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Theoretical Characterization of Ion Channel Blockade: Ligand Binding to Periodically Accessible Receptors†

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With repetitive stimulation, the time course of use-dependent blockade as assessed by peak membrane ion currents can be described by a sequence of blocking relationships that have the form of recurrence equations. The equations of the sequence describe blockade acquired during each interval of a stimulus where the possibly different binding and unbinding rates are assumed constant during each interval. The solution predicts that use-dependent uptake follows an exponential time course. Furthermore, the exponential uptake rate is a linear function of uptake rates associated with the stimulus time intervals. Similarly, the fraction of blocked channels at steady state is a linear function of the interval dependent blockade equilibria. Several novel tests of consistency between the model and observations are derived from these theoretical results. It is also shown that as the stimulus interval increases to infinity, steady state dissociation constants measured by peak membrane currents are theoretically equivalent to those measured with true equilibrium methods such as radioligand binding studies.

Introduction

With the modulated receptor hypothesis, Hille (1977) introduced the formal notion of a state-dependent ion channel affinity for blocking agents. Recently, we investigated the case where the state-dependent variation in channel binding affinity was considered to be the result of channel gate control of receptor access (Starmer *et al.*, 1984; Starmer & Hollett, 1985). Using the Hodgkin-Huxley gate formalism (Hodgkin & Huxley, 1952) in conjunction with a bimolecular first order binding process, we were able to numerically integrate the differential equations describing use-dependent ion channel blockade. However, due to the complexity of the H-H gating coefficients, we were unable to derive a closed form solution.

Recent observations of single channel events using the patch clamp technique show transitions between open and closed conformation to be rapid, and for the most part, channel open times follow an exponential distribution (Grant *et al.*, 1983; Cachelin *et al.*, 1983). Assuming that binding sites become simultaneously accessible and remain accessible for a time equal to the mean conformation dwell time, we have shown that channel blockade associated with simple pulse train stimulation can be described by a sequence of simple algebraic recurrence relations (Starmer & Grant, 1985). Here we will extend and generalize the description to cover more

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than two states and complex pulse protocols. Furthermore, we will derive several new and novel relationships between channel blockade and stimulus protocols that lead to a simple parameter estimation procedure. We will then compare these theoretical predictions with published data for sodium, calcium, and potassium channel blockade.

Continuous Blockade Process

In its most general form, we can describe the modulated receptor hypothesis in terms of a simple bimolecular interaction of unblocked channels, U, and drug, D, to form blocked channels as

$$U + D \underset{l_s}{\overset{k_s}{\rightleftharpoons}} B$$

where k_s and l_s are state (voltage) dependent binding and unbinding rates. Using the law of mass action to describe binding and unbinding processes, blockade is described by

$$\frac{dB}{dt} = k_s D[C_{max} - B] - l_s B \tag{1}$$

where C_{max} is the maximum number of channels. Let b be the fraction of blocked channels ($b = B/C_{max}$). Then for constant binding and unbinding rates, the time course of blockade is described by

$$b(t) = b(0)\, e^{-t/\tau} + b(\infty)(1 - e^{-t/\tau}) \tag{2}$$

where

$$\tau = (k_s D + l_s)^{-1} \quad \text{and} \quad b(\infty) = \left(1 + \frac{l}{k_s D}\right)^{-1}. \tag{3}$$

These relationships describe channel blockade as an exponential process, starting with an initial fraction of blocked channels, $b(0)$, and eventually reaching an equilibrium, $b(\infty)$.

Periodic Two-State Blockade Process

Consider now the bimolecular blockade process in a setting where the channel switches states in response to periodic excitation. Let the periodic excitation of the channel have a period, I, consisting of a rest interval of duration t_r followed by an excited interval of duration t_e. Let the equilibrium block, uptake rate and time constant associated with the excited state be E_∞, λ_e and τ_e and the equilibrium block, uptake rate and time constant associated with the resting state be R_∞, λ_r and τ_r. Thus,

$$E_\infty = \left(1 + \frac{l_e}{k_e D}\right)^{-1} \tau_e^{-1} = \lambda_e = k_e D + l_e \tag{4}$$

$$R_\infty = \left(1 + \frac{l_r}{k_r D}\right)^{-1} \tau_r^{-1} = \lambda_r = k_r D + l_r \tag{5}$$

