PHARMACODYNAMICS OF NON-DEPOLARIZING NEUROMUSCULAR BLOCKING AGENTS

C. J. HULL

When a clinical dose of a competitive neuromuscular blocking drug is given i.v. to a patient, a period of latency is followed by a progressive onset of blockade until a maximum is reached after several minutes. Thereafter, the intensity of block declines gradually. The precise relationship between the dose of drug and the time-course of the effect is complex, being dependent upon both pharmacokinetic and pharmacodynamic factors. Following an examination of pharmacokinetic factors (Miller, 1982), this short review considers the relationship between the plasma concentration of drug and the effect.

Numerous studies have sought to elucidate this relationship, using a wide variety of agents and experimental techniques, but controversy still surrounds the mechanism involved (Kopman, 1980; Feldman, 1981; Stanski, 1981). Accepted concepts will be presented first, followed by those which are arguable or simply speculative.

THE AGONIST-RECEPTOR REACTION

According to A. J. Clark's (1933) occupancy hypothesis, an agonist ligand (D) will interact with its receptor site (R) to form bound complexes (DR); and thereby cause an effect (E)

\[ D + R \xrightarrow{k_1} DR \xrightarrow{k_2} E \]  

(1)

The association and dissociation rate constants are \( k_1 \) and \( k_2 \) respectively. The magnitude of effect is determined by the number of bound complexes, and by a proportionality constant \( k_e \) which is referred to as either efficacy (Stephenson, 1956) or intrinsic activity (Ariens, 1964). According to the law of mass action, the equilibrium equation is:

\[ \frac{[D]}{[DR]} = \frac{k_2}{k_1} = K_D \]  

(2)

where \( K_D \) is the equilibrium dissociation constant.

If \( R_T \) = total concentration of receptors, it follows that

\[ [R] = R_T - [DR] \]  

(3)

and the proportion of receptor sites occupied (\( Y_D \)) is given by

\[ Y_D = \frac{[DR]}{R_T} \]  

(4)

By substitution of equations (3) and then (4), equation (2) can be developed to:

\[ Y_D = \frac{[D]}{[D] + K_D} \]  

(5)

Equation (5) can also be expressed as:

\[ K_D = \frac{[D](1 - Y_D)}{Y_D} \]  

(6)

Inspection shows that when \( Y_D = 0.5, K_D = [D] \). Thus \( K_D \) is numerically equal to the free ligand concentration when half the receptor sites are occupied.

If we accept Clark's assumption that effect is proportional to occupancy, then \( Y_D \) also yields the fractional effect (expressed as a fraction of the effect when all receptors are occupied by ligand).

The free ligand concentration corresponding to occupancies \( Y_D = 0.2 \) and \( Y_D = 0.8 \) can easily be calculated from a further rearrangement of equation (5):

\[ [D] = \frac{K_D \cdot Y_D}{(1 - Y_D)} \]  

(7)

Thus for \( Y_D = 0.2, [D]_{0.2} = 0.25K_D \)

\[ Y_D = 0.8, [D]_{0.8} = 4K_D \]

The concentration for \( Y_D = 0.2 \) must therefore be increased by a factor of 4/0.25 = 16 to increase \( Y_D \) to 0.8.

The relationships between ligand concentration and occupancy can be expressed graphically by
direct or semilogarithmic plots. The direct plot (fig. 1A) shows that [DR] (i.e. bound ligand) plotted against [D] (i.e. free ligand) yields a rectangular hyperbola. Thus [DR] = RT when [D] = \infty, and [D] = KD when [DR] = 0.5RT.

![Fig. 1. The relationship between receptor binding [DR] and ligand concentration according to classical occupancy theory. A = linear; B = semilogarithmic; C = Hill. In each case a horizontal line corresponding to Y_D = 0.5 is dropped to the X-axis. In the linear plot this indicates KD, and in the others, log KD. In each case, KD = 1, since CD is normalized to multiples of KD (i.e. CD = [D]/KD). Following equation (9), the Hill coefficient is 1.]

The semilogarithmic* plot yields a sigmoid relationship (fig. 1B). This presentation has the advantage that a greater range of ligand can be accommodated. If the quantity of ligand bound to receptor sites is plotted on the ordinate, the asymptote yields the total number of sites (RT).

Both these plots have the disadvantage of non-linearity, so that alternative representations are frequently used to linearize the data.

The Hill (1910) plot relates logit Y_D (i.e. \log [Y_D/(1-Y_D)]) to log [D] (fig. 1C). Since Y_D represents fractional occupancy of receptor binding sites, knowledge of RT (i.e. from a preliminary semilogarithmic plot) is a prerequisite.

Examination of figure 1C shows that the line (of gradient s) intersects the ordinate (i.e. when \log [D] = 0) at point \log Q, this representing the value of logit Y_D when \log [D] = 0. The equation for the line is

$$\log\left(\frac{Y_D}{1-Y_D}\right) = s \cdot \log [D] + \log Q \quad (8)$$

The relationship between this equation and Clark's occupancy equation (5) can be readily appreciated by rearrangement of (5) and taking logarithms (Norman, 1979):

$$Y_D = \frac{[D]}{[D] + K_D} \quad (equation \ (5))$$

becomes

$$\frac{Y_D}{1-Y_D} = \frac{[D]}{K_D}$$

Taking logarithms:

$$\log\left(\frac{Y_D}{1-Y_D}\right) = \log [D] - \log K_D \quad (9)$$

in the case where s = 1, -\log K_D is clearly equal to \log Q (fig. 1), and so equation (8) reduces to equation (9).

