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Affinity, Efficacy, and Receptor Classification: Is the Classical Theory Still Useful?

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DEVELOPMENT OF IDEAS ABOUT AFFINITY AND EFFICACY

Receptor Classification

Although receptor classifications have often originated from observations with agonists, there appears to be widespread agreement that the safest method of classification is based on the affinities of selective competitive antagonists. Many people would, for example, now define the muscarinic receptor as a receptor with an equilibrium constant for atropine of about 1 nM. One reason for the success of this approach is that the equilibrium constant is *measurable*, even without direct observation of binding, by the Schild method. If, as is generally supposed, most competitive antagonists merely occlude the receptor but elicit no further conformation change, then it is implicit in this approach that the term *receptor* refers only to the first macromolecule (that which binds the drug) in the chain of molecules that leads from binding to response (or even to just the agonist-binding region of this molecule) (see Stephenson, this volume). If the events to which the first molecule was linked were included in the definition of receptor type then 1) there would be a greatly increased number of receptor types and 2) the classification of these receptors would be incompatible with that based on antagonist affinities. Neither of these consequences seems desirable at present.

Structure-Activity Relationships

Investigation of the relationship between structure and activity is a fundamental goal of pharmacologists. It was, therefore, one of my earliest disappointments to discover that there is little discernible relationship, at least for agonists. It is, of course, a truism to say that the activity of an agonist depends on its structure. The problem is that no general principles have emerged to allow relationships with any substantial predictive ability to be defined between structure and activity. On one hand, one has the complex calculations of *quantum pharmacology*; at the other extreme, one has the primitive constructs that underlie the grandly- named subject of *quantitative structure-activity relationships*. Both have been equally unsuccessful in the predictive sense (which is what matters). In the end, all one is left with is a series of often tortuous post hoc

rationalisations of observed potency measurements.

The Classical Approach

The early attempts to improve this situation were based on the reasonable premise that to make sense of the relation between an agonist's structure and its potency it was necessary to distinguish between the effects of structural changes on 1) the ability of the molecule to bind to the receptor in the first place and 2) its ability to produce a response once bound. The first attempt to do this was the definition of *intrinsic activity* by Ariëns [1954]. It soon became clear that this was unsatisfactory except as a measure of the observed maximum response, a view that eventually (after 10 years of sometimes heated argument) gained wide assent [van Rossum, 1966]. The definition of *efficacy* given by Stephenson [1956] looked much more promising, and his concept has become quite widely used by pharmacologists.

Stephenson supposed that the activity of an agonist could be described by two separate and independent parameters, viz., its affinity and its efficacy. Affinity measured the ability of the agonist to bind to the receptor in the first place, and it was supposed that it could be measured by a single equilibrium constant (which will be defined here as a dissociation constant, denoted K, with molar dimensions). In fact, one constant is not always sufficient (see below), but this complication will be ignored for the moment. The *efficacy* of an agonist was defined as a number, supposed to be quite independent of affinity, that provides a measure of how much response can be produced by each drug-receptor complex once it has formed. Stephenson defined the "fraction of receptors occupied" as p, and he denoted the efficacy as e; he then supposed that the response of the tissue, R, could be written as some function of the product of these quantities, so

$$R = f(ep) = f(S), \qquad (1)$$

where the "stimulus" is defined as $S = ep$. The essential point here is that although e will vary from one agonist to another, as will the occupancy at any given concentration, the function f was supposed to be the *same for all agonists* (for a specified response type and tissue type and under specified conditions).

It has been suggested more recently that the value of Stephenson's concept for the purposes of the classification of receptors in different tissues would be considerably increased if 1) the possibility of variations in the *number* of receptors between tissues were taken into account explicitly as suggested by Furchgott [1966], and if 2) other "tissue-specific factors" were also included explicitly (D.H. Jenkinson, personal communication). For example the "stimulus" could be defined as $S = \theta ne'$, where n is a measure of the number of receptors in the tissue, θ represents other tissue-dependent factors, and e' is a measure of efficacy that is the same for all tissues. These are indeed very sensible suggestions within the general framework of the classical approach, but they do not overcome the problems to be discussed below, so they will not be considered further here.

