RECEPTOR MECHANISMS[1,2]

By Robert F. Furchgott[3]
Department of Pharmacology, State University of New York, Downstate Medical Center, Brooklyn, New York

Introduction

It is common practice to interpret the effect of a given drug on a given effector system in terms of an interaction between the drug and a specific receptive substance or receptor of the cells of the effector system. Such interpretations, especially when they attempt to account for the quantitative relationship between effect and dose (or concentration) of drug, may be said to be interpretations on the basis of "receptor mechanisms." It must be admitted immediately that, with rare exceptions, we can neither identify the receptor as an individual chemical entity nor study the primary chemical or physical change which occurs when drug and receptor interact. Nevertheless, there is impressive indirect evidence, particularly from studies on the quantitative aspects of drug antagonism, for the validity of the concept that receptors are specific and real [Schild (1)].

That the term "receptor" has different connotations, even for pharmacologists, is apparent from the discussion of the use of this term at a recent symposium (2). The term receptor as used in this review, will indicate the postulated specific molecular sites or structures in (or on) an effector cell with which molecules of a specific agonist must react in order to elicit the characteristic response of the cell to the agonist. There may be other sites within a cell which bind molecules of the agonist, and some of these sites may have specialized functions (e.g., enzymatic removal, storage, or transport of the agonist) which modify the concentrations of agonist reaching the receptors, but these sites should not be confused with the receptors proper. The term "drug acceptor" has been suggested as a general term to cover various types of binding sites for a drug (3)—with a receptor being a very distinct type of acceptor.

It would be impossible in a review of this size to cover the recent literature concerned with receptor mechanisms for all classes of drugs. Consideration

[1] The survey of literature pertaining to this review was concluded in June, 1963.

[2] Abbreviations which are used: AcCh (acetylcholine); CNS (central nervous system); DCI (dichloroisoproterenol); DFP (diiso-propylfluorophosphate); DMPP (dimethylphenylpiperazine); EPI (epinephrine); 5-HT (5-hydroxytryptamine); ISO (isoproterenol); log (logarithm to the base 10); NE (norepinephrine).

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will be largely limited to papers bearing on receptor mechanisms for autonomic drugs, histamine and 5-HT on effector systems outside of the CNS. Recent developments in the general theory of receptor mechanism, based largely on the study of such drugs on such systems, will receive major emphasis in this review. Because of this emphasis, and because this is the first article on receptor mechanisms to appear in the *Annual Review of Pharmacology*, it will be necessary, for the purpose of intelligible discussion, to refer to many papers which appeared prior to the last few years.[4]

Agonists and Partial Agonists

Agonists.[5]—In simple classical theory (4, 5) of receptor mechanisms it was assumed that the following relationship held in the case of any agonist, A, acting through combination with the same type of receptor in a given effector system:

$$E/E_m = [RA]/[R_t] = 1/(1 + K_A/[A]). \qquad 1$$

Here the effect, E, is proportional to concentration of receptor-agonist complex, $[RA]$; is equal for the same $[RA]$ regardless of the agonist; and reaches the same maximum, E_m, when $[RA]$ is essentially equal to the total receptor concentration, $[R_t]$ (i.e., when $[A]$ greatly exceeds K_A, the dissociation constant of $[RA]$). The assumptions in this formulation were seriously challenged independently by Ariëns and colleagues (6 to 9), Stephenson (10), and Furchgott (4). It was clearly shown that within a series of agonists acting on the same receptor in the same biological preparation, the maximal effect obtainable with some was below that obtainable with others. To allow for this variation, Ariëns introduced the concept of intrinsic activity, α, so that

$$E_A = \alpha[RA] = \alpha[R_t]/(1 + K_A/[A]) \qquad 2$$

in which E_A is the effect produced by a given agonist, and α may vary from 1 for a full agonist to 0 for a pure competitive antagonist. For two different

[4] Unfortunately, in the literature on general theory of receptor mechanisms, there is no standardized use of symbols. For the sake of consistency in this review, it has been necessary to select arbitrarily a set of symbols. Therefore the symbols used here will often be different from those used in the papers referred to. In particular, it should be noted that the dissociation constant for a drug-receptor complex is used, rather than the affinity or association constant, which is its reciprocal.

[5] Numerous examples can be given of drugs which cause activation of receptors of a given type, not by directly reacting with such receptors, but by somehow causing within the biological test system the liberation, formation or accumulation of a second substance which, then, activates these receptors (4). In so far as the specific receptor system under study is concerned, such drugs are best designated as "indirectly acting drugs rather than agonists. Some drugs appear to activate receptors in a single type of effector cell both by direct and indirect action. The pharmacologist attempting to obtain quantitative data to test some theory of receptor mechanism should always be aware of the possibility that the "agonist" which he is using may exert part or all of its effects by an indirect action.

agonists, A_1 and A_2, with intrinsic activities of α_1 and α_2, the ratio of effects for the same $[RA]$ would be $E_{A1}/E_{A2}=\alpha_1/\alpha_2$.

The original formulation of Ariëns still allowed response to be proportional to concentration of receptor-agonist complex for a given agonist. On the basis of experiments on irreversible competitive antagonism by β-haloalkylamines (4), and experiments on the relative effectivenesses and interactions of different alkyltrimethylammonium compounds (10), the concept of proportionality between E and $[RA]$ was abandoned as one of general applicability. Stephenson (10) proposed the following:

$$E = f(S) = f(e[RA]/[R_t]) = f\{e/(1 + K_A/[A])\} \quad 3$$

where effect is some positive function of the stimulus, S, which, in turn, is equal to the efficacy, e, times the fraction of total receptors occupied by the agonist. With a given type of receptor, e will vary depending on the agonist used. In such a system, it is assumed that essentially the maximal effect attainable by activation of receptors of this type will be reached when S reaches a certain value. If with a given agonist, e is too small for S to reach this value even when $[RA]/R_t]$ approaches 1, then the actual maximum will fall short of the potential maximum. (If e is zero, one has a pure competitive antagonist.) On the other hand, if e is sufficiently high for S to attain this value when $[RA]/R_t]$ is significantly below 1, then the fraction of receptors unoccupied by the agonist at this point may be considered as "spare receptors." Furchgott (4) had also pointed out that in a theoretical approach to account for the shift of log concentration-effect curves in the face of progressive inactivation of receptors by irreversible competitive antagonists, there could be a marked decrease in the fraction of "total receptors still active" without a significant decrease in maximal response.

In recent papers (11 to 13) van Rossum & Ariëns have used a formula rather similar to that of Stephenson (10), namely:

$$E_A/E_m = f(S_A/S_m) = f(\alpha[RA]/[R_t]). \quad 4$$

Here, E_A/E_m is the effect relative to the maximal effect to be obtained with the biological object, and S_A/S_m is the relative intensity of the stimulus. α is now permitted to exceed unity in some situations.

The problem of spare receptors and of the nature of the function relating stimulus and effect will be considered further in later sections of this review, as will be the approach to differences in potencies of agonists on the basis of "rate theory" (14).

Partial agonists.—If two agonists act on the same type of receptor, and one can give the maximal effect obtainable, E_m, while the other, even when saturating the receptors, can give only a maximal effect $E_{A_m} < E_m$, then the latter, by competing for the receptors, can act as an antagonist of the former. This fact was first fully appreciated by Ariëns and colleagues (15, 16), who, therefore, called an agonist of the latter type a drug with "dualism in action." Stephenson (10) called such a drug a "partial agonist," and since this simpler

term has gained wider acceptance, it will be used here. Ariëns and colleagues developed theoretical equations based on mass-action equilibria for the interactions of agonists and partial agonists (the latter having intrinsic activities less than 1 but greater than 0), and showed a number of experimental examples which fitted well with theory.

In the past few years the group at Nijmegen has given many more examples of partial agonists (17 to 25). Their general approach has been to compare the activities of an active agonist and a series of its derivatives with progressive alterations in chemical structure (e.g., progressive elongation of an alkyl side-chain at one position, or step-wise replacement of methyl groups by ethyl groups on a quaternary N). In such a series there is usually a progressive change from strong agonists to competitive antagonists. In the middle of many series there can be found compounds which behave as partial agonists. Series have been studied which act on cholinergic receptors in parasympathetic effectors (17, 18), in ganglia (19, 20), and in skeletal muscle (21); on histamine receptors (22); and on adrenergic receptors of both α- and β-types) (23, 24). In studies on these series many interesting correlations and conjectures about structure-activity relationships have been made (25), but since this is a subject beyond the scope of the present review, the reader is referred to the original articles for contributions to this subject.