Strictly, since K_D is the concentration when Y_D = 0.5, and -\log Q = \log K_D', Q^{-1} = K_D'. Now, by further rearrangement of equation (8), Y_D can be expressed for any value of s.

$$Y_D = \frac{[D]'}{K_D' + [D]'} \quad (10)$$

*The prefix log denotes the natural logarithm (base e) throughout this paper.
Generally known as the Hill coefficient, $s$ indicates the order of reaction between $Y_D$ and $[D]$, and is of considerable significance (although it will always be 1 in the Clark model). If we follow Clark’s assumption that effect is directly proportional to occupancy, then the Hill plot can be constructed in terms of logit $E$ v. log $[D]$, where $E$ is the magnitude of the effect expressed as a fraction of the maximum attainable effect (i.e. at $[D] = \infty$). $E_{\text{max}}$ is easily determined directly, so does not require preliminary computation. The value of log $[D]$ when logit $E = 0$ yields $EC_{50}$, the concentration required to produce a 50% effect (i.e. 0.5$E_{\text{max}}$) (Wagner, 1968).

Thus

$$E = \frac{[D]^s}{EC_{50}^s + [D]^s} \tag{11}$$

Equation (11) is directly equivalent to (10) if Clark’s model is valid.

COMPETITIVE ANTAGONISM

A competitive antagonist (I) will react with free receptor sites, but has no efficacy:

$$I + R \xrightarrow{k_3} IR \xrightarrow{k_4} I$$

Taking into account the presence of antagonist (Ariens, 1966), the occupancy equation for agonist becomes:

$$Y_D' = \frac{[D]}{K_D(1 + [I]/K_I) + [D]} \tag{13}$$

(The prime indicates the presence of a second drug (Mackay, 1966).)

Occupancy equations are more easily expressed with the concentrations expressed as multiples of their respective equilibrium dissociation constants, so

$$C_D = \frac{[D]}{K_D} \quad \text{and} \quad C_I = \frac{[I]}{K_I} \quad \text{(Waud, 1968)}$$

Thus equation (5) becomes

$$Y_D = \frac{C_D}{C_D + 1} \quad \tag{14}$$

and equation (13) is simplified to:

$$Y_D' = \frac{C_D}{C_D + C_I + 1} \tag{15}$$

Clearly, when negligible antagonist is present, the equation reduces to (14). However, as $C_I$ increases, the concentration of agonist required to maintain the same occupancy increases (i.e. its value of $K_D$ appears to increase). Consequently, the semilogarithmic and Hill plots are displaced to the right (fig. 2). Equation (15) can be expressed in terms of antagonist occupancy:

$$Y_I = \frac{C_I}{C_I + C_D + 1} \quad \tag{16}$$

When negligible agonist ($C = 0$) is present, equation (16) reduces to

$$Y_I = \frac{C_I}{C_I + 1} \quad \tag{17}$$

which is directly equivalent to (14).

![Figure 2](image-url)

Fig 2. Figure 1a and c are developed to show the addition of a competitive antagonist in normalized concentrations $C_I = 2, 4$ and 6 (equation (15)). The agonist occupancy curves are shifted to the right, so that at any value of $C_D$, agonist occupancy is progressively diminished.
Thus the semilogarithmic and Hill representations can be applied to antagonists in the same way as agonists, simply by substituting fractional inhibition ($I'$) for fractional effect ($E$). However, since fractional inhibition (note the prime) can never be measured in the absence of agonist, the denominator in equation (16) always includes a term representing the presence of agonist. Thus the concentration of antagonist which results in 50% inhibition ($EC_{50}$) can be readily determined from either semilogarithmic or Hill plots (fig. 3), but cannot be used to yield an estimate of $K_t$.

Equation (16) also indicates that, in the presence of a constant amount of antagonist, an increase in agonist concentration will result in a lower antagonist occupancy $Y'$. Thus equation (12) moves to the left, and some antagonist is “displaced” from binding sites as a new equilibrium is reached.

The rate at which this happens depends upon the association and dissociation rate constants for both agonist and antagonist (equations (1) and (12)).

DYNAMICS OF LIGAND–RECEPTOR INTERACTIONS

Up to this point we have considered only the equilibrium condition, ignoring the manner in which equilibrium is approached. Many drug receptor reactions are very rapid indeed, making a study of unsteady states somewhat academic, but others are much slower. Indeed, the rate of agonist–receptor association and dissociation has been claimed (Feldman and Tyrrell, 1970; Feldman, 1981) to be rate-limiting in the onset and offset of effect, so some consideration of the basic principles is worthwhile.

Following early work by Hill (1909), Clark (1926) showed that, when ligand is added to an isolated muscle preparation, the equilibrium of equation (5) is approached exponentially:

$$Y_D = \frac{C_D}{C_D + 1} (1 - e^{-kt})$$

(18)

where

$$k = k_1 [D] + k_2.$$  (See equation (1).)