Interpretation of "Occupancy" in the Classical Theory

On the assumption that Stephenson's framework provides an adequate description of the action of agonists, it has been possible to derive a considerable number of useful results without many further assumptions. For example, null methods have been derived to estimate the affinities of partial and full agonists and the relative efficacies of agonists without having to know details of the response function, f, defined in equation 1 [e.g., Stephenson, 1956; Jenkinson, 1979] (see also Stephenson, this volume). These methods and their underlying theory will be referred to as the *classical approach* throughout the rest of this chapter.

One problem that has arisen stems from the fact that Stephenson's original paper contains an ambiguity that has proved quite mislead-

ing. This ambiguity concerns what is meant by *occupancy*. Stephenson defined p in equation 1 as the "proportion of receptors occupied"; it has therefore been widely supposed that p is what would be measured in an ideal binding experiment. This clearly cannot generally be so, because the whole point of separating the occupancy factor, p, and the efficacy factor, e, in equation 1 is that the former should reflect only the binding step, and not subsequent events. However, thermodynamic considerations [e.g., Edsall and Wyman, 1958, p 653] suggest that the binding of an agonist to a receptor molecule (as measured in a binding experiment) will generally be influenced by whatever conformation changes (or other molecules) are linked to the receptor to produce the response, just as these subsequent events are influenced by the binding of the agonist molecule (which they are, or the molecule would not be an agonist). In other words, the total measured binding should, in general, reflect not only the *affinity* for the initial binding step but also the *efficacy* as manifested by the nature and efficiency of events subsequent to binding. For the classical theory to make sense the term *occupancy* in equation 1 must be interpreted not as the actual measurable (in principle) occupancy, but as a sort of hypothetical occupancy of receptors that are uncoupled from any subsequent conformation change or other events that lead to the response [Colquhoun, 1973, p 157] (see examples below).

VALIDITY OF THE THEORY

Despite the confusions that may result from interpretation of the term *occupancy*, the null methods (exemplified below) that have been derived from the classical theory nevertheless provide, *given the assumptions of the theory*, estimates of what is required: the true (microscopic) affinity for the binding step (independently of subsequent events) and relative efficacies (independently of the binding step). Therefore, everything turns on whether the assumptions of the theory are, or are not, sufficiently close to being correct to allow useful results to be obtained. The types of verification that might be sought will now be discussed, followed by consideration of the relationship between the classical theory and real mechanisms.

Verification of the Theory

In the case of competitive antagonists, the values for antagonist affinities produced by the Schild approach have been abundantly confirmed as essentially correct by direct measurements of binding first by Paton and Rang [1965], and subsequently in numerous other studies. However, the experimental verification of the classical theory for agonists is not quite so satisfactory.

There are two levels at which verification might be sought. At the more modest level one might ask whether it is possible to find two quite arbitrary parameters, denoted e and K, and a function f (the same for all agonists on a given tissue), such that equation 1 describes observations reasonably well. At this level no attempt would be made to give any physical interpretation to either e or K. There is some reason to think that this may be a reasonable approximation. If this were so, a useful, though entirely "black box", description of agonists would be provided by the theory. However, it is less clear that when two agonists are acting simultaneously their stimuli can be treated as additive. If they were, the various null methods of the classical approach should give the same values for e and K (regardless of how the values are to be interpreted); however, when such tests have been done there is often considerable disagreement among them [for details, see Kenakin, 1984].

At the more ambitious level the additional postulates are made that the parameter K in equation 1 can be interpreted as reflecting only the initial binding step and that it is independent of the subsequent events described by e. Thus, although e must of course remain an arbitrary parameter without mechanistic significance, K is suggested to be a

well-defined physical quantity, a microscopic equilibrium constant. This more ambitious interpretation seems to be closest to what the originators of the classical theory had in mind; but there is little experimental reason to suppose it valid. The examples discussed below show that it may be invalid for quite simple agonist mechanisms. The fact that K is a well-defined physical quantity means that in principle it *could* be determined by methods independent of the classical theory as a check, but I am aware of no case in which this has actually been done. To do so it would be necessary to understand something of the actual mechanisms involved because it is not possible to predict the outcome of, for example, a binding experiment given *only* the values of K and e that are produced by the classical approach.

