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CO-OPERATIVITY IN THE CYCLIC MODEL FOR DRUG–RECEPTOR INTERACTION

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Abstract—The cyclic model for drug–receptor interaction predicts a hyperbolic relationship between response and concentration provided that the drug equilibrates instantaneously with the receptors. It is shown theoretically that if equilibration is not very much faster than desensitization and recovery, the response–concentration relationship appears to exhibit negative co-operativity. On the other hand, if the drug also diffuses relatively slowly to the receptors the relationship may appear to exhibit positive co-operativity.

In several current models for the interaction between drugs and the cholinergic receptors of muscle the receptors are thought of as being present in either of two states: an active conformation, R (ion channels open) or an inactive one, T (ion channels shut). Endplate conductance is said to be directly proportional to the fraction of receptors in the active conformation, whether complexed with drug or not. Consequently a drug which combines preferentially with one or other of the two conformations will alter their relative proportions and hence endplate conductance [1]. The simplest of these two-state receptor models is (Fig. 1):

$$\begin{array}{ccc} X + R & \underset{k_2}{\overset{k_1}{\rightleftharpoons}} & RX \\ k_7 \uparrow\downarrow k_8 & & k_4 \uparrow\downarrow k_3 \\ X + T & \underset{k_5}{\overset{k_6}{\rightleftharpoons}} & TX \end{array}$$

Fig. 1.

where X is the drug. This model predicts that at equilibrium the fraction of receptors in the active conformation (the state function) increases hyperbolically with drug concentration. Since conductance (and thus the state function) may increase with concentration in a sigmoidal manner rather than hyperbolically, the simplest two-state model has been modified by supposing the receptors to be made up of a number of identical subunits. There are several different ways in which such subunits could interact, each giving rise to slightly different state functions [2]. However, the state functions for all these two-state models have an important feature in common: they have been derived assuming that after a drug is added the new equilibrium between the different conformations is attained instantaneously.

A rather different sort of two-state model has been shown by Katz and Thesleff [3] and Rang and Ritter [4] to explain the kinetics of desensitization. This model, which they called cyclic, is formally identical with that in Fig. 1, but differs from it in two crucial ways: (i) the response is directly proportional to the occupancy of the active form of the receptor (i.e. to the amount of RX); and (ii) the transitions between the active forms of the receptor (R and RX) and the inactive or desensitized ones (T and TX) occur slowly compared with the time taken to measure the response. The dissociation steps were assumed to be instantaneous, in which case the maximum response must be a hyperbolic function of concentration. However, if these steps were also relatively slow, it is not at all obvious how the maximum response would be related to concentration. Conceivably the relationship could be sigmoidal, with the result that there would be no need to invoke a multi-subunit structure for the receptor. (A model of this sort would be similar to those explaining the sigmoidal kinetics of single subunit enzymes [5]).

We have therefore explored theoretically the nature of the relationship between response and concentration when the dissociation steps are relatively slow, assuming, first, that the drug diffuses rapidly to the receptors and, secondly, that it diffuses slowly to them. Since the relevant equations cannot be solved algebraically, we have solved them numerically.

THEORY AND METHODS

Model. The cyclic model for the interaction of drug (X) and receptor (R) was shown in Fig. 1. As a first approximation, the slow diffusion of drug to receptor was considered in terms of the "limited biophase" model [6]. In this model the external solution and the compartment bathing the receptors are thought of as being well mixed but separated by a diffusion barrier. The kinetics of drug–receptor interaction are then described by the simultaneous equations:

$$\frac{d(X)}{dt} = \frac{P \cdot A}{V}((X_0) - (X)) + (k_2(RX) + k_5(TX)) - (k_1(R) + k_6(T))(X),$$

$$\frac{d(R)}{dt} = k_2(RX) + k_7(T) - (k_1(X) + k_8)(R),$$

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$$\frac{d(RX)}{dt} = k_1(X)(R) + k_4(TX) - (k_2 + k_3)(RX),$$

$$\frac{d(T)}{dt} = k_5(TX) + k_8(R) - (k_6(X) + k_7)(T),$$

$$\frac{d(TX)}{dt} = k_3(RX) + k_6(X)(T) - (k_4 + k_5)(TX),$$

$$(R_{tot}) = (R) + (RX) + (T) + (TX),$$

where (R_{tot}) is the total concentration of receptor (in e.g. moles·cm^{-3} biophase), (X_0), (X) are the concentrations of drug in the external solution and biophase respectively (in e.g. moles·cm^{-3}), P is the permeability coefficient of the diffusion barrier (in e.g. cm·sec^{-1}), A is its cross-sectional area (in e.g. cm^2) and V is the volume of the biophase (in e.g. cm^3).