The half-time for the association process ($0.693/k$) depends upon both association and dissociation constants (since dissociation begins as soon as some drug is bound) and the free ligand concentration. Thus the higher the concentration, the more rapidly is equilibration approached.

If all ligand is instantaneously cleared from the bath, and dissociating ligand is prevented from reassociation by rapid washing, then the dissociation process depends upon $k_2$ only:

$$Y_D = \frac{C_D}{C_D + 1} e^{-kt}$$

(19)

where $k = k_2$.

Since $K_D$ is the ratio $k_2/k_1$ and determines the degree of binding at equilibrium, it gives no clue as to the rate of equilibration during either onset or offset. If $[D]$ is known, and $k_2$ is determined during washout, $k_1$ can be calculated from the onset data. Early studies made use of this method, and reported that some antagonists dissociated very slowly from receptors. Paton (1961) concluded that atropine dissociated from guineapig ileum with a half-time of 40 min; this undoubtedly gave impetus to the concept that the same might apply to some neuromuscular blocking agents. However, later work (Paton and Rang, 1965; Thron and Waud, 1968) showed that the time taken for atropine to wash out of the tissue was a rate-limiting factor, since the apparent value of $k_2$ diminished dramatically to 4–5 min when isolated, very thin strips of muscle were used instead of intact gut.

In the situation where the drug is not removed instantaneously, the “off” kinetics do not follow...
PHARMACODYNAMICS

equation (19), but simply move to a new equilibrium as the biophase concentration changes (Boynaems and Dumont, 1980).

If the ligand concentration changes from initial condition $C_D^i$ to a final value of $C_D^f$, the occupancy will shift from

$$Y_D^i = \frac{C_D^i}{C_D^i + 1} \quad \text{to} \quad Y_D^f = \frac{C_D^f}{C_D^f + 1}$$

Thus

$$Y_D = Y_D^i + (Y_D^f - Y_D^i) \cdot 1 - e^{-kt} \quad (20)$$

where

$$k = k_1[D]^f + k_2$$

(as in equation (18)).

In the absence of ligand, the same equations apply to a competitive antagonist.

AGONISM AND ANTAGONISM AT THE CHOLINERGIC RECEPTOR

If Clark's model were to apply, an agonist concentration–effect curve would be hyperbolic (equation (5)), and the corresponding Hill plot should be linear with a slope of 1. Unfortunately, this is simply not the case, since there is abundant evidence (Katz and Thesleff, 1957; Jenkinson, 1960; Rang, 1971) that the acetylcholine concentration/membrane conductance curve is sigmoid (fig. 4), yielding a Hill plot of gradient 1.6–1.9 over a wide range of agonists (Changeux and Podleski, 1968). This behaviour (fig. 4) is characteristic of a phenomenon known as positive co-operativity, which is defined as a condition in which the order of reaction is greater than 1. It is characterized by:

1. The ratio $[D]_0/[D]_2$ (see equation (7)) is less than 16, so that the effect develops over a narrower range of concentrations than in the Clark model.
2. The Hill coefficient is greater than 1.

The Hill coefficient is a direct index of co-operativity; hence the utility of this construction. The binding of oxygen to haemoglobin is a classic example of molecular co-operativity (Hill, 1910), where binding of one oxygen molecule enhances the affinity for the next, but co-operativity may also be purely phenomenological, with no direct interaction between receptor sites at all.

Thus in the case of the cholinergic receptor we are faced with several possibilities:

(a) As in a model proposed by Hodgkin and Huxley (1952) for the potassium channel in a squid axon, each ionophore may be associated with a number of ligand-binding subunits, some of which must be occupied simultaneously in order to induce a conformational change in the ionophore and thus open the sodium channel.
The relation between effect and concentration is co-operative as a direct result of the multiplicative probability of \( n \) simultaneous events, and no interaction between subunits is required. There is a good deal of evidence to support the subunit concept, since each ionophore is surrounded by a "rosette" of apparently identical structures, each of which is likely to possess a ligand binding site (Cartaudl et al., 1978; Rash, Hudson and Ellisman, 1978). However, the probability model insists that the Hill coefficient is equal to \( n \), and must therefore be an integer.

(b) A class of allosteric models may be postulated, in which each receptor site can exist in two (or more) states, each of which has a different affinity for the ligand (Karlin, 1967). Thus we can imagine a model consisting of independent subunits, each of which may exist in two conformational states, closed (R) with low affinity for ligand, in equilibrium with open (T) which has high affinity. The equilibrium may be presented as:

\[
\begin{align*}
\text{channel closed} & \quad \frac{K_o}{K_{DRT}} \quad \text{channel open} \\
D + R & \rightleftharpoons T + D \\
DR & \rightleftharpoons DT
\end{align*}
\]

Since the dissociation constant \( K_o \) is large, the majority of units exist in the R state in the absence of ligand. When ligand is added to the system, it binds to both R and T forms in proportion to their affinities. If \( K_{DRT} \) is large, and \( K_{DRT} \) very small, T is consumed to form DT, and the equilibrium \( R \rightleftharpoons T \) shifts to the right, thus creating more high affinity sites. Thus the addition of ligand appears to have caused a conformational change in the receptor subunit (Colquhoun, 1973).