et R_0 be the initial fraction of blocked channels. Let E_i and R_i represent the action of blocked channels at the end of the excitation and rest intervals. Note at the progressive change in block during pulse train stimulation can be described / a sequence of recurrence relations where the fraction of blocked channels at the id of each interval is used as an initial condition for computing block during the ext interval. Thus applying equation (2) to each interval we have

$$R_0 = \text{initial block}$$

$$E_0 = R_0\, e^{-\lambda_e t_e} + E_\infty(1 - e^{-\lambda_e t_e}) \tag{6}$$

$$R_1 = E_0\, e^{-\lambda_r t_r} + R_\infty(1 - e^{-\lambda_r t_r}) \tag{7}$$

$$\vdots$$

$$E_n = R_n\, e^{-\lambda_e t_e} + E_\infty(1 - e^{-\lambda_e t_e}) \tag{8}$$

$$R_{n+1} = E_n\, e^{-\lambda_r t_r} + R_\infty(1 - e^{-\lambda_r t_r}). \tag{9}$$

Combining these, each recurrence relation can be written as

$$E_n = E_{n-1}\, e^{-\lambda} + R_\infty(1 - e^{-\lambda_r t_r})\, e^{-\lambda_e t_e} + E_\infty(1 - e^{-\lambda_e t_e}) \tag{10}$$

$$R_{n+1} = R_n\, e^{-\lambda} + E_\infty(1 - e^{-\lambda_e t_e})\, e^{-\lambda_r t_r} + R_\infty(1 - e^{-\lambda_r t_r}) \tag{11}$$

where

$$\lambda = \lambda_e t_e + \lambda_r t_r \tag{12}$$

s the observed or "apparent" uptake rate determined by the weighted sum of the tate dependent rates. After many pulses, a steady state will be reached where $E_n = E_{n-1}$ and $R_{n+1} = R_n$, so the steady state fraction of blocked channels is

$$E_{ss} = \frac{R_\infty(1 - e^{-\lambda_r t_r})\, e^{-\lambda_e t_e} + E_\infty(1 - e^{-\lambda_e t_e})}{1 - e^{-\lambda}} \tag{13}$$

and

$$R_{ss} = \frac{E_\infty(1 - e^{-\lambda_e t_e})\, e^{-\lambda_r t_r} + R_\infty(1 - e^{-\lambda_r t_r})}{1 - e^{-\lambda}}. \tag{14}$$

The solution to blockade for the nth pulse is then

$$E_n = E_{ss} + (E_0 - E_{ss})\, e^{-n\lambda} \tag{15}$$

and

$$R_n = R_{ss} + (R_0 - R_{ss})\, e^{-n\lambda}. \tag{16}$$

From this we make three observations that can be verified experimentally: (1) channel blockade via a single diffusion path follows an exponential course; (2) the apparent uptake rate, λ, is a linear combination of the uptake rates (λ_r and λ_e) for the resting and excited states; and (3) steady state block is linearly related to the resting and excited conformation equilibria.

Multistate Recurrence

For the bimolecular interaction, let m represent the number of channel conformational states elicited by a multi-voltage stimulus pulse protocol, i represent the ith state, t_i represent the lifetime of the ith state, n represent the nth pulse, $s_{i,n}$ represent the fractional blockade associated with pulse n and state i, and $b_{i,\infty}$ represent the equilibrium block for the ith state. Using equation (2) as a basis, the generalized sequence of recurrence relations for the nth stimulus pulse is described by

$$s_{0,0} = \text{initial block}$$

$$s_{i+1,n} = f(s_{i,n}) \quad \text{for} \quad 1 \le i < m-1 \quad \text{and} \quad s_{0,n+1} = f(s_{m-1,n}) \tag{17}$$

where

$$f(s_{i,n}) = \alpha_{i+1} s_{i,n} + (1-\alpha_{i+1}) b_{i+1,\infty} \tag{18}$$

$$\alpha_i = e^{-t_i/\tau_i} = e^{-\lambda_i t_i} \tag{19}$$

$$\lambda_i = \tau_i^{-1} = k_i D + l_i \tag{20}$$

$$b_{i,\infty} = \left(1 + \frac{l_i}{k_i D}\right)^{-1} \tag{21}$$

and the subscript i takes on the state values $0, 1, \ldots, m-1$ as determined by i mod m.