Mechanisms of Agonist Responses

The binding step. Stephenson's formulation (and most of the work that followed it) assumed simple "Langmuir" binding at equilibrium to independent identical binding sites, as originally formulated by Hill [1909]. In many cases it is known that agonist binding is more complex than this; for example, receptors for fast transmitter molecules such as acetylcholine, glutamate, and GABA are thought to have more than one subunit that binds the agonist, and the responses show cooperativity. Some of the classical theory still holds good for certain cooperative binding mechanisms [Colquhoun, 1973], but the classical theory for agonists (and even the simpler Schild theory for antagonists) will no longer be valid if there are two *nonequivalent* binding sites for the agonist per receptive unit [Colquhoun, 1986; Kenakin, 1984]. There is evidence for nonequivalent subunits in the nicotinic acetylcholine receptor [Sine and Taylor, 1981]; the two α subunits that bind acetylcholine, though the same in primary amino-acid sequence, appear to differ because of their environment (which cannot be the same for both in a pentamer with an $\alpha_2\beta\gamma\delta$ structure) or possibly because of different posttranslational modifications. There is no evidence one way or the other for all other sorts of receptor.

Steps subsequent to binding. In 1956 virtually nothing was known about what happened *after* binding. It is a great virtue of the Schild method that it allows information to be obtained with a minimum of assumptions (and, it has subsequently emerged, with great success) about antagonist affinities despite this ignorance. In the classical approach an attempt is made to apply similar ideas to agonists; null methods, it was hoped, would allow inferences about agonists without detailed knowledge of how binding was linked to response. There are two differences from the Schild case, however, 1) the assumptions that must be made to get this knowledge (in particular, that something with the form of equation 1 can describe the response) are very much more restrictive than is the case with antagonists, and 2) the lack of independent experimental verification of the results for agonists contrasts sharply with the ample verification available for antagonists.

A great deal has been learned since 1956 about events subsequent to binding (and a great deal more remains to be learned). It is now clear that the nicotinic receptor and the responding unit, the ion channel, are all part of the same macromolecule, and when both binding sites are occupied by agonist this macromolecule has a greatly increased chance of undergoing a conformation change to an active state (i.e., opening of the ion channel). Everything that happens seems to take place in a single pentameric macromolecule; this is the simplest sort of response that is known. Responses to muscarinic receptors, to α and β receptors for catecholamines, and to many others are a great deal more complicated than this. They may involve, for example, linkage of the receptor molecule, through a separate (G protein) molecule, to an enzyme that produces cyclic AMP or to a phospholipase system that generates inositol phosphates. These second messengers may then diffuse inside the cell, change internal calcium concentrations, activate other enzymes, and phosphorylate

further proteins eventually to produce a response (the later stages are far from clear in many cases). If one could imagine that someone were to propose now that this whole complex sequence of events could be related to the initial process of binding by a single numerical index, "efficacy," it would seem an unreasonable oversimplification. Perhaps it is only because the idea of efficacy was well- established before much was known of the actual events that the possibility of using such a simple description is countenanced at all.

However, we do not need to consider such complex systems to see the sort of complications that can arise when real mechanisms are considered. Even the very simple agonist mechanism discussed below may not fit into the classical framework.