This added refinement frees the model from the constraint of an integer Hill coefficient.

The model proposed by Monod, Wyman and Changeux (1965) is based on the above principle, but supposes that all subunits change state in a concerted fashion, so that each receptor complex can exist in only two states. The intermediate states in which some subunits are "T" and others "R" are thus excluded for the sake of simplicity.

Koshland, Nemethy and Filmer (1966) proposed a sequential model, in which no such constraint is imposed, but individual subunits within the "rosette" interact with each other. Thus occupation of one subunit induces a conformational change in its immediate "neighbours" to a higher affinity state. Thus a number of hybrid states can occur, depending upon the geometry of the subunit cluster.

In both the above cases, the Hill coefficient will exceed 1, but the Hill plot itself becomes non-linear, so that equation (11) remains valid only over a narrow range of concentrations.

Identification of one of these proposed models with the cholinergic receptor presents great difficulty, since each can yield saturation curves which closely mimic those obtained experimentally. However, there are some clues. The Hill coefficient does not appear to be an integer. Pre-treatment of the Electrophax membrane with dithiothreitol (which reduces disulphide bonds) reduces the Hill coefficient from 1.8 to 1.3, suggesting that the phenomenon of co-operativity is not simply based on occupational probability (Karlin, 1967). More directly, decamethonium in very low concentrations has been shown to reduce the Hill coefficient for carbachol from 1.8 to 1.0 (Changeux and Podleski, 1968), although this finding is open to several interpretations (Podleski, 1973). Recent work has shown that the agonist–receptor interaction involves several (probably three) time constants and affinities, thus favouring the concept of an interactive, allosteric model (Giraudat and Changeux, 1981).

Antagonism

If some kind of molecular co-operativity is postulated for the agonist, then competitive antagonism must be considered in those terms. There are three possibilities (Boynaems and Dumont, 1980):

The antagonist

(a) has greater affinity for the "T" configuration. This would enhance the agonist at low concentrations before inhibiting it at high concentrations, and agonist co-operativity would be sharply increased.

(b) has greatest affinity for the "R" form. This would exert its effect both by direct site competition and by reducing the co-operativity of the agonist.

(c) has no preference for either form. Here, inhibition proceeds according to the classical equation (16), and simply increases the apparent value of \( K_D \).
The author is unaware of evidence that a competitive antagonist may modify the Hill coefficient for acetylcholine, so options (a) and (b) would seem unlikely. If we accept (c), then there is no molecular co-operativity involved in the process of antagonism.

Paton and Waud (1967) showed that in the cat, nearly 80% of receptors must be occupied by tubocurarine before blockade begins, and 90% for complete block. However, this apparent co-operativity can be readily explained by the following evidence: First, each nerve impulse releases much more acetylcholine than is needed to generate the critical degree of end-plate depolarization which triggers an action potential (Paton and Waud, 1967). Second, only a small minority of receptors need be occupied (fig. 5) in order to generate an action potential; the remaining majority constitute "spare receptors" (Ginsborg and Jenkins, 1976). (N.B. that is not to say that those receptors are truly spare, since that would imply that a maximal increase in membrane conductance could be achieved without them.) The release of excess agonist, and the consequent occupation of more receptors than required, gives the system a "margin of safety" (Paton and Waud, 1967), which ensures that the efficiency of neuromuscular transmission is not impaired by variations in acetylcholine output. Thus a good deal of "antagonism" must take place at receptor level before any macroscopic effect is seen. Since the generation of an action potential is an all-or-none process, the degree of block achieved by each successive level of occupancy is determined by the probability distribution of receptor occupancy by both agonist and antagonist, and by differences in firing threshold over a large population of end-plates.

EQUILIBRIUM CHARACTERISTICS OF COMPETITIVE ANTAGONISTS

As the whole binding curve of a classical agonist can be characterized by $K_D$, so the behaviour of an antagonist can be described by equation (16) if we know $K_I$ (and can thereby calculate the normalized concentration $C_I$).

$K_I$ can be determined in isolated preparations by the "dose-ratio" method, in which the ratios of antagonist to agonist required to keep a "response" constant over a range of antagonist concentrations are determined. (Since any competitive agonist yields the same result, carbachol is usually preferred to acetylcholine.) The results are plotted on a Schild plot (Arunlakshana and Schild, 1959), from which $K_I$ can be read directly (fig. 6). Table I lists the values of $K_I$ determined by this method for several neuromuscular blocking agents. Results in man are notable by their absence.

![Fig. 5](image1)  
**Fig. 5.** The margin of safety. The co-operative ($z = 1.9$) agonist occupancy curve of figure 4B is plotted over a limited range (labelled $C_I = 0$), corresponding to an acetylcholine pulse which (say) just reaches an occupancy of 0.5. The effects of increasing normalized concentration of antagonist ($C_I = 2, 4, 6$ and 10) are shown in the lower curves. If an action potential is assumed to follow $Y_D = 0.2$ (horizontal line), then occupancy is considerably in excess of that required. At $C_I = 2$, agonist occupancy is reduced, but is still greater than 0.2. Only when $C_I = 4, 6$ and 10 does $Y_D$ decrease to less than 0.2, and a neuromuscular block develop.

