greater than competitive terms. From Eq. 2.6a, it is evident that the appearance of classical noncompetitive inhibition with a drug that acts at the receptor level can occur only if $c = u/(1 + e)$; this condition cannot occur when there is receptor reserve, with pure competitive, uncompetitive, or noncompetitive inhibition.

3. A FORM FOR UNCOMPETITIVE BLOCKADE THAT CAN MASQUERADE AS COMPETITIVE OR NONCOMPETITIVE

It is possible to account for the apparent competitive component of the blockade by atropine (Fig. 2A) by modifying the previous model of uncompetitive blockade by postulating that agonist may dissociate from the blocked as well as from the unblocked form of the agonist-receptor-ionophore complex. If one assumes that the different states of the receptor can attain thermodynamic equilibrium, then one has, at a minimum, a four-state cyclic system, essentially the same as the second model postulated by Katz and Thesleff (24) to describe densensitization:

$$\begin{array}{ccc} & \overset{K_{a1}}{\rightleftharpoons} & \\ A + R + D & & AR + D \\ K_{d2} \updownarrow & & \updownarrow K_{d1} \\ A + RD & \underset{K_{a2}}{\rightleftharpoons} & ARD \end{array}$$

At equilibrium one has:

$$K_{a1}AR = A \cdot R;\ K_{d1}ARD = AR \cdot D$$

$$K_{a2}\,ARD = A \cdot RD;\ K_{d2}RD = R \cdot D$$

And thus

$$\mathrm{AR/R_t} = [1 + \mathrm{D/K_{d1}} + (1 + \mathrm{D/K_{d2}}) \cdot \mathrm{K_{a1}/A}]^{-1} \quad (3.1)$$

Note that $K_{a1}K_{d1} = K_{a2}K_{d2}$; i.e., a relatively low affinity of the nonactivated receptor for the antagonist D ($K_{d2} \gg K_{d1}$) is associated with a relatively high affinity of RD for A ($K_{a2} \ll K_{a1}$). This scheme is formally tantamount to a mixture of uncompetitive and competitive antagonism with $c = D/K_{d2}$ and $u = D/K_{d1}$; in the presence of receptor reserve the blockade may give the appearance of all three classical forms of receptor inhibition—competitive when D/K_{d2} is large relative to $(D/K_{d1})/(1 + e)$, uncompetitive when the converse is true, and noncompetitive when these two expressions are nearly equal.

The above scheme differs from a simple mixture of competitive and uncompetitive antagonism in that the blocker D is not presumed to attach to receptor at the same site as the agonist A. Thus, a competitive antagonist may be able to combine with either R or RD (with dissociation constants K_I and K_{Id}). In the presence of such an inhibitor, one has:

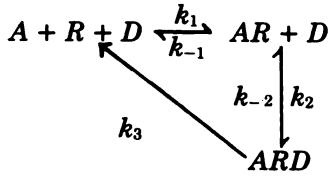
$$AR/R_t = \{1 + D/K_{d1} + (K_{a1}/A) \cdot [1 + I/K_I + (D/K_{d2}) \cdot (1 + I/K_{Id})]\}^{-1} \quad (3.2)$$

which is the same as Eq. 2.3 with

$$c = (D/K_{d2}) \cdot (1 + I/K_{Id}) + I/K_I; \quad u = D/K_{d1}$$

Thus, although the effector blocker may give the appearance of competitive inhibition, the combined effects of the EC_{50} of the effector blocker and a true competitive antagonist will not be simply additive, as it is with two competitive antagonists, where c is the sum of normalized concentrations of both, and a relatively high concentration of one such antagonist occludes the effect of the other. Unless K_{Id} is much larger than K_I, the effect of D to shift EC_{50} cannot entirely be occluded by I. If $K_{Id} = K_I$ there will be no occlusion at all, and if $K_{Id} < K_I$ the effectiveness of the competitor will actually be increased in the presence of D.

It may not always be plausible to suppose that the receptor in the nonactivated state is at all capable of binding uncompetitive antagonist. For example, in the case of the blockade of the nicotinic system of local anesthetics it is the open ionic channel that is considered to be the site of binding of the drug (25–27). A model that gives much the same result as the previous one is obtained by postulating a cycle in which the blocker does not combine with inactive receptor and dissociation of agonist from the blocked state constitutes an essentially irreversible step. This scheme might also be applicable in situations where insufficient time is allowed for a true steady state to be achieved.