A Simple Agonist Mechanism

In 1957, quite independently of Stephenson's paper, del Castillo and Katz proposed a very simple agonist *mechanism*; this was a physical reaction scheme that involved proposed discrete molecular states of the receptor as opposed to the classical "black box" approach. Their scheme was as follows:

$$\underset{\text{occupied}}{R \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} AR} \qquad \underset{\text{open}}{AR \underset{\alpha}{\overset{\beta}{\rightleftharpoons}} AR^*}, \tag{2}$$

where R is the unoccupied receptor, AR is the occupied but shut receptor, AR^* is the active receptor (e.g., the open ion channel), x_A is the agonist concentration, $K_A = k_{-1}/k_{+1}$ is the dissociation equilibrium constant for binding, and $E \equiv \beta/\alpha$ is the equilibrium constant for isomerisation to the active state.

del Castillo and Katz [1957] wrote:

"According to this concept, whether a substance acts as a depolarizer or a competitive inhibitor would depend entirely on the rate constants for the two steps; d-tubocurarine, for instance, may be considered to form a reversible intermediate compound AR without proceeding to the next step. Moreover, a substance which by itself has a relatively weak or slow depolarising action . . . may, at the same time antagonize the depolarisation produced by fast and powerful agents. . . ."

Thus, they independently provided a remarkable description of affinity and efficacy that, if less general than the classical formulation, was more firmly based in physical realities. Even their mechanism is simple only if the response that is measured is directly proportional to the fraction, p_{open} say, of channels that are in the open state (AR^*), e.g., if the response is measured as the current through a voltage-clamped membrane. In this case, the response at equilibrium can be written in the form

$$p_{open} = \frac{E\left[\dfrac{x_A}{x_A + K_A}\right]}{1 + E\left[\dfrac{x_A}{x_A + K_A}\right]}. \tag{3}$$

Clearly, K_A is a measure of the *affinity* of the binding step in isolation (its reciprocal is the affinity constant), and, as suggested by del Castillo and Katz [1957], $E = \beta/\alpha$ is a measure of *efficacy* because it measures the extent to which the agonist-receptor complex tends to be in the active rather than the inactive form. We should, however, note here that $E = \beta/\alpha$ refers to the rate constants for each individual molecule; it contains no reference to the number of receptors present and is, therefore, more directly analogous to the *intrinsic efficacy* defined by Furchgott [1966] as efficacy divided by "receptor concentration." This distinction will turn out to be important in the discussion of the irreversible antagonist method (below).

If we now *define* P as

$$P \equiv \frac{x_A}{x_A + K_A} \tag{4}$$

and

$$S \equiv EP \tag{5}$$

then equation 3 can be written in a form exactly like Stephenson's formulation in equation 1, viz.,

$$p_{open} = f(EP) = f(S), \tag{6}$$

where the particular form of the function, from equation 3, is

$$p_{open} = \frac{S}{1 + S}. \tag{7}$$

Thus, although the Castillo-Katz mechanism specifies a particular *physical* reaction scheme whereas Stephenson's approach made no mention of mechanisms, this particular reaction scheme appears to accord perfectly with Stephenson's formulation (even to the extent that it is scaled according to the convention suggested by Stephenson, that a stimulus of unity should correspond to a 50% response, as is seen to be the case in equation 7). Note, however, that the occupancy P defined in equation 4, in the manner necessary to preserve the Stephenson form, is *not* the occupancy that would be observed in a binding experiment (a particular example of the general conclusion mentioned above). Rather, it is the fraction of *inactive* receptors that is occupied (or, equivalently, the occupancy that would be observed if the receptor were uncoupled from the active form, e.g., if $\beta = 0$). The actual occupancy would be

$$p_{occ} = \frac{x_A}{x_A + K_{eff}}, \tag{8}$$

where

$$K_{eff} \equiv \frac{K_A}{1 + E}, \tag{9}$$

i.e., p_{occ} would depend on both affinity (K_A) and "efficacy" (E). On the other hand P, in the spirit of Stephenson's approach, depends only on affinity.

Although this particular mechanism appears to be perfectly analogous with the classical formulation, we must now enquire whether it fulfills the further assumptions that are necessary to derive useful null methods from that theory. For example, can "stimuli" be regarded as additive, and do the null methods give the results expected of them? In the remainder of this section, three of the classical null methods for estimation of "affinity" will be considered in order to cast light on this question, viz., the "interaction" and "comparison" methods for partial agonists, and the "irreversible antagonist" method for full agonists.