Van Rossum (18) found that on certain parasympathetic effector organs pilocarpine behaved as a partial agonist, as compared with AcCh and furtremethonium ("full" agonists), and suggested that some of the conflicting reports in the literature on the effects of pilocarpine may be due to its partial agonistic properties. He also showed that the tertiary analogues of certain quaternary parasympathomimetics behaved as partial agonists (18).

The moderate stimulatory effects of dichloroisoproterenol (DCI) on heart at dose levels generally used to produce blockade of cardiac adrenergic β-receptors, have been attributed by a number of workers to a partial agonistic effect of this drug on these receptors (26 to 28). On the other hand, the partial inhibition of bronchial smooth muscle tone by DCI, when it is used to block adrenergic β-receptors in this tissue, has been ascribed to a nonspecific inhibition of contraction rather than to its action as a partial agonist (24, 29).

Drug Antagonism

Competitive antagonism.—In classical competitive antagonism the receptor-antagonist complex, like the receptor-agonist complex, can dissociate (i.e., the antagonism is "reversible"). In antagonism of this type, theoretical application of mass action equations to equilibrium conditions show that the sigmoidal log concentration-effect curves of the agonist in the presence of an antagonist (or combination of antagonists) should remain parallel to the sigmoidal log dose-effect curve in the absence of antagonist, regardless of the degree of shift along the log-dose axes. This is so whether the effect is a linear or nonlinear function of the concentration of receptor-agonist complex, as

long as the same concentration of the complex produces the same effect with or without competitive antagonists present (4, 15, 30). The degree of the parallel shift along the log concentration axis should be given by log $(1+[B]/K_B)$, where $[B]$ is the concentration of antagonist and K_B is the dissociation constant of the receptor-antagonist complex. Many experimental examples of extensive parallel shifts of log concentration-effect curves for different agonists in the presence of competitive antagonists are available in recent papers (13, 16 to 19, 21, 22, 32, 33).

The use of pA_x values, first introduced by Schild (31), has continued to prove helpful in the analysis of competitive antagonism. (The pA_x value for a given agonist-antagonist pair is the negative log of $[B]$ at which the ratio of equiactive doses of agonist in the presence and absence of antagonist is x.) If the receptor-agonist complex is bimolecular, it can be shown that

$$\log (x-1) = \log 1/K_B - npA_x \qquad 5$$

where n is the number of antagonist molecules in the complex with receptor. A plot of log $(x-1)$ against pA_x should give a straight line of slope $(-n)$, which intersects the pA_x axis at a point corresponding to pA_2. If $n=1$, then $-\log K_B = pA_2$, and $pA_2 - pA_{10} = 0.95$. Although the $pA_2 - pA_{10}$ difference was originally used to test for competitive antagonism, a plot of log $(x-1)$ against pA_x for a wide range of antagonist concentrations is preferable (32). This is particularly true in situations where there is a large component of "spare receptors" (*vide infra*); for in such situations the results with certain types of noncompetitive antagonists (11), and even those with irreversible competitive antagonists (4, 11, 12, 34, 35), may sometimes resemble those expected with a reversible competitive antagonist, if concentrations of antagonist no greater than those equivalent to pA_{10} are tested. Arunlakshana & Schild (32), Jenkinson (36), Paton (14), and Ariëns & Simonis (33) have presented plots of log $(x-1)$ against pA_x (or $-$ or $+$ log $[B]$) for competitive antagonism at cholinergic and histaminergic receptors in isolated muscle preparations, in which the maximal concentrations of antagonist used have been equivalent to pA_x values in the range of 100 to over 1000. The plotted points fit remarkably well along straight lines with slopes extremely close to unity. As pointed out by van Rossum & Ariëns (12), a slope of unity is consistent with a bimolecular reaction for agonist and receptor and for antagonist and receptor, and thus provides evidence for a 1:1 agonist-receptor complex, even when the shape of the dose-response curve with the agonist gives no such evidence.

If pA_x values (or apparent K_B values) are accurately determined by the method of Schild (30, 32) or by some other satisfactory method (37), using a wide range of concentration of the competitive antagonist, they can help in the classification of drugs according to the receptors on which they act in a given tissue. If different agonists give the same pA_x values for the same antagonist on a given tissue, it is strong provisional evidence that they act on a

common receptor. By this criterion histamine, pyridylethylamine and pyrazolethylamine act on the same receptors in guinea pig ileum, since they give the same pA_x values with the same antihistamines (30, 32). Other examples of classification of drugs by this procedure are available in the literature (17, 36). Comparison of receptors in different tissues which are activated by the same agonist can also be made by comparing pA_x or K_B values for a common antagonist (30, 37). Jenkinson (36) found very similar values for K_B for *d*-tubocurarine as an antagonist of AcCh in both slow muscle fibers and twitch muscle fibers of the frog, and was able to conclude that the cholinergic receptors were similar in both types. (This is a particularly striking example since contracture was measured in the first type, and end-plate depolarization in the second). Schild & Arunlakshana (32) pointed out the remarkably similar pA_x values for atropine against receptors for AcCh in preparations as varied as frog heart, chick amnion, guinea pig lung, and mammalian intestine. As might be expected, the pA_x values in frog rectus muscle were different.

Furchgott (4) has cautioned that the K_B estimated even under "steady-state" conditions, is not, strictly speaking, the true K_B since it is calculated on the basis of concentrations of antagonist in the extracellular fluid (aqueous phase) rather than in the region of the tissue containing the receptors (biophase). It may sometimes be equal to the true K_B times the "partition coefficient" for the antagonist between the two phases. Jenkinson (36) has also pointed out that if the receptor can also combine with cations of the bathing fluid in competitive fashion, the estimated K_B may differ from the true K_B. If M is considered as one such cation which associates with the receptor to form the complex RM, then a modified form of equation (5) results, namely:

$$\log (x - 1) = \log \frac{1}{K_B(1 + [M]/K_M)} + \log pA_x \qquad 6$$

where K_M is the dissociation constant of RM. The estimated constant would then be $K_B(1+[M]/K_M)$, and would approach K_B only if $[M]/K_M \ll 1$.

Noncompetitive antagonism and autoinhibition.—A noncompetitive antagonist can be broadly defined as a drug which antagonizes the response to an agonist by reacting with some site other than the site on the receptor with which the agonist reacts. In 1955 this reviewer stated that there was no experimental evidence for either reversible or irreversible noncompetitive antagonism (4). Since this statement has been challenged (21), it may be appropriate to indicate that the statement referred to antagonism in which inactivation of a specific receptor is produced by the antagonist combining or reacting with a site on the receptor (molecule or molecular complex) other than the site with which the agonist combines (analogous with noncompetitive inhibition of enzymes). No convincing evidence for this type of antagonism, which may better have been referred to as "specific" noncompetitive antagonism, yet exists to my knowledge. Examples of noncompetitive an-

tagonism in which the site of action of the antagonist is not on the specific receptor, have been plentiful. In this type of unspecific noncompetitive antagonism different agonists acting on different receptors are blocked equally well by the antagonist. For reversible noncompetitive antagonism (assumed to be unspecific unless proven otherwise), various approaches have been used for quantitating the potency of the antagonist. Schild & Arunlakshana (32) proposed the use of a pA_h value, where pA_h is defined as the negative logarithm of the molar concentration of antagonist which reduces the maximal effect obtainable with the agonist to one-half. Ariëns and colleagues (22, 38) assign pD_2' values to reversible noncompetitive antagonists, by use of the equation

$$pD_2' = pD_x + \log (x - 1) \qquad 7$$

where x is the ratio of the response (usually the maximal response) to a given dose of the agonist in the absence and presence of a concentration of antagonist, the negative logarithm of which is pD_x'. If one were dealing with specific noncompetitive antagonism and there were, as in classical theory, proportionality between response and concentration of drug-receptor complex, the calculated pD_2' would be independent of agonist concentration and antagonist concentration and would be a measure of the negative logarithm of the dissociation constant (K_B) of the antagonist-receptor complex (30, 38); but actual situations appear to be far from this neat theoretical situation (32).