In effect the blocker now acts to shorten the average lifetime of AR. Such a scheme is compatible with the observed time course of miniature end-plate currents at the mouse neuromuscular junction in the presence of local anesthetics or barbiturates (see ref. 28). The steady-state solution for this model is the same as Eq. 3.1, with

$$K_a = k_{-1}/k_1; \quad K_{d1} = (k_3 + k_{-2})/k_2; \quad K_{d2} = K_{d1} \cdot k_{-1}/k_3$$

As before, the antagonism may masquerade as classically uncompetitive, noncompetitive, or competitive.

It should be pointed out that in the nonequilibrium model, k_3 may present a route by which the blocked

receptor is converted into a form which only slowly reconverts to the form that can be activated by acetylcholine. This would be analogous or equivalent to a "desensitized" receptor; it has been observed that many local anesthetics and similarly acting agents appear to increase densensitization (23, 29). As may readily be verified, the introduction of another form of receptor in the above model, in the irreversible k_3 path between ARD and R, acts to increase the fraction of total receptor that is in equilibrium with ARD and thus increase u. This merely alters the range of k_{-1}/k_3, where the antagonism will appear as "noncompetitive."

It has already been remarked that with the reversible cyclic model a shift of EC_{50} (K_a') by the blocker cannot entirely be occluded by a true competitive inhibitor. This is also the case with the non-equilibrium model. The solution corresponds to Eq. 3.2 with $K_I = K_{Id}$; i.e., the uncompetitive antagonist will shift the response-log agonist curve to exactly the same extent in the presence of large I/K_I as in the absence of I.

With the irreversible cycle, K_{a2} is a function of k_{-1} as well as of k_3. With the nicotinic acetylcholine receptor the duration of the ionic channel associated with activated receptor varies considerably from one agonist to another (30–32). It would therefore appear likely that k_{-1} may in general be different for different agonists acting on the same receptor. Hence, with this scheme, both the potency and apparent nature of the antagonism by an uncompetitive antagonist may be expected to vary with the agonist against which it is tested. Such a result has recently been reported for the near steady state for ionic channel blockade by a light-activated drug acting on nicotinic receptors (33). The blockade of the inhibitory action in cerebellar cortex of *N*-methyl amino acids, by strychnine and picrotoxin (34), also suggests agonist-dependent antagonism. With the reversible cyclic model, on the other hand, only the uncompetitive component of the antagonist's action can be agonist-dependent.

4. PARTIAL AGONISTS

It is usual to account for the phenomenon of partial agonism by supposing that the amount of "stimulus" produced by a certain receptor occupancy varies with the agonist. A mathematically equivalent formulation is obtained by supposing that the occupied receptor is not continuously associated with a stimulus (see ref. 35). This can be written in the form of an oscillation of the occupied receptor between inactive (AR') and active forms (AR).

$$A + R \rightleftharpoons AR \rightleftharpoons AR'$$

Formally, this is exactly equivalent to uncompetitive inhibition (but in the absence of any inhibitor) and therefore leads to a constant term included in u in the above equations; when this term is appreciable with respect to $e + 1$, the maximal response to the partial agonist will be less than that to a full agonist. The same result is obtained by supposing that the direct combination of agonist with receptor forms first the inactive complex, which subsequently isomerizes to the active form (see ref. 36). Such a step must occur with any agonist-receptor interaction (see refs. 37 and 38), but with full agonists the ratio of AR to AR' is presumably large, and the corresponding term in u is small.

Both models are also equivalent to a nonproductive binding model (37; see also ref. 38), i.e.:

$$A + R \rightleftharpoons AR \qquad K_{a1}$$

$$A + R \rightleftharpoons AR' \qquad K_{a2}$$

where only AR is an active receptor. This can be viewed as a "collapsed" version of a Monod-Wyman-Changeux allosteric model in which the partial agonist stabilizes both inactive and active conformations of the receptor (39, 40). Here the agonist itself is acting as a competitive inhibitor, and one can therefore simply substitute A/K_{a2} for I/K_I in Eq. 2.1, and omit J/K_J, to obtain

$$AR/R_t = [1 + (1 + A/K_{a2})K_{a1}/A]^{-1} \qquad (4.1)$$

$$= [1 + K_{a1}/K_{a2} + K_{a1}/A]^{-1}$$

Again, this constitutes a constant increase of u in Eq. 2.3.