These equations cannot be solved algebraically to find the maximum value of (RX), RX_{max}, and thus of the response. However if the various constants are given numerical values, the equations can be solved numerically and the relationship between response and concentration defined, at least for that set of values.

Values of constants. The basic set of values used was: $(P \cdot A/V) = 100$, $k_1 = 100$, $k_2 = k_5 = 10$, $k_3 = 2$, $k_4 = 0$, $k_6 = 10{,}000$, $k_7 = k_8 = 1$. The relationships between k_1–k_8 are roughly those estimated for the interaction of acetylcholine with frog sartorius muscle [3], but there do not appear to be any reliable estimates of the absolute values of k_1, k_2, k_5 and k_6. We therefore arbitrarily made k_2 and k_5 about an order of magnitude greater than k_3 and k_7. (R_{tot}) was set to unity, so at equilibrium in the absence of drug $(R) = (T) = 0.5$.

Numerical solutions. The equations were solved by using the digital computer program CHEK [7, 8]. Programs were run on an IBM 370/158 at the Edinburgh Regional Computing Centre.

RESULTS

The nature of the relationship between maximum response (RX_{max}) and drug concentration $((X))$ depended on the rate of diffusion of the drug. When this was rapid $(P.A/V = 10^6)$, the relationship was as shown in Fig. 2 (dashed line). The curve does not have a point of inflexion (i.e. it is not sigmoidal) but it is not a rectangular hyperbola, for a double reciprocal plot of the data was concave downwards, and the slope of a Hill plot was less than unity (=0.95). These results might be (mis-) interpreted as evidence for negative co-operativity [9]. On the other hand, when diffusion was slow $(P.A/V = 100)$, RX_{max} increased sigmoidally with concentration (Fig. 2, continuous line). In this instance the Hill plot was curved, with a maximum slope of 1.2, and might again be interpreted incorrectly in terms of interacting subunits [10]. (What Fig. 2 does not show is that at lower concentrations still the plot is concave downwards—i.e. it has two points of inflexion). At both rates of diffusion the time at which the maximum response was observed increased as the drug concentration decreased (Fig. 3).

In other respects the model behaved in a similar manner to the special case analyzed by Katz and Thesleff [3] and Rang and Ritter [4], even when diffusion was slow. For example, at equilibrium the total amount of drug bound $(=(RX) + (TX))$ increased hyperbolically with concentration; the onset of desensitization was an exponential process with a rate constant that increased asymptotically with concentration; and after the removal of drug, sensitivity $(=(R))$ returned approximately exponentially with a rate constant that was independent of concentration.

These results were quantitatively, but not qualitatively, dependent on the exact values chosen for the rate constants. As long as diffusion was fast, none

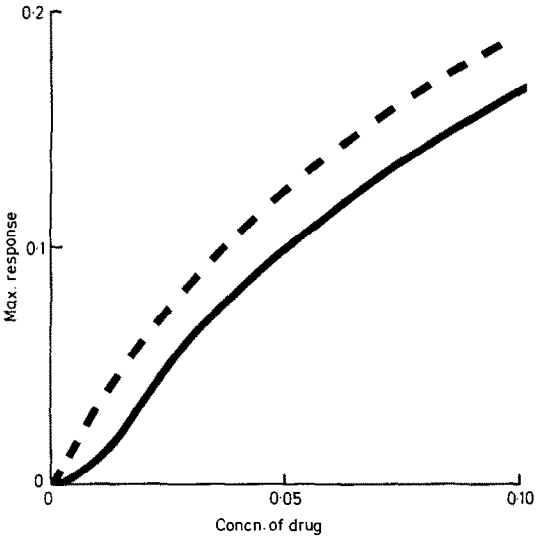

Fig. 2. The relationship between maximum response and drug concentration when diffusion is rapid ($P.A/V = 10^6$, dashed line), and when it is slow ($P.A/V = 100$, continuous line). The asymptotic value of the maximum response is close to 0.5.