In some reported cases of reversible noncompetitive antagonism, pD_2' does appear to be practically independent of the dose of agonist (e.g., certain antagonists against cholinergic agents on frog rectus muscle (11, 21, 38), and papaverine against NE on rat vas deferens (11)). However, in most reported cases of noncompetitive antagonism the pD_2' value decreases as the agonist concentration increases. This variation is most likely attributable to a nonlinear relationship between receptor-agonist complex and effect, and the presence of "spare receptors" (11). Arunlakshana & Schild (32) have derived an equation for calculating the K_B value of a complex between a noncompetitive antagonist and a receptor, which is independent of the relationship between receptor occupation by agonist and response. However, in applying this equation to noncompetitive antagonism of AcCh by cinchonidine in guinea pig ileum, they obtained such variable values for K_B that they doubted that "true noncompetitive antagonism" could be assumed.

Van Rossum & Ariëns (21) found that sufficient elongation of alkyl chain-length in certain derivatives of quaternary ammonium agonists for frog rectus muscle led to the formation of compounds with strong noncompetitive antagonist properties on this same muscle. Ariëns & Simonis (22 to 24), have also shown that derivatives of NE and sympatol or of pyridylethylamine (a histamine-like agonist) in which large alkyl or alkylaryl groups have been attached to the amino N, behave as noncompetitive antagonists. This behavior appears to be associated with the strong "surface" activity of the

derivatives. Compounds have also been found which behave as competitive antagonists at lower concentrations and as noncompetitive ones at higher concentrations (18).

Intermediate derivatives in some of the homologous series studied by the Nijmegen group on frog rectus muscle had the properties of agonists or partial agonists exhibiting "autoinhibition." With increasing concentration of such derivatives there was first an increase in response and then a decrease, so that the resulting log concentration-effect curves were, more or less, bell-shaped (21, 39). This autoinhibition has been attributed to a noncompetitive antagonism at a second site by these compounds, counteracting their own agonistic action at the receptor site, and comparisons have been made between the actual curves and curves to be expected on the basis of mass action theory. Somewhat similar curves have been obtained with certain cholinergic ganglionic stimulants, including nicotine and dimethylphenylpiperazine (DMPP), on guinea pig ileum (20, 40). However, analysis of the shift of the bell-shaped log concentration-effect curve of nicotine by the competitive antagonist, hexamethonium, would indicate, according to van Rossum (20), that autoinhibition in this case is not due to noncompetitive antagonism at a second site. He criticized the conclusion of Feher & Borki (41) that autoinhibition of stimulation of the superior cervical ganglion with high doses of AcCh is due to occupation of the receptor by a second molecule of the agonist (in analogy with the concept of autoinhibition of specific cholinesterase by AcCh).

Bijlsma et al. (42) have carefully studied the autoinhibition exhibited by valerylcholine on the guinea pig intestine. They provide convincing evidence that the contractile response to this agent results from stimulation of intramural ganglia, leading to release of AcCh which acts on the smooth muscle parasympathetic receptors, and that the autoinhibition at high concentrations is due to a successful competitive antagonism at these receptors by valerylcholine itself.

Irreversible competitive antagonism or nonequilibrium antagonism.—The terms "irreversible competitive antagonism" (4) and "nonequilibrium antagonism" (35) have both been used to designate the type of antagonism exerted by certain β-haloalkylamines (dibenamine and phenoxybenzamine being well-known examples) against drugs acting on a number of different types of receptors. Nickerson (35) has reviewed the evidence for differentiating this type of antagonism from that of classical competitive antagonism. In this type of antagonism the final complex formed between receptor and antagonist does not reversibly dissociate (probably because linkage is through covalent bonds) (43 to 46), and therefore mass action equilibrium, such as is assumed to occur in classical competitive antagonism, is not possible. Evidence for the "competitive" nature of the antagonism, despite its irreversibility once established, was provided by Furchgott (47) in "protection" experiments on rabbit aortic strips. When epinephrine (EPI), 5-hydroxy-

tryptamine (5-HT), histamine or AcCh were present in very high concentration during an exposure to dibenamine, there was partial protection against blockade of response for the agonist used but not for the others. Also, "cross-protection" against irreversible dibenamine blockade was demonstrated in the case of a series of sympathomimetics (EPI, NE, ISO, and phenylephrine), indicating a common receptor for their stimulatory action (47). Finally a reversible competitive antagonist against a given agonist could protect against irreversible dibenamine blockade of the response to the agonist.

In two recent papers Ariëns and colleagues (11, 33) have given interesting examples of the capacity of reversible competitive antagonists to protect specific receptors against irreversible dibenamine blockade on other biological preparations. One of the protecting agents for the adrenergic α-receptors was *d*-isoproterenol, which, unlike its *l*-isomer, is not an agonist on such receptors, but a pure competitive antagonist (24, 48).

The use of "cross-protection" experiments against irreversible phenoxybenzamine blockade has been applied by Innes in an attempt to differentiate receptors in certain smooth muscle preparations (49, 50). On rabbit aortic strips, dog retractor penis, and rabbit uterus, EPI as well as ergometrine in high doses protected against blockade of contractile responses to themselves as well as to ergotamine (49). High doses of AcCh, histamine, and 5-HT afforded only specific self-protection. These findings, along with other pharmacological evidence, allowed Innes to conclude that the contractile responses to ergotamine and ergometrine in these preparations were due to activation of adrenergic α-receptors. (It would be interesting to repeat such experiments on preparations depleted of catecholamines by reserpine treatment in order to ascertain whether the activation of adrenergic receptors by these ergot alkaloids is a direct action or an indirect one mediated by released catecholamines.) In studies on isolated strips of cat spleen high doses of EPI and 5-HT gave "self-protection" as well as "cross-protection" against phenoxybenzamine blockade (50). Tyramine behaved similarly to 5-HT. On strips from reserpine treated animals, high doses of 5-HT and tyramine still gave protection of EPI, even though the stimulating potencies of these two agents were markedly reduced by such treatment. On the basis of these findings and others, Innes concluded that 5-HT produces its contractile effect on cat spleen through a "dual action, viz., a major action due to release of stored noradrenaline and a minor direct action on adrenaline receptors." Although his evidence is convincing for release of NE (indirect action) and for an appreciable occupation of adrenergic receptors by 5-HT at high concentrations, this reviewer is doubtful as to whether this occupation leads to any contractile response. The possibility does not seem to have been excluded that the response with very high concentrations of 5-HT in reserpinized preparations was due to an action of this drug on receptors other than adrenergic receptors (51).

Waud (52) has recently criticized the use of "cross-protection" experiments for differentiating receptors. He points out that: (*a*) the agonist used may have some affinity for receptors other than the specific type through which it initiates a response (for possible experimental examples, see (17, 47)); and (*b*) an agonist in sufficient concentration may still elicit a marked response even when a major fraction of its receptors are irreversibly blocked (see section on "spare receptors"). He argued that partial protection of response to a second agonist by a first agonist is not convincing evidence that both elicit responses through a single type of receptor. His criticism is well founded. However, to this reviewer, it appears that the cross-protection method is still useful in differentiating receptors if it is used quantitatively, and with an understanding of possible pitfalls.

Bevan et al. (53) reported that the contractions produced by excess K^+ on the rabbit aortic strip were markedly antagonized following exposure of such a strip to high concentrations of phenoxybenzamine for one hour. They demonstrated that this antagonism was not due to a nonspecific depression of the smooth muscle (angiotensin still retained practically its full stimulating activity) nor to the irreversible blockade of adrenergic α-receptors.

Although phenoxybenzamine and related β-haloalkylamines do not block adrenergic β-receptors in cardiac muscle, they do appear to react in an essentially irreversible manner with certain sites which are involved in the uptake and storage of NE in this tissue (54).

According to classical theory (see section on *Agonists*), a given decrease in active receptors should produce a proportionate decrease in maximum and in slope of the conventional sigmoidal log concentration-effect curves, but no shift of the curves along the log concentration axis. However, progressive inactivation of receptors by β-haloalkylamines leads to a shift as well as to a decrease in maximum and slope, and the shift is often appreciable before there is any detectable decrease (4, 34). This shift provides decisive evidence against the concept that effect is proportional to the concentration of the receptor-agonist complex, and for the concept of "spare receptors."

Efficacy, Intrinsic Activity and Spare Receptors

In the section on *Agonists* the related equations of Stephenson (10) and of Ariëns and co-workers (11 to 13), were presented, in which effect, E, is an undefined function of the stimulus, S, which in turn is equal to the fraction of receptors occupied by the agonist ($[RA]/[R_t]$) times a constant (efficacy, e, or intrinsic activity, α) for the particular agonist.