![Fig. 6](image2)  
**Fig. 6.** The Schild plot. Over a range of agonist concentrations, the concentrations of antagonist required to maintain a constant effect are determined. A plot of log (dose ratio - 1) vs. log $[I]$ will, in the case of true competitive antagonism, yield a linear function of gradient 1. The intercept with the horizontal axis yields log $K_I$. 

---

**Table I**

<table>
<thead>
<tr>
<th>Agent</th>
<th>$K_I$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tubocurarine</td>
<td>2.1</td>
</tr>
<tr>
<td>Neostigmine</td>
<td>1.3</td>
</tr>
<tr>
<td>Physostigmine</td>
<td>0.5</td>
</tr>
<tr>
<td>Pancuronium</td>
<td>0.1</td>
</tr>
<tr>
<td>Gallamine</td>
<td>0.05</td>
</tr>
</tbody>
</table>

---
TABLE I Estimates of $K_i$ by the "dose-ratio" method for different muscle relaxants in the guinea pig

<table>
<thead>
<tr>
<th>Agent</th>
<th>Species</th>
<th>$K_i$(nmol)</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tubocurarine</td>
<td>Guineapig (diaphragm)</td>
<td>108</td>
<td>Lu (1970)</td>
</tr>
<tr>
<td>Tubocurarine</td>
<td>Guineapig (lumbrcal)</td>
<td>110</td>
<td>Waud, Cheng and Waud (1973)</td>
</tr>
<tr>
<td>Tubocurarine</td>
<td>Guineapig (lumbrcal)</td>
<td>105</td>
<td>Waud (1977)</td>
</tr>
<tr>
<td>Pancuronium</td>
<td>Guineapig (lumbrcal)</td>
<td>25.1</td>
<td>Waud, Cheng and Waud (1973)</td>
</tr>
<tr>
<td>Fazadinium</td>
<td>Guineapig (lumbrcal)</td>
<td>377</td>
<td>Waud (1977)</td>
</tr>
</tbody>
</table>

Several authors (Lund and Stovner, 1970; Matteo, Spector and Horowitz, 1974; Agoston et al., 1977) have attempted to estimate $K_i$ by simply determining the plasma concentration of drug at 50% inhibition of twitch height. For this method to be valid, the following conditions must be met: First, the whole kinetic system must be at equilibrium, so that equilibration time constant (i.e. equations (18) and (19), and those for transport to the receptor) do not distort the result. Second, the "free" plasma concentration with which the biophase presumably equilibrates must be determined. This is only possible if the degree of plasma protein binding is measured as part of the study. Finally, the effect of agonist concentration upon the apparent value of $K_i$ must be known. Inspection of equation (16) shows that the presence of agonist is a determinant of antagonist occupancy. It can be argued that when neuromuscular transmission is studied by single twitches at 10-s intervals the antagonist occupancy between stimuli is the principal determinant, and that acetylcholine pulses at such long intervals will not disturb the occupancy equation (i.e. $C_D$ is effectively zero); thus equation (16) reduces to:

$$Y_i = \frac{C_i}{C_i + 1} \quad (22)$$

However, the random release of acetylcholine quanta between stimuli makes (22) an unsafe simplification; and it has been shown by Blackman, Gauldie and Milne (1975) that tubocurarine dissociates quickly enough to be significantly displaced during the period of a very short ionophoretic pulse of acetylcholine (in the frog). This and other evidence (see below) suggests that

TABLE II Estimates of EC$_{50}$ and Hill coefficient (s) for different muscle relaxants in man. Results marked * were not reported, and have been calculated from the experimental results

<table>
<thead>
<tr>
<th>Agent</th>
<th>EC$_{50}$</th>
<th>$s$</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(μg ml$^{-1}$)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tubocurarine</td>
<td>0.37</td>
<td>2.53</td>
<td>Sheiner and others (1979)</td>
</tr>
<tr>
<td>Tubocurarine</td>
<td>0.45</td>
<td>3.48*</td>
<td>Matteo, Spector and Horowitz (1974)</td>
</tr>
<tr>
<td>Pancuronium</td>
<td>0.25</td>
<td>4.18--17</td>
<td>Shanks, Somogyi and Triggs (1979)</td>
</tr>
<tr>
<td>Pancuronium</td>
<td>0.113</td>
<td>8.17*</td>
<td>Agoston and others (1977)</td>
</tr>
<tr>
<td>Pancuronium (bolus)</td>
<td>0.10</td>
<td>5.63*</td>
<td>Agoston, Feldman and Miller (1979)</td>
</tr>
<tr>
<td>(infusion)</td>
<td>0.14</td>
<td>3.63*</td>
<td></td>
</tr>
<tr>
<td>Pancuronium</td>
<td>0.29</td>
<td>7.75</td>
<td>Hull, English and Sibbald (1980)</td>
</tr>
<tr>
<td>Fazadinium</td>
<td>1.005</td>
<td>4.46</td>
<td>Hull, English and Sibbald (1980)</td>
</tr>
</tbody>
</table>
confidence in an undisturbed antagonist equilib-rium is misplaced, and that the simple “clinical” method may approach the truth only by mutual cancellation of numerous errors (table II).