The Interaction Method for Partial Agonists

The concentration-response curves for a full agonist (denoted A) in the presence and absence of a fixed concentration of the partial agonist (denoted B) are compared [Stephenson, 1956; Colquhoun, 1973; Jenkinson, 1979]. We define concentrations x_A and x'_A such that equal responses are produced by a concentration x_A of the full agonist acting alone and by a concentration x'_A of the full agonist in the presence of a fixed concentration (x_B, say) of the partial agonist. The classical procedure is to plot x_A against x'_A and to use the slope of this line to make an estimate (K_{est}, say) of the binding constant from

$$K_{est} \equiv \frac{x_B}{(1/slope) - 1}. \tag{10}$$

(Note that this plot, and those described for other methods below, represent the classical procedures; they cannot be recommended as methods of parameter estimation in practice.)

Classical theory. The classical theory, when stimuli are assumed to be additive, implies that the plot of x_A against x'_A will be linear and that equation 10 provides an estimate of

$$K_{est} = \frac{K_B}{(1 - e_B/e_A)}. \tag{11}$$

This will be a good estimate of K_B, the equilibrium constant for binding of the partial agonist, if the full agonist used has a sufficiently high efficacy, so $e_B \ll e_A$.

Castillo-Katz mechanism. As long as the active state produced by the full agonist (AR^*) and the active state produced by the partial agonist (BR^*) are *equally effective* (e.g., they are open channels with the same conductance and reversal potential) then "stimuli," as defined in equation 5, are indeed additive, and the predictions have exactly the same form as in the classical theory. The plot of x_A against x'_A will be linear, and equation 1 will provide an estimate of

$$K_{est} = \frac{K_B}{1 - E_B/E_A}. \qquad (12)$$

Thus, we can obtain a good estimate of the binding constant, K_B, of the partial agonist, independent of its efficacy, as long as the full agonist is much more "efficacious" than the partial (i.e., $E = \beta/\alpha$ is much smaller for the partial agonist, B, than for the full agonist A). Suppose, however, that the active complexes are not equally effective for the full and partial agonist. In the simple example of ion channels, for example, this would be the case if the two agonists opened channels of different conductance and/or reversal potential. This is not actually so for nicotinic receptors [Gardner et al., 1984], but for other channels it may happen (e.g., because of selective activation of particular conductance sublevels) [see Hamill et al., 1983]. In any case, for more complex systems it may very well be true that the initial active state is not equally effective for different agonists (there is little evidence about this). A similar effect is produced by desensitization or self-block (see below).

To take a simple example, suppose that the conductances of the channels produced by full and partial agonists are γ_A and γ_B. The response will be proportional to γp_{open} for each agonist. In this case too a plot of x_A against x'_A should be linear, so the experimental results would show no sign that anything was wrong. The slope of this plot would, however, have an interpretation rather different from that in equation 12; in this case equation 10 would provide an estimate of

$$K_{est} = \frac{K_B}{\left[1 - \frac{\gamma_B E_B}{\gamma_A E_A} + E_B\left(1 - \frac{\gamma_B}{\gamma_A}\right)\right]} \qquad (13)$$

$$\simeq \frac{K_B}{1 + E_B\left(1 - \frac{\gamma_B}{\gamma_A}\right)} \text{ if } E_B \ll E_A. \qquad (14)$$

Thus, even if the full agonist is much more efficacious than the partial so that $E_B \ll E_A$ (or, perhaps more appropriately, $\gamma_B E_B \ll \gamma_A E_A$), the conventional expression, equation 10, does *not* give correctly the affinity of the partial agonist, as measured by K_B, but, as shown by equation 14, it gives a *mixed* measure of both affinity, K_B, and "efficacy," E_B (i.e., the value of β/α for the partial agonist). The results could be quite misleading even in this very simple case (though the linearity of the plot would give no indication of invalidity). The error in estimation of K_B is clearly likely to be much smaller for a weak partial agonist than for a stronger agonist.

If the null methods can fail in such a simple case, how can any reliance be placed on their validity in much more complex cases such as the muscarinic receptor or the α-adrenoceptors?