If E is some undefined nonlinear function of S, how does one determine the relationship between E and S (including the value of S which first gives an essentially maximal effect, E_m), and how does one separately evaluate e (or α) and K_A for the agonist? Stephenson, in comparing dose-effect curves of a homologous series of alkyl trimethylammonium ions, arbitrarily set S as unity for E equal to one-half of the E_m obtainable with butyltrimethylam-

monium, the most potent "full" agonist of the series.[6] Then, making the assumption (probably valid) that over the low concentration range required to attain E_m with this agonist, $[RA]/[R_t]$ (and, therefore, S) was proportional to the concentration, he obtained a graphical presentation of E as a function of S. His method of evaluation of e and K_A for partial agonists in the series was fairly convincing; but his evaluation of e for the full agonists depended on unproven assumptions about the relationship between alkyl chain length and K_A, and was therefore open to criticism (11), as he himself admitted.

Van Rossum & Ariëns (12) have discussed the possibility of a nonlinear relationship between $[RA]$ and E and the existence of spare receptors (or a "receptor reserve") in two different theoretical situations. In the first they assume an all-or-none response for each individual unit of the effector system, which occurs when the stimulus (S_A/S_m) for that unit reaches a critical value, τ; and biological variation for the assembly of units so that there is a frequency distribution of τ, with $\bar{\tau}$ being the mean and σ the standard deviation. The slope of the concentration-effect curve depends on σ, and a threshold should exist. In the second situation these workers (12) assume a chain of intermediate steps between the primary stimulus (S_A/S_m) and the final step which is responsible for the observed effect. Each intermediate step produces a stimulus, the value of which determines the value of the stimulus produced by the next step. E_A becomes a monotonous nonlinear function of the primary stimulus. The slope of the dose-effect curve depends on the nature of the functions relating successive steps and on the number of steps. In both theoretical situations, if the intrinsic activity, α, is sufficiently high for a given agonist, a stimulus level capable of eliciting an essentially maximal effect, E_m, may be attained with only partial occupation of receptors.

To evaluate the relative values of α for a series of agonists acting on a type

[6] In a special case E may be a hyperbolic function of $e[RA]$, as follows:

$$E/E_m = e[RA]/(1 + e[RA]).$$

In this case, if S is equated with $e[RA]$, then E/E_m is equal to 0.5 when S equals 1. If $[RA]$ is related to $[R_t]$ by the mass-law equation,

$$[RA] = [R_t]/(1 + K_A/[A]),$$

then it can be shown that:

$$\frac{E}{E_m} = \frac{[A]e[R_t]/(1 + e[R_t])}{K_A/(e[R_t] + 1) + [A]}$$

E/E_m is, therefore, still a hyperbolic function of $[A]$. If $[R_t]$ is arbitrarily given a value of unity and e is much greater than unity, the maximal effect, E_{Am}, obtainable with A, is essentially the same as E_m. If e were equal to 1000, then E would be 0.5 E_{Am} when $[A]$ was only 0.001 K_A (occupation of 0.1 per cent of receptors), and 0.99 E_{Am} when $[A]$ was only 0.1 K_A (occupation of 9 per cent of receptors); and an inactivation of 99 per cent of the original receptors would only cause a 9 per cent fall in E_{Am}, along with a shift of the log dose-effect curve by 2 log units.

of receptor which can be blocked irreversibly by an antagonist such as dibenamine, van Rossum & Ariëns (12) obtain cumulative dose-effect curves for each agonist following successive incubation periods during which the biological test system is exposed to a fixed concentration of the antagonist. They assume that the inactivation obeys first-order kinetics, so that the fraction of total receptors still in active form after a total incubation time of t, is given by e^{-Bkt}, where B is the concentration of antagonist and k is a rate constant. They estimate the total incubation time required to reduce the maximal possible effect with each agonist to the same submaximal level, namely, 0.5 E_m. From their equation relating S_A/S_m to $\alpha[RA]$, it is then possible to show that:

$$\alpha_1 : \alpha_2 : \alpha_3 \cdots = e^{Bkt_1} : e^{Bkt_2} : e^{Bkt_3} \cdots \qquad 8$$

where each corresponding α and t refer to the intrinsic activity and required incubation time for reduction of maximum for a given agonist. (Unfortunately, in the paper cited (12), the above equation was written without the Bk term.) To obtain relative intrinsic activities it is necessary to find Bk (or $1/t_a$, where t_a is the time constant and the reciprocal of Bk) and insert it into the equation. Van Rossum and Ariëns show how they estimate t_a from their experimental data. However, to this reviewer it appears to be an erroneous method. Moreover, even when using it with the authors' data, I obtain different relative intrinsic activities than those reported by the authors for a series of parasympathomimetics on rat intestine and a series of histamine-like agonists on guinea pig intestine. Despite this criticism, this new approach for evaluating relative intrinsic activity is interesting and could be quite useful if some suitable procedure for estimating an accurate t_a value were found. To evaluate relative K_A values for different agonists from data obtained in experiments of the type described above, the authors (12) use the relationship:

$$K_{A_1} : K_{A_2} : K_{A_3} \cdots = [A_1] : [A_2] : [A_3] \cdots \qquad 9$$

where $[A_1]$, $[A_2]$, etc. are equiactive concentrations of the agonists, after the maximal effect obtainable with each has been reduced to 0.5 E_m.

Shape of Dose-Effect Curves and Thresholds

The shape of actual dose-effect curves of agonists varies considerably (10, 55, 56). In the usual plot of effect against log dose, the maximum slope is frequently much greater than that predicted in classical receptor theory which has E proportional to $[RA]$ (10). Correspondingly, a plot of $1/E$ against $1/[A]$ (Lineweaver-Burk plot) does not usually give straight lines, but upward curving lines as $1/[A]$ increases (11). Early attempts to explain these curves within the framework of classical receptor theory involved assumptions of receptor-drug complexes of the type RA_n, where n was different from 1 (usually higher). However, with the growing evidence that effect is not proportional to the concentration of receptor-agonist complex, attempts to

explain the shapes of curves by assuming other than a bimolecular complex are now rarely seen. Occasionally dose-effect data for agonists do fit a rectangular hyperbolic curve predicted by classical theory very well, but this is probably fortuitous (4, 10), and K values calculated from such data should not be taken as the dissociation constant, K_A, of the receptor-agonist complex.

The recording conditions will sometimes modify the shape of the dose-effect curve. Paton (14) found that the curves for histamine and AcCh on guinea pig ileum were close to theoretical rectangular hyperbolas when recording was with an auxotonic lever (one in which shortening and tension both increase with increasing contraction) or an isometric transducer; but with an isotonic lever there was an initial "foot" to the curves followed by a slope which was much steeper than in theoretical curves. In contrast to this finding, Rocha e Silva (37) found excellent agreement between experimental and theoretical curves for histamine on guinea pig ileum with an isotonic lever, both in the absence and presence of antihistamines. The reason for this discrepancy is not clear.

A "foot" on a dose-effect curve often indicates a threshold phenomenon. If one extrapolates the curve back to the effect axis, it intersects this axis below the dose axis. According to Kirschner & Stone (57), the distance of the intersection below the dose axis is a measure of threshold stimulus, a stimulus which must be exceeded before any effect manifests itself. In their work on cholinergic agonists and antagonists on frog rectus muscle, plots of $1/E$ against $1/[A]$ did not fit straight lines, but plots of $1/(E+a)$ against $1/[A]$, where a is the estimated threshold stimulus, fitted straight lines very well. Ariëns & van Rossum (11) have applied the same type of correction for threshold in plotting data obtained with cholinergic agents and histamine on intestinal preparations and with NE on the rat vas deferens, and have obtained fairly satisfactory fitting of straight line plots both in the absence and presence of antagonists.