With all of these models the actions of antagonists on responses to the partial agonist are also readily predicted. (a) Competitive antagonists will shift the curve of response versus log agonist concentrations in the same way as with full agonists. (b) Irreversible competitive blockers must act immediately to depress the maximal response, since the pre-existing u is already appreciable with respect to e (see Eq. 2.6e); K_a' will continue to increase until u becomes large relative to e. (c) An uncompetitive antagonist, unless it can combine with AR', will contrast with a true noncompetitive or irreversible competitive antagonist in that it will appear no more potent in depressing the maximal response to partial agonists than in depressing the response to full agonists. This arises because the effect is small until the contribution to total u by the uncompetitive antagonist becomes substantial relative to the term already introduced by the partial agonist.

In general, published experimental observations on partial agonists acting on a variety of preparations and receptors are fully in accord with the above formulation. Derived values for affinity of partial agonists should by any of the above models appear to be the same whether they are studied as agonists or as competitive inhibitors of full agonists (e.g., refs. 12, 41, and 42). However, it should be noted that by any of these models this affinity is composite in nature, depending upon the rate constants involved in the generation of both active and inactive agonist receptor combinations.

5. COMPETITIVE ANTAGONISTS MASQUERADING AS NONCOMPETITIVE

It has been observed in several systems that agents which appear to act purely competitively when tested against full agonists reduce the maximal response to partial agonists. There are many examples involving partial muscarinic agonists and competitive antagonists such as hyoscine and atropine (e.g., ref. 6); the slow rate of loss of atropine from muscarinic receptors (43) makes it possible that this arises because of a failure to reach equilibrium in the time allowed for responses to take place (6). A "noncompetitive" action of atropine, nor-

mally masked by receptor reserve, is unlikely in view of the data of Spero (8); in rabbit stomach fundal strips, doses of atropine that reduced maximal responses to the partial agonists pilocarpine and heptyltrimethylammonium had no effect on maximal responses to carbachol even after receptor reserve had been largely eliminated by pretreatment of strips with the irreversible competitive antagonist dibenamine. These data suggest that partial agonists themselves may have an action to depress contraction, exerted either on the receptor itself or at some point in the effector process.

At nicotinic receptors, decamethonium is a partial agonist to which the maximal response (increase of conductance) is about 15% that produced by full agonists such as carbachol (19), although individual ionic channels activated by decamethonium have at least 80% the conductance of those opened by full agonists (31). Thus, the low maximum for decamethonium is related to the production of inactive receptors rather than to a reduction in response to each individual activated receptor; this conforms to the models of partial agonism discussed above. It is observed that (+)-tubocurarine, at concentrations where it behaves only as a competitive antagonist (44) of full agonists, clearly reduces the maximal response to decamethonium (7) (Fig. 3) or pentyltrimethylammonium (9). Since (+)-tubocurarine can dissociate from receptors at a rate of at least 10/sec (see ref. 45), it is unlikely that this arises from insufficient time being allowed for equilibration (about 1 min in ref. 7). It will be shown below that the phenomenon may be explained by a model in which partial agonists themselves act uncompetitively on the activated receptor-ionophore system. Such an action of decamethonium on nicotinic receptors,