The fractional blockade relating two successive stimuli, n and $n+1$, for any state, j, is described by

$$s_{j,n+1} = f(f(f(\ldots f(s_{j,n}) \ldots))) \tag{22}$$

$$= s_{j,n} \prod_{i=0}^{m-1} \alpha_i + (1-\alpha_j) b_{j,\infty} + \sum_{i=j+1}^{m+j-1} (1-\alpha_i) b_{i,\infty} \prod_{k=i+1}^{m+j} \alpha_k \tag{23}$$

where subscripts i and k are taken mod m. At steady state, when $s_{j,n+1} = s_{j,n}$

$$s_{j,ss} = \frac{(1-\alpha_j) b_{j,\infty} + \sum_{i=j+1}^{m+j-1} (1-\alpha_i) b_{i,\infty} \prod_{k=i+1}^{m+j} \alpha_k}{1 - \prod_{i=0}^{m-1} \alpha_i}. \tag{24}$$

As the residence time in state t_j becomes large, the value of α_j becomes negligible, so that in the limit $s_{j,ss} = b_{j,\infty}$, which is the equilibrium value associated with continuous access binding. The solution to the recurrence relation can now be written as

$$s_{j,n} = s_{j,ss} + (s_{j,0} - s_{j,ss}) \left(\prod_{i=0}^{m-1} \alpha_i\right)^n. \tag{25}$$

The apparent uptake rate, λ, described in equation (12) for the two state case, is generalized here, where

$$\lambda = -ln \prod_{i=0}^{m-1} \alpha_i = -\sum_{i=0}^{m-1} ln\, \alpha_i. \tag{26}$$

inactivation could be described by defining the state-dependent binding and unbinding rates in terms of the channel gate conformations. We determined the state-independent binding affinities by removing gate and membrane voltage effects from the state-dependent rate coefficients. For the sodium channel, we defined $k(V) = m^3 h k_c$ and $l(V) = m^3 h l_c \, e^{-zVF/RT}$ for the hydrophilic path and $k(V) = m^3 h k_n$ and $l(V) = l_n$ for the hydrophobic path. Here, m^3 and h are the H-H gating variables, V is the receptor voltage, z is the drug charge, and F, R, and T have their usual meaning. The apparent inactivation for a neutral hydrophobic agent is then

$$h^* = h\left(1 + \frac{m^3 k_n D}{l_n}\right)^{-1} \tag{35}$$

and the dose-response curve is described by

$$b_\infty = \left(1 + \frac{l_n}{m^3 k_n D}\right)^{-1}. \tag{36}$$

Both of these expressions illustrate voltage dependence of the apparent equilibrium dissociation constant as derived from the voltage dependence of the activation gate, m^3. In particular, hyperpolarizing membrane potentials shift the dose-response curve to the right, reflecting the reduced availability of channels (m^3 small).

Results

From the relationships derived above, one can apply several tests for consistency with observations derived from pulse train stimulation of excitable cells in the presence of channel blocking agents. When state transitions are fast enough for one to disregard the transition time, the sampled (i.e. from peak channel current) uptake curve will follow an exponential time course described by $\exp(-n\lambda)$ where λ is the uptake rate and n is the stimulus number $(0, 1, \ldots n)$. Thus, a first test is to fit the uptake curves to an exponential of the form

$$i_{n,x} = i_{0,x}[1 - b_{ss} - (b_0 - b_{ss})\, e^{-n\lambda}] \tag{37}$$

where $i_{n,x}$ is the peak current of channel type x associated with the nth stimulus pulse. As derived above, under appropriate conditions λ can be linearly related to a stimulus interval or the drug concentration. This apparent uptake rate is defined by the weighted sum of the state-dependent time constants over the m states:

$$\lambda = \sum_{i=0}^{m-1} \lambda_i t_i = \sum_{i=0}^{m-1} (k_i D + l_i) t_i \tag{38}$$

$$= D \sum_{i=0}^{m-1} k_i t_i + \sum_{i=0}^{m-1} l_i t_i. \tag{39}$$

Holding the stimulus intervals constant and varying the drug concentration will produce a linear change in λ. For blockade described by this protocol, a plot of λ against D produces a straight line with an intercept of $\sum l_i t_i$ and a slope of $\sum k_i t_i$. Alternatively, the drug concentration can be held constant and the various intervals,