Kinetics of Drug-Receptor Interaction

Del Castillo & Katz studied the time-course of action of drugs on a single end-plate of frog sartorius muscle, using the elegant method of applying the drugs ionophoretically from a micropipette (58 to 61). Their results suggested "that the time course of drug potentials, due to AcCh and carbachol and probably also nicotine and succinylcholine, depend on . . . the kinetics of local diffusion and hydrolysis, rather than on the kinetics of drug-receptor interaction." With respect to decay of antagonism following brief ionophoretic application of *d*-tubocurarine (mean half-decay time of 1.4 sec), these authors favored the idea that it was indicative of slow dissociation of the drug-receptor complex, but acknowledged the possibility that escape of *d*-tubocurarine through a diffusion barrier separating receptors from surrounding bath may have been the rate-limiting step. Goldsmith (62) studied

the action of bath-applied *d*-tubocurarine and erdrophonium on end-plate depolarization produced either by nerve stimulation or AcCh. He concluded that when the rate of perfusion was sufficiently fast, the rate-limiting step for the onset of action of each of these drugs was diffusion to its site of action. Creese et al. (63), using ^{131}I-labeled iodocholinium (which acts like decamethonium) on rat diaphragm, found that the slowly developing "phase II" neuromuscular block was correlated with a slow uptake and concentration of this agent in the preparation, probably in an intracellular location. The rate of development of both block and uptake were slowed in the presence of *d*-tubocurarine. Bevan (64) investigated the time-course of EPI contractions on rabbit aorta strips at different temperatures. He proposed that the rate-limiting step for onset of action was the transfer of EPI molecules across a diffusion barrier to the receptors; and that a certain energy of activation was required for this transfer.

Controversial views have been expressed about the rate-limiting step for the development of and recovery from blockade with reversible competitive antagonists on isolated smooth muscle preparations. The following scheme (4) will help illustrate the point of controversy:

$$\begin{array}{c|c} \text{Aqueous Phase} & \text{Biophase} \\ B_a \underset{k_b}{\overset{k_a}{\rightleftarrows}} & B_b + R \underset{k_2}{\overset{k_1}{\rightleftarrows}} RB \end{array}$$

B_a and B_b, respectively, represent the antagonist in the aqueous (extracellular) phase and biophase (region of tissue containing receptors); and the vertical dividing line can be considered as a diffusion barrier.

If $k_1 \ll k_a$ and $k_2 \ll k_b$, then the *rate-limiting steps are at the level of drug-receptor interaction.* Onset of blockade (formation of RB) is given by (14):

$$[RB]/[R_t] = \frac{[B_a]}{[B_a] + [K_B]} [1 - \exp \{-(k_1[B_a] + k_2)t\}] \qquad 10$$

where $[B_a]$ is the concentration of agonist added at $t=0$, and K_B is equal to k_2/k_1. Offset of blockade (reappearance of free receptors, R_f) is given by:

$$[R_f]/[R_t] = 1 - [RB]/[R_t] = 1 - ([RB]_0/[R_t]) \exp(-k_2 t) \qquad 11$$

where $[RB]_0$ is $[RB]$ at zero time. Both the onset and offset are simple exponential functions of time, but the rate constant for onset is greater than that for offset by the amount, $k_1[B_a]$.

If $k_a \ll k_1$ and $k_b \ll k_2$, then the *rate-limiting step is at the level of the aqueous phase-biophase barrier.* Assuming simple first-order kinetics for diffusion across the barrier, the equation for onset is (4)[7]:

$$[RB]/[R_t] = \frac{(k_a/k_b[B_a]\{1 - \exp(-k_b t)\}}{K_B + (k_a/k_b)[B_a]\{1 - \exp(-k_b t)\}} \qquad 12$$

[7] Equation 12 is obtained by combining equations 11 and 13 of reference (4); equation 13, by combining equations 12 and 13 of the same reference.

And the equation for offset is:

$$[R_f]/[R_t] = \frac{K_B}{K_B + [B_b]_0 \exp(-k_b t)} \qquad 13$$

Some time ago Rocha e Silva & Beraldo (65 to 67) investigated the time-course of the restoration of response to histamine and to AcCh in guinea pig ileum following temporary exposure of the preparation to competitive antagonists. The response to a fixed small dose of agonist at any time after washout of antagonist was expressed in per cent of the control response before antagonist, and it was assumed that this percentage response measured the fraction of free receptors. Using a wide range of concentration with each antagonist, they found that response to the agonist, once it did begin to recover, always recovered along a sigmoidal curve rather than the "expected" exponential one (as predicted by equation 11). They presented an empirical "autocatalytic" equation to explain their results. Since equation 13 gives a recovery curve which is symmetrically sigmoidal once it begins to rise, the present reviewer reanalyzed the results of Rocha e Silva & Beraldo, and found them in rather good agreement with the results predicted by that equation (4). Moreover, these workers (66, 67) had found that if various concentrations of an antagonist were used, the time for recovery of the response of the agonist to 50 per cent of the control (called R_{50}) was a linear function of the logarithm of the antagonist concentration. If percentage response is a fair measure of the fraction of free receptors, then this relationship is also in accord with equation 13, which predicts that:

$$R_{50} = (2.3/k_b)(\log [B_b]_0 - \log k_b). \qquad 14$$

On the basis of the results of Rocha e Silva and Beraldo, as well as on the basis of results of experiments on the time course of recovery of sensitivity of rabbit aortic strips to various agonists following washout of reversible competitive antagonists, this reviewer supported the view that escape from the biophase was the rate-limiting step in recovery. (4).

More recently, Paton (14) in developing his "rate theory" of drug action, reinvestigated the time course of recovery from hyoscine, mepyramine, and atropine blockade in the guinea pig ileum using the "dose-ratio" method. In this method if dr is the ratio of equiactive doses of agonist with and without blockade, then the fraction of blocked receptors, $[RB]/[R_t]$, is given by $(dr-1)/dr$. According to his analyses, recovery of free receptors does, indeed, follow the exponential time course predicted by equation 11. Moreover, he found onset of antagonism to be in agreement with equation 12; and the ratios of calculated values for k_1 and k_2 were in fairly good accord with the dissociation constants ($K_B = k_2/k_1$) found under equilibrium conditions. He criticized the basic assumption of Rocha e Silva & Beraldo (65) that percentage response in their experiments was a measure of free receptors, and suggested that the sigmoidal shape of their recovery curves for percentage re-

sponse reflects the shape of their dose-response curves, since they were recording with an isotonic lever (see preceding section).

Paton has undoubtedly presented a very strong case for dissociation of the antagonist-receptor complex being the rate-limiting step in offset of blockade from long lasting competitive antagonists in guinea pig ileum. This reviewer, however, still feels that the question is not fully settled. In our work with aortic strips the "dose-ratio" method was used and gave results in much better agreement with escape from a biophase being the limiting step (4). Perhaps different tissues vary. Perhaps in some with some antagonists k_2 and k_b are of the same order of magnitude and intermediate type recovery curves will result.[8] The statement of Paton (14) that "a diffusion barrier should yield symmetrical onset and offset curves" is misleading. It is true that the curves for accumulation and for elimination of the antagonist in the biophase would both be exponential with the same time constant, k_b. However, a comparison of equation 12 and 13 indicates that the curves for occupation of receptors during onset and freeing of receptors during offset could be very different; for as $[B]$ is progressively increased relative to K_B, the time during onset for occupation of a given fraction of receptors occupied at equilibrium decreases; although the time during offset for freeing the same fraction of previously occupied receptors increases.

Paton estimates the half-time for dissociation of the antagonist-receptor complex in guinea pig ileum to be about 40 min for both hyoscine and atropine, and about 10 min for mepyramine. If he is correct, then there would be little dissociation during the course of a typical short test (usually less than 1 min) to a strong agonist (e.g., AcCh or histamine). Thus, the potent reversible competitive antagonist would be acting essentially as an irreversible competitive antagonist (a nonequilibrium antagonist) during such a test. If this is so, then the ability of AcCh to give an essentially maximal effect in the presence of hyoscine or atropine at concentrations over $1000 \times K_B$, would indicate that only about 0.0001 of the total receptors are required for such an effect, and about 0.9999 would be "spare receptors" [Schild (1)]. This may be so, but experimental results with irreversible competitive antagonists, such as dibenamine, do not reveal such a tremendous fraction of spare receptors. In progressive blockade with dibenamine, the maximal shift in parallel fashion of log dose-effect curves of the agonist prior to the onset of a progressive decrease in maximum and slope, may be taken as an index of the fraction of spare receptors (see section on this subject). However, when progressive blockade with a potent reversible competitive antagonist is produced by

[8] Paton does point out that when the ileum is exposed to high concentrations of hyoscine for several minutes or more, the time course of recovery from blockade is not a simple exponential one. He attributes this variation to a significant intracellular uptake of the antagonist during exposure, with gradual outward diffusion from intracellular sites providing a high concentration at receptor sites for some time after washout.

raising its concentration, the parallel shift of log dose-effect curves appears to exceed greatly that found on progressive blockade with dibenamine. This "discrepancy" is clearly seen if one compares the reversible competitive antagonists, dihydroergotamine and phentolamine, with dibenamine with respect to their effects on the log dose-response curves of sympathomimetics in rabbit aortic strips (4). Similar "discrepancies" can be observed in the experiments of Ariëns et al. on progressive blockade of parasympathomimetics and histamine on intestinal segments by reversible competitive antagonists and by dibenamine [e.g., compare Fig. 2b of (33) and 7a of (11)]. One might argue that the appearance of a decrease of maximum and slope with dibenamine results from a nonspecific inhibition which is separate from its blockade of receptors, but strong evidence against this possibility is provided by the finding that the decrease in the case of different agonists on the same preparation occurs after different extents of treatment with dibenamine.