The Comparison Method for Partial Agonists

The comparison method uses the concentrations of full agonist (x_A) and partial agonist (x_B) that produce the same response when each drug is given on its own [Barlow et al., 1967; Waud, 1969]. The classical procedure is to plot $1/x_A$ against $1/x_B$ (again, this form of plot is not recommended in practice). Then an estimate (K_{est}, say) of the binding constant is found as

$$K_{est} \equiv (slope)/(intercept). \quad (15)$$

The predictions of this procedure are exactly the same as for the interaction method discussed above. According to the classical theory the plot will be linear, and equation 15 will provide an estimate of equation 11. According to the Castillo-Katz mechanism, equation 15 will provide an estimate of equation 12 as long as the active state is equally effective for both full and partial agonists. And again, if the active states are *not* equally effective then, although the plot is still linear, equation 15 will yield an estimate of equation 13, so the initial binding step is again not properly separated from subsequent events.

The Irreversible Antagonist Method for Full Agonists

The irreversible antagonist method was used by Waud [1963], Furchgott [1966], Mackay [1966], and Stephenson [1966]. The concentration-response curve is determined for the full agonist; the tissue is then treated with an irreversible antagonist for sufficient time to depress the maximum response, and the concentration-response curve is determined again. The classical procedure is to determine concentrations that produce the same response before (x_A) and after (x'_A) the application of the antagonist; then $1/x_A$ is plotted against $1/x'_A$. An estimate of the binding constant is found as

$$K_{est} \equiv (slope - 1)/intercept. \quad (16)$$

The classical theory. It is predicted that plot will be linear and that equation 16 will give an estimate of

$$K_{est} = K_A \quad (17)$$

exactly. The slope of the plot will be

$$slope = 1/q, \quad (18)$$

where q is the fraction of receptor that are *not* blocked by the antagonist and are therefore free to bind agonist (with the same K_A as before treatment). If we were to calculate simply *slope/intercept* as in the comparison method, we would estimate $K_A/(1-q)$, a result directly analogous to that found by the comparison method (eq. 11). This is as might be expected from the Furchgott formulation [1966], according to which efficacy is directly proportional to the number of receptors present, so q represents the relative efficacy of the agonist after and before treatment and hence replaces e_B/e_A in equation 11.

The Castillo-Katz mechanism. The simplicity and exactness of the result in equation 17 for the classical theory can be viewed as originating from the fact that the irreversible antagonist is supposed, in the classical theory, to affect efficacy (via the number of receptors) but not affinity. As soon as coupling of response and binding are considered, as in the Castillo-Katz mechanism, this simplicity is lost. An irreversible antagonist should affect neither K_A nor $E_A = \beta/\alpha$ if its action is simply to reduce the number of receptors, and the contrast with the classical theory is much stronger than it is with the methods for partial agonists. The plot of $1/x_A$ against $1/x'_A$ should still be linear for the Castillo-Katz mechanism, but the classical procedure (eq. 16) yields

$$K_{est} = \frac{K_A}{1 + E_A} = \frac{K_A}{1 + \beta/\alpha} = K_{eff}. \quad (20)$$

This is the *effective* binding constant (see eq. 9), i.e., that which would be observed in an ideal binding experiment. It depends on both affinity and efficacy (it will be equally affected by changes in either for a strong agonist) and so fails entirely to separate the initial binding step from subsequent events. A similar result has been shown to apply more generally, at least as an approximation, to cooperative mechanisms that involve independent subunits [Colquhoun, 1973]. However, other sorts of cooperative mechanisms, such as the Monod-Wyman-Changeux type, would yield more complex and less interpretable results, even

for "simple" ion channel responses. No physical mechanism is known, at present, for which the irreversible antagonist method would separate properly the initial binding affinity from subsequent events.