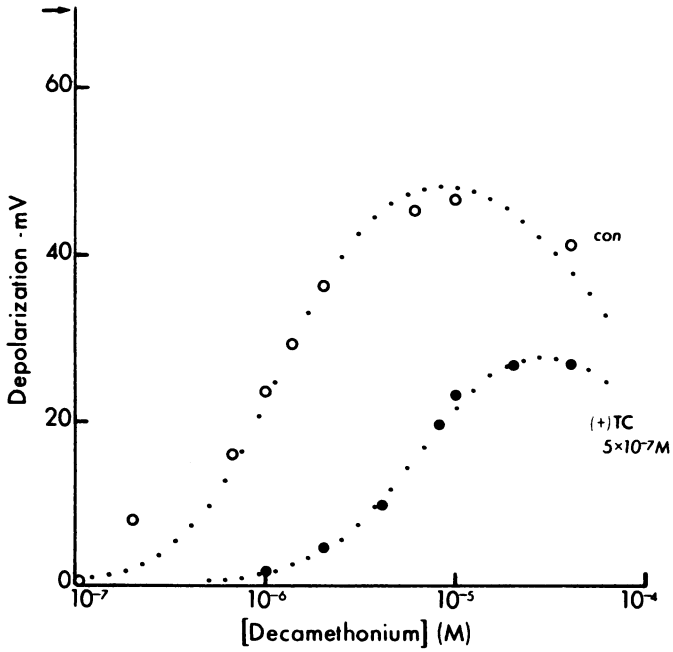


FIG. 3. *Apparent noncompetitive action of (+)-tubocurarine [(+)TC] in electroplax when decamethonium is the agonist*

Graphs were replotted from the data of Podleski (7). *Dotted lines* are drawn according to Eq. 6.1 combined with Eq. 2.5a, with parameters given in the text.

to block opened ionic channels in the same way as local anesthetics, has recently been reported by Adams and Sakmann (46), and presumably also accounts for the autoinhibition observed with decamethonium (7, 47) and alkyltrimethylammonium salts in the series *n*-butyl through *n*-decyl (18).

With the assumption that, as with local anesthetics and atropine, the blocked channel conformation produced by the partial agonist can dissociate to yield a closed channel and free agonist, without passing through the open channel conformation, one can write the appropriate equation simply by substituting A for D in Eq. 3.2.

$$\begin{aligned} AR/R_t &= \{1 + A/K_{d1} \\ &\quad + (K_{a1}/A)[1 + I/K_I \\ &\quad + (A/K_{d2})(1 + I/K_{Id})]\}^{-1} \qquad (5.1) \\ &= [1 + A/K_{d1} + (K_{a1}/K_{d2})(1 + I/K_{Id}) \\ &\quad + (K_{a1}/A)(1 + I/K_I)]^{-1} \end{aligned}$$

The same result is obtained with the irreversible cycle, but with

$$K_{Id} = K_I.$$

In this equation, u (see Eq. 2.3), which has the effect of reducing the maximal response, now depends upon the concentration of *competitive* antagonist. As before (see Eq. 2.6), only if the absolute increase of u is sufficiently large relative to receptor reserve will the maximal response be appreciably reduced by the competitive antagonist.

Both of these models also predict that at very high doses of the partial agonist the response should be reduced, eventually to zero (i.e., autoinhibition), even in the absence of inhibitors, since u includes a term proportional to A. This is often observed (e.g., refs. 4, 7, 18, and 47). Even where it it not seen, it would not necessarily negate the model, since K_{d1} may well be so large that autoinhibition will not occur except at very high concentrations of the agonist.

It should be emphasized that it is in general possible to substitute inhibition in the effector chain for an uncompetitive action at the receptor level by the partial agonist, as here postulated, to account for a spurious noncompetitive action of a competitive inhibitor. However, in the case of decamethonium acting at the nicotinic receptor, the measured response (depolarization) is sufficiently close to the primary event produced by receptor activation (channel opening) as to obviate such a possibility.

It is conceivable that at least in some cases partial agonism may itself be attributable entirely to the above mechanism, which requires only that K_{a1}/K_{d2} (see Eq. 5.1) be appreciable relative to e, the term for receptor reserve, in order to account for both a low maximal response and depression of this maximum by a competitive antagonist. Alternatively, the "partialness" of the partial agonist may stem from both mechanisms—(a) formation of a simple inactive receptor complex (AR') and (b) an "impure" uncompetitive action associated with a new route of dissociation of the active agonist-receptor combination. An action in the effector chain is

a third mechanism that could exactly mimic mechanism b. With different partial agonists, either one or the other mechanism might predominate. If this is the case, the apparent potency of competitive antagonists in reducing the maximal response to a partial agonist will vary with the partial agonist. The extent to which the partial agonist acts as an uncompetitive antagonist should be visible in terms of (a) a shift to the right of the response-log dose curve to a full agonist, by the partial agonist, that is not occluded by a true competitive antagonist (see Eq. 3.2) and (b) a reduction of the maximal response to full agonist, by the partial agonist, after elimination of receptor reserve by an irreversible competitive antagonist (also see Eq. 3.2).