The difference in character of progressive blockade with potent reversible and irreversible competitive antagonists is not consistent with Paton's view that half-times for dissociation of receptor complexes with the former are of the order of many minutes. Further work to explain this difference is required.

Rate Theory of Drug Action

Paton (14) recently introduced an ingenious and provocative new theory of the mechanism of drug action. The basic concept of the older theory is that response to an agonist is some positive function of the number of receptors occupied by the agonist. Paton calls this "occupation theory." The basic concept in his new theory is that response is a function of the rate of association between drug molecules and receptors. In this "rate theory" each association gives a quantal stimulus for response. Occupation of a receptor following the act of association produces no stimulus for response; and, indeed, the receptor is not free for another association until it is unoccupied.

The rate of association A is given by:

$$A = k_1[D](1 - p) \qquad 15$$

where $[D]$ is the drug concentration and $(1-p)$ is the fraction of free receptors (p being the fraction of occupied receptors). After establishment of equilibrium conditions (association rate equal to dissociation rate):

$$A = \frac{k_2}{1 + (k_1/k_2)/[D]}. \qquad 16$$

Comparison of this equation with equations for occupation theory (equations 3 and 4) shows them to be analogous except that a stimulus term is replaced by A, and efficacy (or intrinsic activity) is replaced by the dissociation rate constant, k_2, of the receptor-drug complex. However, rate theory gives a specific meaning to the stimulus and to the proportionality factor relating stimulus and receptor occupation at equilibrium. The latter is k_2, and maxi-

mal steady-state rate of association approaches k_2 as values of $[D]$ are raised much above the dissociation constant, k_1/k_2 (equivalent to K_D). Immediately, one is able to conclude that for equilibrium conditions drugs with very high k_2 values will be strong agonists, those with very low k_2 values will be essentially pure competitive antagonists, and those with k_2 values in a certain intermediate range will be partial agonists. If k_2 is sufficiently high, then the maximal response is limited by the effector system's characteristics rather than by k_2. Thus, there can be "spare receptor capacity" rather than "spare receptors," as in occupation theory.

However, there is no experimental method of differentiating between the two theories under equilibrium conditions. To differentiate one must study the kinetics of offset and onset of response when one adds and removes the drug. Paton presents equations which show what would happen *if there were no diffusion barrier to delay drug receptor interactions*, and if response were proportional to receptor occupation (p) (simple occupation theory) or to rate of association (A) (simple rate theory). In occupation theory onset of response and offset of response would both be exponential, with rate constants of $-(k_1[D]+k_2)$ and $-k_2$, respectively. In rate theory onset would be characterized by a maximal response at zero time equal to $k_1[D]$, followed by an exponential "fade" of the response (rate constant, $-(k_1[D]+k_2)$) to the equilibrium value given by equation 16; and offset would be immediate and complete on removing the drug. Among other things, the theory predicts that even drugs which appear to be pure competitive antagonists at equilibrium should give a transient stimulation at the very beginning of onset of action.

Paton first investigated onset, offset, and equilibrium conditions for the antagonistic action of hyoscine against AcCh in guinea pig ileum. He could detect no initial transient stimulatory effect of hyoscine. However, he was able to obtain kinetic and equilibrium data from which he calculated what appear to be k_1 and k_2 values, as noted in the previous section. Although the k_2 value was very low, in keeping with rate theory, the findings can be fitted just as well into occupation theory as into rate theory. To investigate the crucial matter of kinetics of onset and offset, he used the series of alkyltrimethylammonium compounds from C_6 to C_{12} ,which act on cholinergic receptors. In this series, tested under equilibrium conditions, there is a progressive change with chain length from fairly strong partial agonists to pure antagonists (against AcCh). He attempted to obtain k_1 and k_2 values for members of this series from kinetic experiments as well as k_1/k_2 ratios from equilibrium experiments. It is impossible in this review to consider his results, calculations, and reasoning in detail, but he concluded that the results were in good accord with rate theory and not with occupation theory.

The crucial experimental finding with these compounds up to C_{10} or C_{11} is that they do exhibit the phenomenon of fade on the guinea pig ileum. But this very decline of response from an initial peak to an equilibrium level during onset of action may well be due to some other action of these agents

than the "self-blockade" of receptors as equilibrium is approached. One first thinks of the possibility of an unspecific noncompetitive antagonism by these long chain alkyltrimethylammonium ions developing after the stimulatory action of the cholinergic receptors, with maximal degree of antagonism increasing with increasing length of chain. Tests of this possibility should not be difficult. Secondly, even though hexamethonium was found not to interfere with their responses, the fade phenomenon with these agents may still be associated with nerve plexus-muscle interactions within this complex tissue. Other possible arguments might be made, but the main point from this reviewer's standpoint, is that, despite a rather remarkable agreement of constants calculated on the basis of different types of experimental data, there is too great a possibility that the phenomenon of fade with the agents studied is due to some action other than that to which rate theory ascribes it.

This criticism does not mean that the reviewer wishes to discard any consideration of rate theory. It is an intriguing theory which is, perhaps, just as plausible as occupation theory. Further critical testing, particularly on systems where there is the smallest possible diffusion barrier (such as the skeletal muscle end-plate) is certainly required to see which theory best satisfies experimental data. An initial attempt by Paton & Waud (68) to study kinetics of action of various quaternary compounds on the neuromuscular junction of cat skeletal muscle *in vivo* was not satisfactory because of difficulties inherent in the procedures and biological preparations used. Apparently these workers are now turning to micro-methods of drug application and recording at the muscle end-plate in order to "outflank cardiovascular and diffusional barriers to kinetic study" (68).

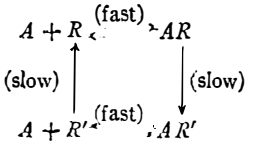
It would be odd, but not impossible, if rate theory were valid for some receptors and occupation theory for others.

Desensitization to Agonists

On continued exposure to a depolarizing dose of AcCh (or to certain related agents), the muscle end-plate repolarizes despite the continued presence of the agonist. Neuromuscular transmission remains blocked despite the repolarization. This "desensitization" phenomenon was first studied by Thesleff (69) with "bulk" application of AcCh to the isolated frog sartorius muscle, and later by Katz & Thesleff (70) using ionophoretic application through micropipettes to the end-plate region. The time required for full desensitization to depolarization and also that for recovery of sensitivity after termination of exposure to AcCh, were of the order of 10 to 30 min in the case of "bulk" application, but of the order of only a few seconds in the case of "micro" application. The "second phase" of blockade by nicotine at autonomic ganglia may also represent a desensitization (repolarization) following the initial depolarizing action of this agent (71).

Analysis of the kinetics of events in the case of "micro" application to the muscle end-plate region led to the development of several hypothetical

schemes (61, 70) involving receptor deactivation. One such scheme proposed the cycle:



in which R is the reactive form of the receptor and R' is a nonreactive form. Conversion of R to R' amounts to "desensitization" of the receptor. In the continued presence of AcCh (or another depolarizing agent) most of R accumulates in AR', and the concentration of free R to form the activating complex AR becomes negligible. Removal of AcCh is followed by recovery of sensitivity as freed R' is converted to R. More recently, Thesleff (72, 73) has speculated that the AcCh-receptor combination only causes depolarization when a substance formed inside the fiber is utilized in the reaction, that desensitization results from a depletion of this substance, and that recovery occurs when the membrane is replenished from the inside by diffusion or synthesis of this substance. He believes that such a system would explain the much slower recovery from desensitization after "bulk" application of AcCh as compared with "micro" application.