Observations Based on Electrophysiologic Measures

In general, channel blockade cannot be observed first hand but must be assessed ndirectly by measuring current flow through a population of channels. For elec-rophysiologic measures, we modify Ohm's law to reflect the loss of conductance lue to blocked channels. Following the Hodgkin-Huxley formalism (1952), we vrite the sodium, potassium and calcium channel currents in terms of the unblocked raction of channels, $1-b_x$, as

$$i_{Na} = g_{Na}(1-b_{Na})(V-V_{Na}) \tag{27}$$

$$i_K = g_K(1-b_K)(V-V_K) \tag{28}$$

$$i_{Ca} = g_{Ca}(1-b_{Ca})(V-V_{Ca}) \tag{29}$$

where g_x reflects the available conductance based on channel gate conformations. Therefore, with pulse train stimulation, the state dependent channel current is described by

$$i_x = g_x(V-V_x)[1-E_{ss}-(E_0-E_{ss})\,e^{-n\lambda}] \tag{30}$$

For inactivating channels, such as the sodium and calcium channels, the apparent nactivation can be derived from the true inactivation. Let h be the fraction of non-inactivated channels. Then the fraction of apparent non-inactivated channels, h^*, is determined by the true h as reduced by the unblocked conducting channels $(1-b)$, so that

$$h^* = h(1-b). \tag{31}$$

In cases of long conditioning pulses where an equilibrium is reached,

$$h^* = h\left(1+\frac{k(V)D}{l(V)}\right)^{-1} \tag{32}$$

where $k(V)$ and $l(V)$ are the voltage and state-dependent rate coefficients for the prepulse associated state. At a drug concentration of zero, $h^* = h$, but values of D greater than zero result in a shift in apparent inactivation in the hyperpolarizing direction. Voltage sensitive states also introduce a voltage sensitivity into equilibrium dose-response relations, as described by

$$b_i^\infty = \left(1+\frac{l(V)}{k(V)D}\right)^{-1} \tag{33}$$

which is characterized by a voltage (state) dependent equilibrium dissociation constant

$$k_D = \frac{l_i(V)}{k_i(V)}. \tag{34}$$

Special Case: Receptor Guarding

Recently (Starmer *et al.*, 1984; Starmer & Hollett, 1985; Starmer & Grant, 1985), we demonstrated that use-dependent blockade and shifts in apparent channel

$_i$, varied. For blockade described by this type of stimulus protocol, each of the rate onstants, λ_i, can be estimated.

When the stimulus protocol is based on only two intervals, t_e and t_r and one is ield constant, λ is linearly related to the other by

$$\lambda = \lambda_e t_e + \lambda_r t_r = (k_e D + l_e) t_e + (k_r D + l_r) t_r \tag{40}$$

For multiple stimulus rates (variations in t_r with t_e fixed), a plot of λ against t_r roduces an intercept of $(k_e D + l_e) t_e$ and a slope of $k_r D + l_r$. In general, the observed requency dependent uptake rates provide the means for estimating the state specific iptake rates, λ_i.

To estimate individual rate constants, another relationship must be coupled to he estimates of λ_i. This is provided by the steady state blockade equation (13), vhich provides a means for estimating the equilibrium block parameters, R_∞ and E_∞. Equation (13) can be written as

$$y = c_1 R_\infty + c_2 E_\infty \tag{41}$$

vhere

$$c_1 = \frac{(1 - e^{-\lambda_r t_r})\, e^{-\lambda_e t_e}}{1 - e^{-\lambda}} \tag{42}$$

$$c_2 = \frac{1 - e^{-\lambda_e t_e}}{1 - e^{-\lambda}} \tag{43}$$

$$y = E_{ss} \tag{44}$$

Since

$$R_\infty = \left(1 + \frac{l_r}{k_r D}\right)^{-1}, \qquad E_\infty = \left(1 + \frac{l_e}{k_e D}\right)^{-1} \tag{45a}$$

ind

$$\lambda_r = k_r D + l_r, \qquad \lambda_e = k_e D + l_e \tag{45b}$$

ach pair of state-dependent rate constants can be estimated.

Sufficient published data exists to test these concepts for sodium, calcium and otassium channels. Courtney *et al.* (1978) studied the use-dependent uptake of tidocaine by sodium channels with a three voltage stimulus (rest, hyperpolarizing otential and test potential) and provided data to test the multistate results. Gintant *et al.* (1983), in studies of QX222 uptake by sodium channels, noted a dose-dependent iptake rate. Sanguinetti & Kass (1984), in studies of dihydropyridine uptake by alcium channels, noted use-dependence and also investigated the interaction etween steady state blockade and stimulus interval. As a final example, Yeh *et al.* 1976) studied "reverse" use-dependent binding of *n*-aminopyridine to potassium hannels. Unlike lidocaine and its derivatives and the dihydropyridines, rapid xcitation of the K^+ channel in the presence of aminopyridines reduces blockade n a use-dependent manner.