6. COOPERATIVE MODELS

The fact that real response-dose curves often do not fit a rectangular hyperbola may sometimes be related to complexity in the effector chain, but in many cases it is now clear that the source of complexity is at the level of agonist-receptor interaction. For nicotinic receptors there exists very convincing evidence that two agonist molecules must cooperate to produce the open-channel state (48, 49). Response-dose curves for a number of probable neurotransmitters similarly show Hill coefficients greater than unity (e.g., refs. 50–52) when tested in a manner where the measured response is related by a known function to ionic channel opening.

Introduction of cooperativity gives rise to a general equation, corresponding to Eq. 2.3, of the form

$$A_nR = R_t/[1 + u + (1 + c_1)K_1/A + (1 + c_2)K_2/A^2 + \cdots + (1 + c_n)K_n/A^n]$$

where u, c_1, c_2, $\cdots$, c_n are polynomial functions of concentrations of antagonists. The complexity of this equation does not alter the main conclusions reached above, namely, that blockade within the effector chain may mimic mixed competitive and uncompetitive action at the receptor level, and that a mainly uncompetitive action may produce the same kind of shift of dose-response curves as would a competitive irreversible antagonist when there is low receptor reserve, i.e., resemble classical noncompetitive blockade despite the existence of substantial receptor response. It is notable that the use of the Furchgott relationship (Eq. 2.4c) in conjunction with an irreversible competitive antagonist will in general give q_a close to $q^{1/n}$, where q_a is the apparent and q the true fraction of receptors remaining when n molecules of agonist activate the receptor. If the irreversible antagonist binds equally to all agonist binding sites, then q_a is in fact the fraction of binding sites remaining unoccupied. With regard to receptor reserve, the two methods determining e (see Section 2) will give slightly different values for e_a (apparent e) such that $e_a + 1 \simeq (e + 1)^{1/n}$ or $e_a + 2 \simeq (e + 2)^{1/n}$, respectively; i.e., by both methods $e_a \simeq e^{1/n}$. It can be argued that $1/(2 + e_a)$ does indeed represent (fairly closely) the fraction of receptor binding sites (as distinct from receptors) that are occupied by agonist when response is half-maximal, provided the agonist binds independently to the binding sites.

It may also be pointed out that a good fit to a simple rectangular hyperbola of response-dose data (e.g., Fig. 2E) does not exclude the existence of cooperativity in agonist-receptor interaction.

In general, all of the equations in Sections 2–5 have their counterparts for models in which cooperativity is incorporated. To the extent that there exist different models of cooperativity, and whether or not more than one antagonist molecule may attach to receptor, these equations may take different forms (53). For example, a system corresponding to the reversible cycle scheme considered in Section 3, but with two agonist molecules necessary to activate receptors, is

$$\begin{array}{ccccccccc} 2A + R & + D \rightleftharpoons & A + AR & + D \rightleftharpoons & A_2R & + D \overset{L}{\rightleftharpoons} & A_2R^* & + D \\ \downarrow & & & & & & & \\ 2A + RD & \rightleftharpoons & A + ARD & \rightleftharpoons & A_2RD & \swarrow & & \end{array}$$

Here we distinguish A_2R, an inactive form, from A_2R^*, the activated receptor, in order to account at least in part for partial agonism. With the simplifying assumption that sites normally involved in receptor activation have always the same affinity for agonist (K_a^{-1}) and for competitive inhibitor (K_I^{-1}), one obtains the equation

$$(A_2R^*/R_t)^{-1} = 1 + (1 + D/K_d)[1 + (K_a/A)(1 + I/K_I)]^2/L \quad (6.1)$$

In Fig. 3, the observed data of Podleski (7) for the apparent noncompetitive action of (+)-tubocurarine on responses of electroplax to decamethonium is fitted to the above equation, with $D = A$ (see Section 5), together with Eq. 2.5a to take into account the relationship between conductance and measured response (depolarization). The fitting parameters used were $K_I = 10^{-7}$ M, $K_a = 3.3\ \mu M$, $K_d = 6.6\ \mu M$, $V_{max} = 90$ mV, $L = 2.4$, and $(g'R_t/G) = 3.25$. The last value is observed maximal conductance to carbachol, relative to resting conductance, in electroplax (54).