Paton (14) recently reinvestigated the partial desensitization of guinea pig ileum to both AcCh and histamine following large doses of either of these agents. He has proposed that such desensitization results from a loss of intracellular K^+. He speculated that each association between agonist ion and receptor in the membrane is effective in producing a quantal stimulus (see previous section) only if the receptor is initially complexed to a potassium ion which can be displaced by the agonist ion (ion exchange concept). The displaced K^+ goes into the extracellular phase. Once the agonist ion has dissociated from the receptor, the latter can combine with another K^+ of intracellular origin, which in turn can be displaced by an agonist ion. Thus, the agonist increases the efflux of K^+. Loss of too much intracellular K^+ would lead to a reduction in concentration of receptors combined with K^+ (activatable receptors) and to a decline in sensitivity.

Two laboratories (74, 75) have investigated the desensitization of isolated cardiac preparations to AcCh on continued exposures to rather high concentrations of this agonist. Furchgott et al. (74) using the guinea pig left atrium, found that gradual desensitization for both the negative inotropic effect and the shortening effect on action potential of AcCh, occurred simultaneously. Also the desensitization was specific, insofar as adenosine still produced its negative inotropic effect after desensitization to AcCh. More recently, Baumann & Waldvogel (76), using the rat left atrium, have found that desensitization to the inotropic effect of AcCh occurs more rapidly than to its effect on action potential.

The cause or causes of desensitization of certain effector systems to presumably directly acting agonists on continued exposure to such agonists, is not yet clear. However, the evidence at present appears to be compatible with an alteration in configuration or composition of the receptor which renders it unreactive.

Innervation and Receptors

Since the embryonic chick heart exhibits a high sensitivity to AcCh and EPI prior to innervation, it may be concluded that the genesis of receptors for autonomic transmitters in this organ does not depend on innervation (77, 78).

Thesleff (72, 79) and Miledi (80) have both reviewed their very interesting independent work on the spread of sensitivity to AcCh to all areas of the membrane of skeletal muscle fibers following chronic denervation. On reinnervation, sensitivity again becomes restricted to the end-plate region. Both investigators agree that the local sensitivity at the end-plate is not significantly increased by denervation, and that it is the "induction" of receptors in areas away from the end-plate which accounts for the marked supersensitivity of denervated skeletal muscle to AcCh and related cholergic agents. Thesleff (81) found that chronic blockade of transmitter release from motor nerves with botulinum toxin also caused a spread of sensitivity to AcCh over the whole muscle fiber membrane. Diamond & Miledi (82) found that rat fetal muscles fibers were also sensitive to AcCh over their entire surface, and that restriction of sensitivity to the end-plate region developed only after birth.

Thesleff (72, 79) has speculated that the skeletal muscle membrane is normally covered with receptors for AcCh, but that the AcCh-receptor combination only causes depolarization when a substance formed inside the fiber can be utilized in the reaction (concept of "silent receptors"). He has proposed that in an innervated fiber this auxiliary substance is somehow restricted to the end-plate region (probably as a result of continual transmitter release from motor nerve terminals), but that in the denervated fiber or in the fiber before or in the early phase of innervation, the substance is throughout the length of the fiber. Miledi (80) also believes there is a neural receptor-controlling factor, but considers it different from the transmitter substance. He has suggested that the appearance of receptors on regions not normally sensitive to AcCh is dependent on a spread, or migration, of receptors to such regions from the end plate, in which region they are produced. He assumes that receptors are not permanent entities but have a limited life span. The neural factor would somehow inhibit an effective spread of receptors, possibly by influencing their rate of destruction.

Whether the increased sensitivity of autonomic effectors following denervation involves the induction of new receptors is not clear at present. It has been proposed by a number of investigators that supersensitivity to NE in

sympathetically innervated effectors following denervation is due to degeneration of nerve fibers which normally function as a very effective uptake and removal system for this catecholamine (54, 83 to 85). However, in the case of the nictitating membrane, it has been shown that denervation produces a greater degree of supersensitivity to NE than does cocaine (84, 86), a substance which is thought to be highly effective in inhibiting uptake and removal of NE by adrenergic nerve endings (84, 87). Thus, it appears likely that part of supersensitivity on denervation of this organ is due to some change other than the loss of a system for uptake and removal of NE. Perhaps this part of denervation supersensitivity may be equated with the supersensitivity developed after deprivation for 7 to 14 days of tonic nervous impulses to the nictitating membrane either by decentralization or various pharmacological means (88). It is interesting that such deprivation of sympathetic nervous impulses also sensitizes to AcCh. Also chronic sympathetic denervation of the membrane sensitizes it to AcCh and 5-HT, although not so much as to NE (89). In like manner, parasympathetic denervation (mainly decentralization) of the submaxillary gland supersensitizes the gland not only to parasympathomimetics but also to sympathomimetics (90). Emmelin (90), in his recent review on supersensitivity of effectors following "pharmacological denervation," has discussed in detail the supersensitivity of the submaxillary gland to EPI following chronic blockade of parasympathetic receptors (with atropine or other antagonist) or of ganglia with chlorisondamine. Thus, there is considerable nonspecificity in the development of supersensitivity in autonomic effectors following denervation, decentralization or "pharmacological denervation." Whether such supersensitivity, specific or nonspecific, is due in whole or in part to induction of new receptors, as in the case of skeletal muscle, will be extremely difficult to ascertain.

Volle & Koelle (91) have reported the unexpected finding that denervation of the cat superior cervical ganglion leads to no increase in sensitivity of the postsynaptic cells to AcCh and to a 26-fold decrease in sensitivity to carbachol. These results, along with others on the action of diisopropylfluorophosphate (DFP), led these workers to propose that threshold doses of carbachol, and possibly of AcCh, activate only the presynaptic terminals, causing them in turn to liberate the neurohumoral transmitter, which then activates the postsynaptic receptors (91).

Differentiation and Classification of Receptors

A number of examples of differentiation and classification of receptors, using antagonists as pharmacological tools, have been given in the section on "Drug Antagonism." Other examples of special interest will be briefly considered here. A large number of recent papers [e.g., (92 to 101)] have provided further evidence for two different types of cholinergic receptors through which AcCh can cause excitation of the postsynaptic membranes of sympathetic ganglia. The "nicotinic" type is blocked by hexamethonium (93), by tubocurarine (92), or during the "second phase" (nondepolarizing) or block-

ade by nicotine (98). The "muscarinic" type is blocked readily by atropine (99, 101). Agonists for the "nicotinic" type include nicotine and DMPP; agonists for the "muscarinic" type include muscarine (99), pilocarpine (93, 100), 4-(*m*-chlorophenylcarbayloxy)-2-butyltrimethylammonium chloride (McN-A-343) (94, 96, 98, 100), N-benzyl-3-pyrrolidyl acetate methobromide (AHR-602) (97, 98), and possibly neostigmine (95, 96). The ganglionic stimulating actions of 5-HT (102) do not appear to be mediated through either type of cholinergic receptor.

The inability of either phentolamine or dibenamine (antagonists of adrenergic α-receptors) or of DCI (antagonist of adrenergic β-receptors) to effectively block the inhibitory effect of NE and EPI on isolated rabbit duodenum led this reviewer (27) to propose a third type (δ-type) of adrenergic receptor for intestine. However, Ahlquist & Levy (103) showed in the intact dog that a combination of an α-receptor antagonist and DCI, could effectively block the intestinal inhibitory effect of these two catecholamines; and postulated that activation of either α- or β-receptors in intestine elicit an inhibitory response. To block an agonist acting primarily on only one type of receptor in the intestine (e.g., phenylephrine on the α-type or ISO on the β-type), only a single appropriate antagonist is required; but in the case of an agonist acting on both types (e.g., NE or EPI), antagonists for both types of receptors must be present simultaneously. In subsequent work on isolated rabbit duodenum, this reviewer (104) confirmed the results of Ahlquist and Levy on the intact dog, and withdrew his proposal of a δ-type of adrenergic receptor for intestine. The question of location of the intestinal α-receptors and β-receptors mediating inhibition is still unsettled. It appears most probable that the β-receptors are on the smooth muscle cells; but, in this reviewer's opinion, the α-receptors may well be on nerve cells in the intestinal plexuses; for if the latter receptors were on the smooth cells, their activation might be expected to elicit a motor response, similar to that observed in many other smooth muscles.

Vane (51) presented evidence, partly obtained with the use of antagonists, that the stimulatory effect of amphetamine and tyramine on certain gastrointestinal preparations, results from the action of these drugs on the same receptors through which 5-HT produces a stimulatory action ("tryptamine receptors"). Both of these drugs appear to owe their sympathomimetic activity to release of stored catecholamines (105). The possibility exists that the persisting responses to them in certain smooth muscle preparations depleted of catecholamines (86, 87, 106), may result from their action on "tryptamine" receptors (51); but, the possibility of a direct "partial" agonistic action on adrenergic α-receptors is not completely excluded.