The exact result (eq. 17) obtained with the classical theory is obtained essentially because efficacy is supposed [following Furchgott, 1966] to be directly proportional to the number of receptors present, so the saturation of the response as the number of receptors is changed (the intrinsic efficacy being constant) has exactly the same form as the saturation of the response as the intrinsic efficacy is changed (the number of receptors being constant). In other words, the same function f (eq. 1) is supposed to be applicable either to changes in receptor number or to changes in intrinsic efficacy. This assumption appears to be entirely arbitrary, and it is untrue for simple cases in which there is coupling between response and binding such as the Castillo-Katz mechanism. For the latter the response (p_{open}) is a hyperbolic function (eqs. 3 and 7) of intrinsic efficacy (i.e., of β/α) when the number of receptors is constant. But clearly the response, i.e., the number of open channels, is directly proportional (rather than hyperbolically related) to the number of unblocked receptors in the Castillo-Katz mechanism (and for many related mechanisms). This is why the result (eq. 20) is so different from that found in the classical case (eq. 17). It may also be noted that the null nature of the experiment ensures that the nonclassical result in equation 20 would still be obtained even if the observed response were a saturating function of the fraction of active receptors (e.g., open channels) rather than being directly proportional to it (e.g., if the depolarization produced by the agonist were measured rather than the flow of current through the open channels in a voltage-clamp experiment).

Self-Block and Desensitisation

The ion channels opened by nicotinic agonists are themselves blocked by every agonist that has been tested, including acetylcholine itself [e.g., Adams and Sakmann, 1978; Ogden and Colquhoun, 1985]. This causes the concentration-response curve to flatten off (and come down again) prematurely and gives the appearance of partial agonism despite the fact that methods that allow for block suggest that most of the agonists actually have quite high efficacy [Colquhoun and Sakmann, 1985; Ogden, 1985; Marshall and Ogden, 1986]. It is not known whether analogous phenomena occur in other tissues, but some sort of "side effect" of this sort is quite likely at high agonist concentrations.

The phenomenon of "desensitisation" seems to be a universal property of agonists. At the end-plate, for example, the equilibrium response in acetylcholine is only about 1 or 2% of the peak response that can be obtained before much desensitisation occurs.

Both of these phenomena can give the appearance of partial agonism. Analysis of simple models for these processes shows that, as in the example discussed in detail above, the classical null methods would not correctly distinguish the initial binding affinity from subsequent events.

AFFINITY AND EFFICACY IN PRACTICE

Research on Fundamental Mechanisms

The classical approach was empirical, or descriptive, in the sense that it avoided any reference to the actual mechanisms that link agonist binding to response. This was its great advantage at a time when little was known about mechanisms, but equally it means that these classical methods can tell us nothing about mechanisms and are irrelevant to the current great efforts that are being made to discover the actual mechanisms involved.

The Search for New Drugs

The discovery of useful new drugs has not always been dependent on previous detailed investigations of molecular mechanisms. It has in the past often been possible to discover useful compounds by the rational use of a more empirical form of structure-action rela-

tionships, and such approaches are likely to continue to be useful, in conjunction with molecular studies. The question that must be asked, then, is whether quantitative measurements of affinity and efficacy form a useful part of such a search. They certainly should be useful if they allowed a distinction to be made between the structural features that contribute to binding and the structural features that contribute to effectiveness of the drug-receptor complex. The arguments presented above make it improbable that a genuine separation can be achieved. Nevertheless, it is conceivable that even if it were permissible to use only the less ambitious interpretation discussed above (in which e and K are both treated as entirely arbitrary descriptive parameters), useful results might still be obtained. It is possible, for example, that trends in the values of these parameters over a series of compounds might allow extrapolation to predict useful new compounds [see, for example, results cited by Kenakin, 1984]. There does not, however, appear to be much experimental evidence to suggest that this approach would be any *more* fruitful than simpler observations of potency and maximum response.