Variation in the results of different investiga
tors may also be related to the methods used. In particular, variation in stimulus frequency (Ali and Savarese, 1980), the choice of e.m.g. or mechanographic recording, muscle pre-tension (Donlon, Savarese and Ali, 1979) and previous exposure of the preparation to such interfering agents as depolarizing muscle relaxants (Katz, 1971), halogenated anaesthetic agents (Hughes and Payne, 1979), some antibiotics (Sokoll and Gergis, 1981) or even i.v. anaesthetic agents (Kraunak, Pleuvry and Rees, 1977) will have profound effects upon the results obtained. Some investigators (e.g. Payne and Hughes, 1981) have chosen to study the phenomenon of tetanic fade on the grounds that it studies neuromuscular transmission under more physiological conditions than does the single twitch. This is quite true, but almost certainly measures prejunctional as well as postjunctional effects of the neuromuscular blocking agent. Since drugs differ widely in this respect (Williams, Webb and Calvey, 1980), the added complication appears unwelcome to this author.

**Kinetics in the Biophase**

It will be appreciated that a static treatment of the neuromuscular junction is almost meaningless, since the effects of antagonists appear to be determined by the rates at which processes occur. The question is: which rates?

For many years Feldman has argued (Feldman and Tyrrell, 1970; Feldman, 1981) on the basis of Paton's (1961) rate theory that the duration of action of a competitive blocking agent is primarily determined by the rate at which it dissociates from the receptor (i.e. up to 1 h or more at each receptor site). His argument is founded upon isolated arm experiments in which, after application of a tourniquet, a small dose of relaxant was injected to the dorsum of the hand in order to perfuse the muscle by retrograde spread, and after a short delay, the tourniquet released and recovery measured using evoked twitch responses in adductor pollicis. Table III summarizes the results of his and other comparable studies. Since these authors assumed that plasma concentration must be negligible, and receptor-capillary diffusion rapid, slow drug-receptor dissociation was thought to offer the only rational explanation for the observed delay in the recovery of neuromuscular transmission.

Feldman and Tyrrell also showed that, after a paralysing dose of tubocurarine, the block in one arm (stimulated with single twitches at 0.2 Hz) recovered more slowly than that in the other (stimulated with tetanic trains of stimuli once per minute). They argued that receptor-bound tubocurarine molecules are displaced by physical bombardment with acetylcholine, so that tetanic stimulation would be expected to accelerate recovery. The results of this experiment were claimed to substantiate the “dissociation theory” for the termination of the effect of tubocurarine.

Waud (1975) suggested that the isolated arm results could be equally well explained by limitation of access of drug to the extracellular space of the muscle (and therefore the biophase), with blood flow being the rate-limiting factor. This explanation was contested by Heneghan and his colleagues (1978), who showed that rate of recovery (in the dog) from paralysing doses of pancuronium was not influenced by large changes in blood flow.

Feldman's assertion that plasma concentration had little to do with the rate of recovery was difficult to reconcile with numerous studies (Matteo, Spector and Horowitz, 1974; Agoston et al., 1977; Shanks, Somogyi and Triggs, 1978) in which recovery of twitch height correlated well with the decline of the logarithm of the plasma concentration. Steady state studies (e.g. Shanks, Somogyi and Triggs, 1978) showed a similar relationship. Other authors (Hull et al., 1978; Sheiner et al., 1979; Hull, English and Sibbald, 1980) have approached the problem by developing pharmacodynamic models which are based upon the concept that the biophase may be visualized mathematically as an elementally small

<table>
<thead>
<tr>
<th>Agent</th>
<th>Rate of recovery (°, per min)</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tubocurarine</td>
<td>3.2</td>
<td>Feldman and Tyrrell (1970)</td>
</tr>
<tr>
<td>Gallamine</td>
<td>5.2</td>
<td>Feldman and Tyrrell (1970)</td>
</tr>
<tr>
<td>Pancuronium</td>
<td>5.0</td>
<td>Agoston, Feldman and Miller (1979)</td>
</tr>
<tr>
<td>Fazadinium</td>
<td>5.05</td>
<td>Hashimoto and others (1979)</td>
</tr>
</tbody>
</table>
pharmacokinetic compartment the concentration of which will follow (by means of first-order rate constants) that in the plasma, and the Hill transformation between concentration and effect (equation (11)). These authors and others (Shanks, Somogyi and Triggs, 1979) using the same method have shown that after a bolus dose of tubocurarine, pancuronium or fazadinium, plasma concentrations of drug can be fully reconciled with twitch depression throughout the time-course of the drug. This method is not without its difficulties, and Shemer and others (1979) have been described (with some cause) as having reached “a new zenith of incomprehensibility” (Feldman, 1980). Agoston, Feldman and Miller (1979) later compared the recovery rate after isolated arm experiments with that after a continuous infusion and found that, at 50% recovery, the plasma concentrations in the post-infusion series were about three times greater than those in the isolated arm series. The isolated arm subjects also recovered significantly (four to six times) more rapidly. The results after a bolus dose were intermediate. They interpreted their results as suggesting a compromise theory: if the plasma concentration decreases very slowly, removal of drug from the synaptic cleft is the limiting factor. If, on the other hand, concentration in plasma decreases abruptly (or is very low to start with), then receptor dissociation is rate-limiting.