Nickerson & Chan (107) carefully re-examined the question of whether antagonists of α-receptors can block the inotropic and chronotropic effects of EPI on mammalian cardiac muscle. They found no specific blockade (although nonspecific depression occurred with some antagonists at high concentrations). Only DCI gave specific blockade.

Brown and co-workers (108) have extended their earlier work on the ability of antagonists of adrenergic α-receptors to increase markedly the output of NE in the venous outflow from cat spleen stimulated through its sympathetic nerve supply at ten shocks per second. They interpret their results to indicate that α-receptors are directly involved in the removal of NE released by nerve stimulation, and that blockade of such receptors permits a greater overflow of this transmitter into the venous effluent. The reviewer, despite earlier objections (27), must admit that the results are reasonably consistent with this interpretation, but feels that differences in the distribution of blood flow in the stimulated spleen in the absence and presence of α-receptor blockade may be a major contributing factor.

Gyermek, in his comprehensive review on 5-HT antagonists (109), has discussed the use of such antagonists for the differentiation and classification of receptors for this amine.

Ions and Receptors

Evidence, based on effects of extracellular pH on activity, favors the view that the ionized species of pilocarpine (110) and nicotine (111) are the species which react with receptors in intestine and skeletal muscle, respectively. In general, the concept appears to be accepted that for drugs which are cations (either quaternary ammonium compounds or ionized amines) the corresponding receptors contain anionic sites, so that ionic bonding is possible (25, 46, 112 to 115).

From a plot of the concentration of histamine required for a half-maximal response at different pH values, Rocha e Silva (116) concluded that the receptor contains an ionizable group (possibly an imidazol residue) with a pK_a of about 7.0. Segre (117) concluded from somewhat similar studies that the receptors for both AcCh and histamine in this tissue have two ionizable groups, and that the active form of each receptor is that in which only one group is ionized. In addition, he felt that the effects of pH indicated two distinct types of receptors for histamine in this tissue.

Schild (110) found that polyethyleneimine or diazotized sulfanilic acid caused a differential depression of response to AcCh, as compared with that to tetramethylammonium on the guinea pig ileum. He suggested that this effect may be due to inactivation by the former agents of one site (B site) of the receptor, leaving another site (anionic A site) intact, since AcCh might be expected to react with both sites and tetramethylammonium only with the A site. He proposed that the hypothetical B site might contain an imidazole group.

Only a few of the many recent interesting papers on the effects of cholinergic and adrenergic drugs on the trans-membrane fluxes of ions, can be cited here. Bülbring & Kuriyama (118), extending the earlier work of the Oxford laboratory on the interactions of ions and drugs on guinea pig taenia coli, concluded that AcCh exerts its stimulating effect by a nonselective increase of membrane permeability, leading to depolarization and action potentials;

and that EPI affects chiefly the Na^+ conductance during the active and resting state of the membrane, and modifies the movement of Na^+ across the membrane (activation of Na^+ pump). Jenkinson and colleagues (119 to 121) have demonstrated that even on muscles (taenia coli of guinea pig and denervated rat diaphragm) depolarized by bathing in potassium-rich solution cholinergic agonists, such as carbachol and AcCh, increase permeability to a number of ions. They suggested that the contractures produced by these agents under such conditions are a consequence of entry of calcium into cells, following the increase in membrane permeability. [For a similar view see (122, 123).] Frank (124) believes that such agents not only increase permeability of the muscle membrane to ions, but may also release calcium from binding sites in or on muscle fibers. Douglas & Rubin (125) from a study of the perfused adrenal glands, suggested that AcCh also causes an increased permeability of medullary cells to calcium ions, and that entry of such ions triggers the catecholamine secretion. A somewhat similar situation may apply in the case of AcCh and NE stimulation of salivary glands (126).

Localization, Quantification and Isolation of Receptors

There is much evidence that the receptors for many agonists (especially cationic agonists) are located on or in cell membranes. Effects of agonists on electrical and permeability properties (see preceding section) of cells are in accord with this view. The complete lack of effect of AcCh or carbachol injected into a muscle fiber just under the end-plate (127) indicates that the cholinergic receptors are located on the exterior of the end-plate membrane. Even adrenergic β-receptors, with which catecholamines react to activate the enzyme system forming cyclic 3,5-AMP (128, 129), may very well be located in cell membranes (130).

Waser and his colleagues have continued to report on their work using autoradiographic techniques in the localization and quantification of receptors for cholinergic agents in the mouse diaphragm (131 to 133). Using ^{14}C-curarine, which concentrates in the end-plate regions, he calculates a binding of about 3×10^6 molecules per muscle fiber, and equates this with the number of receptors per end-plate for this blocking agent. With labeled decamethonium and muscarone, there is more spread away from the end-plate regions, and a much larger number of receptors for these depolarizing agents is calculated per muscle fiber. It has been proposed that these latter agents may diffuse into the fibers through the end-plates. Although the specificity of this method for quantification of receptors to the exclusion of nonspecific binding sites is yet to be rigorously proven, it is, nevertheless, an ingenious and interesting new approach.

Although the acidic mucopolysaccharide first isolated by Chagas and co-workers from the electroplax of the electric eel does not appear to be the actual cholinergic receptor in this organ, it still serves as an interesting model for investigating competitive binding of different basic compounds which exert pharmacological actions on this organ (134, 135). Ehrenpreis (136)

reviewed his work with Nachmansohn on the attempted isolation of the AcCh receptor from this organ. Although the former now feels that the protein fraction isolated is not the AcCh receptor, the latter (137) still believes that it may be so. Turpajev et al. (138) reviewed their work on the attempted isolation of the AcCh receptor from cardiac muscle. They believe it to be a sulfhydryl-containing protein, precipitable with $HgCl_2$.

Nature of Receptors: "Models"

Cavillito (115) has speculated about the nature of the anionic sites (carboxyl or phosphate groups) in cholinergic receptors of the muscle end-plate, and has considered how such sites might interact with cationic agonists and antagonists. Belleau (46) has proposed that the anionic site of the adrenergic α-receptor is a phosphate group, possibly contained in ATP or something related to it; but appears to have withdrawn his earlier suggestion (45) that the ATP which is converted to cyclic 3,5-AMP when catecholamines act on β-receptors, actually constitutes the binding site of β-receptors. He has speculated on the possibility of a single comprehensive adrenergic receptor, containing both an anionic site, for triggering responses attributed to α-receptors, and a site which interacts with the catechol nucleus, for triggering responses attributed to β-receptors.

Geiger & Mandel (139) have speculated on the presence of a sulfhydryl or other type of group, in the AcCh receptor of smooth muscle, which may be trans-acetylated by AcCh when the latter activates it. Williams (140) and Cavallito (115) have proposed models for the AcCh receptor in which hydrolysis of this ester is an integral part of activation. The objection to acetylation by, or hydrolysis of, AcCh in activation of the receptor is that so many compounds chemically incapable of such reactions also activate receptors for AcCh. Cavallito has argued that such compounds could act by releasing AcCh from binding sites, but such an indirect action in most cases appears unlikely to this reviewer.

There has been speculation by many that AcCh, in reacting with a receptor in a cell membrane, produces a change in the molecular configuration of the receptor, and that this change leads to the widening of a membrane "pore," and thus to greater permeability of the membrane to ions (141). In Waser's model (130 to 132) of the receptor area in the muscle end-plate, the receptor sites for AcCh (and "depolarizing" blocking agents) are on the outer membrane surface surrounding the exits of pores, and the receptor sites for curarine are directly at the exits of the pores. A curarine molecule could thus physically block a pore which had been opened by the binding of AcCh with its receptors ("functional" rather than a true competitive antagonism).

Paton (14), in connection with his development of ideas on "rate theory" and desensitization phenomena, proposed an "ion exchange" model for receptors for AcCh and histamine (see section on "Desensitization to Agonists"). Mackay (142) has suggested a "flux-carrier" hypothesis for depolar-

izing agonists, in which the net rate of flux (extra- to intracellular) of the cationic agonist through the cell membrane would determine the extent of depolarization, and in which the agonist diffuses through the membrane in association with a "carrier." He has shown how such an hypothesis could be applied to problems of "partial" agonistic action, antagonism and desensitization.

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