Affinity, Efficacy, and Receptor Classification

There can be no doubt at all that the proper classification of receptors *has* been exceedingly useful for the development of new drugs. Many of the most important results in this field have been concerned with antagonists and have relied on the Schild analysis rather than analysis of affinity and efficacy. Nevertheless, the development of agonists specific for particular receptor subtypes is a field of great interest, and of course in many cases (such as the adrenoceptors) the receptor classification was originally based on observations with agonists rather than antagonists, despite the much greater problems of interpretation posed by agonists. Many of the methods that have been used are much less subtle (from the point of view of the receptor classification) than the classical analyses. For example, prenalterol was first classified as a β_1-selective agonist on the basis of tissue-selectivity studies in whole animals [Carlsson, et al., 1977]. The thorough analysis of this problem by Kenakin and Beek [1980] by the classical methods showed quite clearly that there was no evidence that prenalterol was specific for the β_1 receptor. Rather, it seemed that the observed tissue selectivity arose from differences between tissues of the stimulus-response relationship. The chance that the complex mechanisms of β receptor-mediated response can really be described by the Stephenson formulation seems slim, so the numerical values for affinities and relative efficacies given by such studies cannot be taken seriously in the sense that they may well not reflect exclusively the initial binding and the characteristics of the drug-receptor complex, respectively. Nevertheless, given that the entire response mechanism is not understood, this sort of analysis may be the best that can be done at present, and the fact that the best available analysis indicates that there is no evidence for selectivity of prenalterol for β_1 receptors means that we must, for the present, suppose this conclusion to be right. The classical analysis is therefore quite useful in this sort of case, but mainly in the negative sense of indicating that there is no evidence for a proposition. The use of numerical values from this sort of analysis to provide positive evidence for receptor specificity would be much more dubious, because the evidence that two receptors differ in themselves (rather than in the postreceptor events to which they are coupled) depends crucially on the interpretation of K in equation 1 as a genuine microscopic binding constant rather than as an empirical descriptive parameter. This is exactly the part of the classical theory about which the greatest doubt exists.

CONCLUSIONS

The 1950s and early 1960s saw the fruitful development of quantitative ideas about antagonists and about agonists based on the ear-

lier foundations laid by A.V. Hill, A.J. Clark, J.H. Gaddum, and others. Of these ideas, only the Schild analysis has really withstood the test of time to the present day. The ideas about the much more complex problems of agonist action, on the other hand, developed into what became known as "receptor theory," a subject that, viewed from a present-day standpoint, became rather sterile and separate from rigorous experimental verification (indeed most of it, being based on a "black box" approach rather than on real physical mechanisms, was not really testable in a useful sense). At the same time research was conducted on the link between binding and response and on the structure of receptors themselves. Much of this, initially at least, was done by biochemists and biophysicists, who often had no knowledge of the earlier pharmacological theories. The latter approach is obviously one that pharmacologists must now follow.

Another limitation of the classical approach is that it deals only with equilibria. More recently much effort has been expended on trying to determine the *rates* of receptor-mediated events for the very good reason that it is quite impossible to understand the effects of some drugs (those, for example, that are involved in, or interfere with, fast synaptic transmission) from equilibrium data alone.

The distinction between affinity and efficacy remains a most important qualitative idea, but it seems to me to be no longer useful to regard this approach as a quantitative pharmacological "theory" of agonist action. Certainly the classical analyses may remain useful in a purely descriptive way, but their use for receptor classification requires the more ambitious interpretation of the "affinity" parameter not just a descriptive constant but also as a genuine microscopic equilibrium constant; the examples cited show that this interpretation is highly dubious, especially for strong agonists (the errors are likely to be smallest for weak partial agonists, as the Schild case is approached). This may seem a rather negative way to regard ideas that have had such a great influence on pharmacological thought, but is probably the ultimate fate of most "black box" theories.

Attempts to extend further the classical approach under the guise of "functional" or "operational" analyses of receptor mechanisms seem more likely to mislead than to enlighten, because these analyses have even more restrictive and untested assumptions than those of their predecessors.

The real answers about receptor subtypes will come not from analysis of efficacies, but from knowledge of receptor structures. Although statements of this sort were sometimes made in the 1960s, they seemed at that time like mere pie in the sky. Now it is really happening [see Takai et al., 1985; Sakmann et al., 1985]. Of course it is likely that measurements of agonist responses (which, after all, are what ultimately matters) will remain important in receptor classification in the foreseeable future, but it seems that we are still far from the stage where such measurements can be interpreted unambiguously.

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