Clearly, clinically based studies could continue for ever without proving anything, since each author’s results simply serve to reinforce his own point of view. Other, more direct evidence must be sought regarding the rate-limiting factors, and for this we must return to more fundamental studies on isolated preparations.

The first question is very simple: how fast do muscle relaxants associate and dissociate? If we discard the fanciful notion that bound tubocurarine molecules can be displaced wholesale by direct collision with acetylcholine, and retain our belief in the law of mass action, then determination of $k_1$ and $k_2$ in equation (20) should resolve the matter.

Waud (1967) attempted to measure these constants in the frog end-plate, but found that, as the experiment refined, the constant appeared to accelerate. He concluded that, although drugs were applied directly to the end-plate by ionophoresis through a micropipette, diffusion to and from the receptors was rate-limiting. Even so, the onset and offset half-times were of the order of a few seconds. He pointed out that the shape of the saturation curves for acetylcholine and tubocurarine would result in much slower onset and offset of effect with the latter. A 20% (approximately) receptor occupancy by an agonist can initiate an action potential. This (fig. 5) can be achieved by a small change in agonist concentration, since the steep part of the occupancy curve is involved and the interaction is co-operative. On the other hand, diffusion of antagonist into the cleft must achieve 80% occupancy before any inhibition of effect is seen, and 90% for full inhibition, because of the agonist’s “margin of safety”. Thus onset will be delayed despite a high antagonist plasma concentration, and offset takes place along the very flat upper zone of the antagonist occupancy curve (fig. 3), where a large change of concentration must take place to achieve a 10% reduction of occupancy. Waud argued that this change in concentration depends upon the rate of decline in plasma concentration and the rate at which drug is washed out of the muscle.

More recently, Armstrong and Lester (1979) have reported a series of studies on the frog neuromuscular junction, using acetylcholine as the agonist. They found that by positioning a double-barrelled micropipette very close to the cleft, recovery after pulses of tubocurarine occurred exponentially with a time constant of 2s. The onset of inhibition varied with concentration as expected (equation (18)), but was at least 10 times faster. Although rapid, these reactions are very slow compared with the response to acetylcholine pulses (on and off in 20 ms).

To determine whether the “slow” response was a result of receptor binding or of diffusional delay, Armstrong and Lester performed several further experiments:

1. By the application of cobra α-toxin, which irreversibly occupies receptor sites, a controlled proportion of the receptor population was occluded. Consequently, the recovery time constant increased threefold, suggesting that recovery rate depends upon receptor density.

2. After collagenase treatment, part of the nerve terminal could be displaced from the postsynaptic membrane. Following this disturbance, end-plate potentials could be produced and inhibited as before, but with the “off” time constant reduced to approximately 0.1s. This
observation suggests that slow onset and offset are dependent upon the integrity of end-plate architecture.

(3) Over the range 14–29 °C, the “on” and “off” time-constants are only weakly temperature-dependent.

If receptor dissociation were determining the recovery time constant, partial occlusion by a α-toxin and disruption by collagenase would have had no effect, whereas a considerable temperature-dependence would have been expected. The authors concluded that the receptor dissociation rate must have been much faster than the fastest rates observed, with diffusional factors responsible for the difference.

Convincing though Armstrong and Lester’s findings might be, acceptance that the receptor rate constants $k_3$ and $k_4$ for tubocurarine are very much faster than had previously been supposed raises a new, and equally fundamental question: “If receptor dissociation is not a rate-limiting factor, how can diffusional factors be responsible, since Eccles and Jaeger (1958) estimated that, on a dimensional basis, tubocurarine should diffuse in and out of the cleft with a time constant of one millisecond?”

Buffered diffusion

Furchgott (1955) proposed an access-limited biophase model in which diffusion to the receptor site played an important part. However, for the sake of simplicity, receptor uptake was assumed to be negligible. Realizing that Furchgott’s model could not possibly account for very slow diffusion, as must be the case here, Rang (1966) relaxed Furchgott’s “nil-uptake” assumption to form the “limited biophase” model, in which receptor uptake has a profound effect upon biophase concentration. This is particularly applicable to the neuromuscular junction, where the concentration of receptors reaches 3000 per μm² on junctional crests (Fertuck and Salpeter, 1974; Rash, Hudson and Ellisman, 1978). Here, where the “diffusion only” time constant is $\tau_d$, the biophase has a volume of $V_{bio}$litre containing a receptor population of $R_{bio}$mol, the apparent diffusional time constant at low antagonist concentrations becomes

$$\tau_{bio} = \left(1 + \frac{R_{bio}}{K_1} \cdot V_{bio}\right) \tau_d \quad (23)$$

Armstrong and Lester estimated that, with likely values substituted into equation (23) ($R_{bio}/V_{bio} = 300 \mu$mol and $K_1 = 0.5 \mu$mol), $\tau$ would increase to about 1 s. This is not surprising when it is appreciated that at 50% occupancy by tubocurarine, only 1 drug molecule in 300 is likely to be free, so that the “apparent” volume of the biophase is 300 times greater (when I = $K_1$) than the anatomical value. At lower concentrations the effect is greater; at higher concentrations, less. Thus at low occupancies, a newly dissociated molecule of antagonist is much more likely to recombine with a receptor site for a further period than to diffuse out of the cleft. Equation (23) shows that the apparent diffusion rate is now a function of $K_1$, since high affinity antagonists will be more “buffered” than low affinity agents with large values of $K_1$.