SODIUM CHANNELS

Courtney *et al.* (1978) noted a use-dependent exponential uptake and release of etidocaine in sodium channels in frog node of Ranvier. At a stimulus interval of 1 s, the uptake rate was 0·16 pulse^{-1}; while at a stimulus rate of 0·5 s, the uptake rate was 0·10 pulse^{-1}. Incorporation of a tertiary amine can be characterized by

$$U + D \underset{hl\, e^{-zVF/RT}}{\overset{m^3hk}{\rightleftharpoons}} B$$

At the stimulus voltages used by Courtney *et al.* ($V_r = -80$ mV, $V_e = -20$ mV), the uptake rate is approximated by

$$\lambda = kDt_e + h_r l t_r\, e^{-zV_rF/RT} \tag{46}$$

where we assume a resting value of $h_r = 0{\cdot}6$ and negligible blocking during rest and prepulse periods. Solving for k and l using the two observed rates yields values of $k = 3{\cdot}85 \times 10^3$ M^{-1} ms^{-1} and $l = 8{\cdot}15 \times 10^{-6}$ ms^{-1}. Adding a hyperpolarizing prepulse ($V_p = -125$ mV) to the stimulus results in a modified λ described by

$$\lambda = kDt_e + h_p l t_p\, e^{-zV_pF/RT} + h_r l t_r\, e^{-zV_rF/RT} \tag{47}$$

where we assume the binding site sees the full membrane potential and $h_p = 1{\cdot}0$ and $h_r = 0{\cdot}6$ are the fractions of non-inactivated channels at the prepulse and resting potentials. Using the values of k and l estimated above and the prepulse and resting intervals and potentials specified by Courtney *et al.* yields an estimated λ of 0·213 pulse^{-1} in substantial agreement of the observed rate of 0·22 pulse^{-1}.

Gintant *et al.* (1983) noted an exponential uptake of QX222 in cardiac Purkinje fibers when pulse train stimulation was incorporated. Furthermore, they noted a linear relationship between uptake rate and drug concentration while holding the stimulus parameters constant. In fact, they stated, "a linear relationship appears to adequately describe changes in the rate of development of use-dependent block associated with different QX222 concentrations." For QX222 blockade, we (Starmer *et al.*, 1986) recently showed that the uptake rate is determined by

$$\lambda = (l_e t_e + l_r t_r) + (k_e t_e + k_r t_r) D \tag{48}$$

which describes a linear relationship between λ and D with a positive slope determined by the binding rates and stimulus intervals.

Courtney *et al.* (1978) and Gintant *et al.* (1983) noted shifts in measured channel availability as a function of holding potential. More recently, Yatani & Brown (1985) noted similar shifts in apparent sodium channel inactivation in the presence of the dihydropyridine nitrendipine. Since the procedure for measuring channel inactivation is based on peak currents as determined by the unblocked channels, one would always expect a concentration dependent shift in apparent inactivation, h^*, in the hyperpolarizing direction, as specified by $h^* = h(1 - b)$.

CALCIUM CHANNELS

Sanguinetti & Kass (1984), in studies of dihydropyridine uptake by calcium channels, noted both frequency-dependent uptake rates and frequency-dependent

steady state fractions of blocked channels. In particular, they observed the effect of changes in the excitation interval, t_e, on uptake of nisoldipine. From their uptake data for nisoldipine (their Fig. 6), they observed an increase in use-dependent blockade with increasing values of the excitatory interval, t_e. From their data, we found the use-dependent uptake of nisoldipine to be characterized well by single exponentials with uptake rates of 0·132 pulse^{-1} for $t_e = 20$ ms, and 0·161 pulse^{-1} for $t_e = 200$ ms, as shown in Fig. 1. While holding the excitation interval fixed at

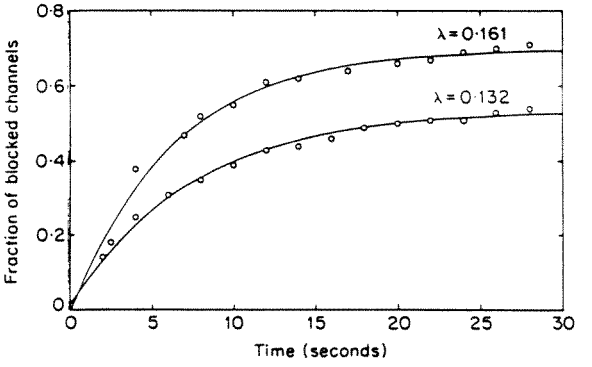

FIG. 1. Time course of use-dependent uptake of nisoldipine by calcium channels (from Fig. 6 Sanguinetti & Kass, 1984). The solid lines represent the least squares fit to a single exponential. The upper curve resulted from a 200 ms stimulus pulse, while the lower curve resulted from a 20 ms stimulus pulse.