Rather better fits to these data could be obtained by allowing the possibility of negative or positive cooperativity in binding, in particular, by ascribing to the ARD form an increased affinity for the competitive inhibitor. However, it was unclear whether the improvement arose merely from the availability of more parameters that could freely be adjusted. Nevertheless, it has indeed been observed in binding studies that a local anesthetic (prilocaine) can increase the affinity of nicotinic receptors for dimethyltubocurarine (55).

7. DISCUSSION

In recent years it has become apparent that the activated state of a receptor represents a target for drug action distinct from the resting state, presumably because the conformational changes associated with effector function result in the exposure of new binding sites for drugs. When drug binding of this kind prevents receptor function, the result will be blockade. At nicotinic cholinergic receptors a diversity of "nonspecific" agents, including local anesthetics (e.g., ref. 28), have been shown to act in this way. Formally, this kind of blockade is equivalent to simple "uncompetitive" antagonism, if the blocking drug combines only with the activated state of the receptor and dissociation of the antagonist returns the receptor to the active state. In principle, the presence of receptor reserve should not disguise the nature of such

an antagonism, but will reduce the apparent potency of the antagonist. The rather small shift of apparent affinity produced by an uncompetitive antagonism (see Fig. 1B) may account for the virtual absence of any reports of "uncompetitive" shifts of response-agonist curves in pharmacology. With models of uncompetitive antagonism modified so that the agonist-receptor-antagonist complex can dissociate with loss of agonist prior to loss of antagonist, the equilibrium responses to agonist are altered in a manner corresponding to a mixture of uncompetitive and competitive antagonism, which is also characteristic of the type of blockade to be expected with "functional" antagonism, i.e., an antagonism exerted at some point in the effector system between the activation of receptor and measured response. Because of their resemblance, these types of antagonism might well be lumped together as "effector blockade"—uncompetitive inhibition is then the special case where a drug acts on the activated "effector state" of the receptor. Depending upon the extent of receptor reserve and the particular parameters (rate constants) of the system, effector blockade may mimic classical competitive or noncompetitive inhibition. In particular, effector blockade may give the appearance of classical noncompetitive antagonism even when, as a consequence of receptor reserve, a true noncompetitive (or irreversible competitive) antagonism of equivalent degree would not reduce the maximal response to agonist. Since examples of this exist in the literature for a variety of receptor systems, it would appear likely that effector blockade may be a common phenomenon which, given the prevalence of receptor reserve in biological systems, should be suspected whenever a drug produces a reduction of maximal response without producing a large change in the EC_{50} of an agonist. The kinetic equations indicate that effector blockade may generally be distinguished from true competitive or noncompetitive blockade by examining the interaction with known competitive and irreversible competitive antagonists. With some models the kinetic equations also predict that the apparent potency of an effector blocker acting on a particular receptor system may not be independent of the agonist against which it is tested. Going one step further, it becomes conceivable that a receptor system may or may not be blocked by an antagonist, depending upon the agonist used to produce a response. Thus, the existence of distinct agonist-antagonist pairs need not in itself imply the existence of distinct receptors, even when the antagonists superficially appear to act competitively.

The concept of effector blockade has interesting consequences if one accepts as a general proposition that some agonists can themselves act as effector blockers, as is the case for decamethonium at the nicotinic receptor (46). Not only can this give an at least partial explanation for the phenomenon of partial agonism, but it predicts that purely competitive antagonists may reduce the maximal response to a partial agonist and thereby give the appearance of acting noncompetitively. Just such a phenomenon evidently does indeed exist with respect to both nicotinic receptors (9) and is possible with respect to muscarinic receptors (7, 8). Thus, to the caveat that the appearance of classical competitive antagonism is no guarantor of a competitive mechanism, because of receptor reserve, one must add the caveat that an action to reduce the maximal response to an agonist does not preclude the possibility that the antagonist is in fact acting only competitively.

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