The recent findings of Minsaas and Stovner (1980) that the artery-to-muscle onset latencies for pancuronium ($K_1 = 25$ nmol), tubocurarine ($K_1 = 110$ nmol) and fazadinium ($K_1 = 377$ nmol) were 31.9, 21 and 8.6 s respectively would appear to support this hypothesis.

Buffered diffusion explains the difference between receptor interactions on a millisecond timescale and the intact-junction results of Armstrong and Lester. It does not provide a rationale for the slow recovery from an isolated-arm experiment.

In the author’s view the arguments for fast dissociation and buffered diffusion are irresistible, so a third question is raised: “If all that is true, how do you explain Feldman’s isolated-arm results, which others have confirmed, and the different recovery rates in the two-arm experiment?”

If we accept the concept of buffered diffusion, then the relatively large time-constants which appear to be interposed between plasma concentration and inhibition of effect must be explained. The saturation curve itself may (as suggested by Waud) play a major role, since it intensifies the effect of access-limitation. There must also be an appreciable time lag between capillary and junctional cleft; note the appreciable difference between the results of Waud (1967) and those of Armstrong and Lester (1979) who simply placed their pipette a few microns nearer the end-plate. The role of perfusion must also be considered. Henehan’s study (Henehan et al., 1978) did not exclude the influence of perfusion, since the rate-limiting factor in his experiment was almost certainly plasma concentration, in which case the negative results were predictable.
Pharmacokinetic factors are also involved, since Feldman's early assumption that after a small (3-mg) dose of tubocurarine into the isolated arm, plasma concentration would be negligible, is simply not tenable. All non-depolarizing agents have small apparent volumes of distribution, so that recirculation after tourniquet release would be expected to yield concentrations which would considerably reduce the concentration gradient between biophase and plasma. The biophase model of Hull, English and Sibbald (1980) for fazadinium predicts that, if the small dose into an isolated arm does not produce a 100% block, the plasma concentration after tourniquet release may actually exceed that in the biophase for a few circulation times, so that drug will diffuse into the biophase, and the block will intensify. Inspection of the fazadinium study by Hashimoto and his colleagues (1979) shows that this was indeed the case. The tubocurarine study of Feldman and Tyrrell (1970) shows a similar effect, which was not commented upon by those authors.

No significant block of other muscles would be expected, since the plasma concentration, being in its $\alpha$-decay phase, decays too quickly for biophase concentrations to reach effective values.

Differential recovery from the two-arm experiment can also be explained (Hull, 1980). If dissociation of antagonist from receptors is rapid then, as observed by Blackburn, Gauldie and Milne (1975), a pulse of acetylcholine will (momentarily) increase the "free" drug concentration in the junctional space. Some of this newly dissociated drug will recombine with receptors when the agonist-antagonist equilibrium is restored, but some will diffuse outwards, and enter the diffusional pathway to the local capillary. After a tetanic burst, the same applies, but much more "displacement" of antagonist occurs, and for much longer. Thus diffusion from the cleft is greatly accelerated during the tetanus. If the plasma concentration is lower than that in the cleft, then return is unlikely. Therefore it follows that an arm subjected to tetanic stimuli will recover more rapidly than one subjected only to single twitches. It should be noted that the "displacement" of antagonist is more likely to occur as a result of rapidly shifting equilibria than by physical bombardment.

The different results following isolated arm bolus and infusion administration (Agoston, Feldman and Miller, 1978) are not unexpected when one considers the differing concentration gradient between biophase and plasma in the three cases.

These authors' results are quite in keeping with the behaviour of the pharmacodynamic models of both Hull and Sheiner (except that the isolated arm case cannot be modelled by the latter).

How, then, can we finally relate plasma concentration with neuromuscular blockade? It is evident that receptor occupancy by antagonist is dependent upon passive diffusion from the nearest capillary, although receptor buffering makes this process quite slow. Drug concentration in the peri-capillary e.c.f. will be in close equilibrium with free (i.e. not protein-bound) drug in plasma. pH partition need not be considered since all quaternary ammonium compounds are 100% ionized at pH 7.4. Thus there is a relationship between plasma concentration and effect at all times, but that relationship is multifactorial, non-linear and subject to one or more first-order lag-factors (fig. 7). Failure to appreciate the nature of these processes has led some authors to refute the relationship, or propose more unlikely explanations. In order to sustain such arguments it is no longer enough to claim simply that one is right; the elegant experiments of Waud, Armstrong, Lester, Rang and others must be shown to be erroneous.

**Fig. 7** Factors which influence the relationship between the plasma concentration of a competitive neuromuscular blocking agent and the effect upon neuromuscular transmission. Factors influencing agonism are excluded.
REFERENCES
Clark, A. J. (1926) The reaction between acetylcholine and muscle cells. J. Physiol. (Lond.), 61, 530.
— (1910). The possible effect of the aggregation of the molecules of haemoglobin on its dissociation curves. J. Physiol. (Lond.), 40, 190.


---