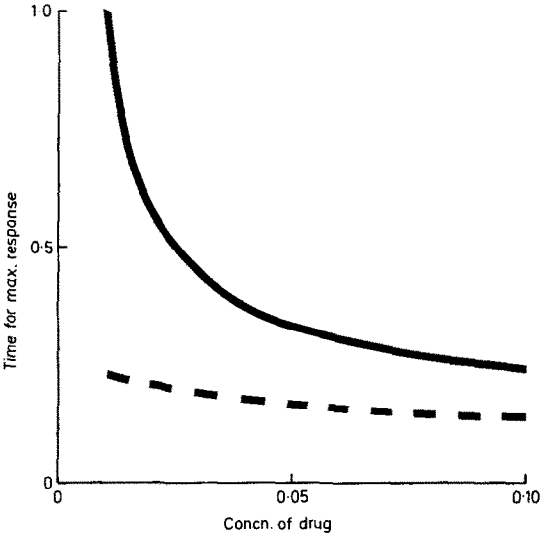

Fig. 3. The relationship between the time at which the maximum response is observed and drug concentration when diffusion is rapid ($P.A/V = 10^6$, dashed line), and when it is slow ($P.A/V = 100$, continuous line).

of the sets tested gave a sigmoidal response–concentration curve (not even the limiting case of $k_2 = k_4 = k_5 = k_6 = k_7 = 0$). When diffusion was slow and the equilibrium concentration of R relative to that of T was reduced 100-fold (k_8 being increased proportionately), the sigmoidal behaviour was still observed, although at higher concentrations. And when diffusion was made slower still (i.e. when $P \cdot A/V$ was reduced below 100) the Hill coefficient increased.

DISCUSSION

Our results have shown that, in certain circumstances, the cyclic receptor model [3, 4] can account, qualitatively at least, for nonhyperbolic plots of response against concentration and for the observed hyperbolic binding [2]. If the drug were to diffuse rapidly from its point of application to the receptors such plots could appear to indicate negative co-operativity, whereas if diffusion were slow they could be sigmoidal. Thus, as in enzyme kinetics [5], non-hyperbolic plots can be explained without recourse to subunit models. However, the receptor model is less general than the enzymic one because its positive co-operativity only becomes apparent when diffusion is slow.

Although the cyclic model can therefore explain co-operativity on a qualitative basis, it may not be so successful quantitatively. Further, some of its predictions may not be fulfilled in practice. For the sets of rate constants we tried, positive co-operativity was observed when the value of X was less than that of R_{tot}. This does not necessarily imply that the concentration of drug in the "biophase" has to be less than that of the binding sites for it (i.e. the receptors), because the effect on the overall kinetics of increasing X can be nullified by a proportionate decrease in the rate constants $P \cdot A/V$, k_1 and k_6. However, if $R_{tot} = 3 \times 10^7$ sites and $V = 450\,\mu m^3$, the concentration of sites is about $100\,\mu M$, which is indeed an order of magnitude greater than that of the acetylcholine [1]. Similarly, it is possible to estimate an upper limit for the diffusion rate constant $P \cdot A/V$, and compare that with expectation. If k_3 and k_7 are about $1.0\ sec^{-1}$ [3], then $P \cdot A/V$ (which is equal to the rate constant for the equilibrium of drug between external medium and "biophase") should be not more than about $100\ sec^{-1}$. It has been calculated [1] that free diffusion ought to clear the synaptic cleft of acetylcholine with a half-time of 2 msec, which implies that the rate constant for diffusion of drug out of (and presumably also into) the synaptic cleft is about $350\ sec^{-1}$—rather greater than the minimum required by the cyclic model. (Of course, larger values of $P \cdot A/V$ could be accommodated by increasing k_1 and k_6 and reducing X). The value of the Hill coefficient for the sigmoidal plot shown in Fig. 2 ($= 1.2$) is less than the 1.5–2.0 observed in practice [1]. However, larger values of the coefficient can be obtained by altering the relative magnitude of the various rate constants (e.g. by reducing $P \cdot A/V$).

The model makes at least three predictions which can be tested experimentally. The first is that desensitization is a necessary counterpart of co-operativity. Although both phenomena are often observed (e.g. [3]), it has been shown that γ-aminobutyric acid acts on locust muscle in a co-operative manner without desensitizing it [11]. The second prediction is that the time taken to reach the maximum response should increase as the concentration of drug decreases. This inverse relationship has been seen in some experiments [4] but not in others [12]. Finally, the time for maximum response should not be very much less than that for desensitization. This appears to be true for drugs applied either iontophoretically [3] or via a bathing medium [4], but the absolute values for the times depended strikingly on the way in which the drug was applied [4].

It is clear that the "limited biophase" model for diffusion is only a first approximation to reality. A better approximation would be to consider diffusion as occurring across several barriers in series. Nevertheless this is unlikely to alter our main conclusion, which is that the cyclic model can account for a sigmoidal response–concentration relationship together with a hyperbolic binding–concentration one (as well as for the kinetics of desensitization [13]). On the other hand the model does not seem to be able to explain all the kinetic characteristics of drug–receptor interaction.

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