50 ms and varying the recovery interval, t_r, they observed increasing steady state blockade with decreasing t_r. Using steady state calcium current values from their Fig. 1, we computed the frequency-dependent values of steady state block. On the basis of equation (13) and assuming negligible resting block ($R_\infty \approx 0$), we would predict a linear relationship between steady state block and $(1-e^{-\lambda})^{-1}$. Although uptake rates were not provided in their paper, theory predicts a linear relationship between λ and the rest interval, t_r, assuming the excitation interval is held constant. We can approximate $e^{-\lambda}$ by $1-\lambda$ with the result that equation (13) is approximated by

$$E_{ss} \approx \frac{\text{constant}}{\lambda} = \frac{\text{constant}}{\lambda_e t_e + \lambda_r t_r} \tag{49}$$

or

$$\frac{1}{E_{ss}} = \frac{\lambda_e t_e + \lambda_r t_r}{\text{constant}} \tag{50}$$

which describes a straight line with an intercept proportional to $\lambda_e t_e$ and a slope of λ_r. The derived values are plotted against stimulus interval in Fig. 2, and the resulting linear relationship is consistent with the predicted relationship.

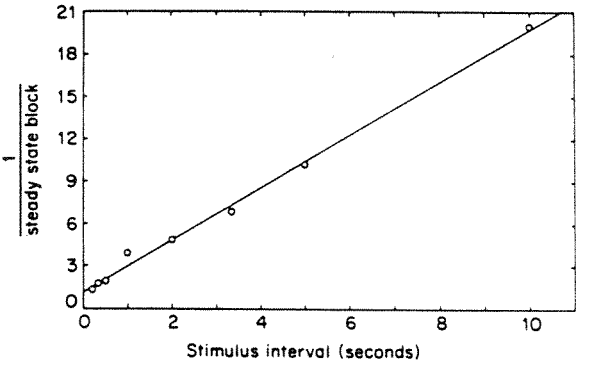

FIG. 2. Relationship between reciprocal steady state block of calcium channels by nisoldipine and stimulus interval (derived from Fig. 1 of Sanguinetti & Kass, 1984). A linear relationship is predicted by equation (50) which approximates equation (13).

Uehara & Hume (1985), in studies of D-600, verapamil, and diltiazem blockade of calcium channels, also observed exponential use-dependent uptake as well as hyperpolarizing shifts in apparent channel inactivation. Again, the shift in apparent channel inactivation is consistent with a drug induced reduction in conducting channels. As shown in Fig. 3, the shift in apparent inactivation as computed with equation (35) is dependent on the drug concentration. For these computations, the slow inward gating model described by Bristow & Clark (1982) was used.

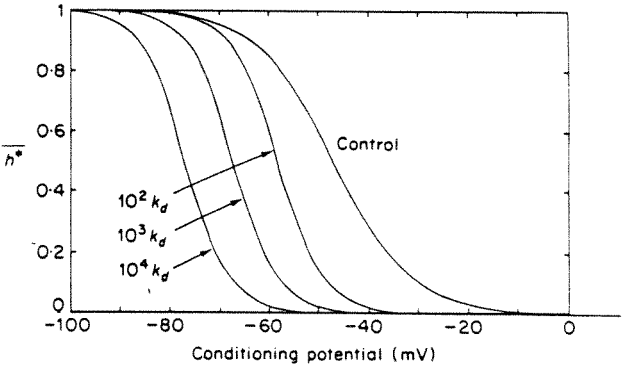

FIG. 3. Predicted shifts in ion channel availability. The apparent inactivation is defined as $h^* = h(1 - b)$, where h is the true inactivation and b is the voltage sensitive fraction of blocked channels. Note the left shift in curves as the drug concentration is increased from 100 k_d to $10^4 k_d$.

POTASSIUM CHANNELS

Since the potassium channel is a non-inactivating channel, it has been possible to study many channel blocking agents under conditions where blockade could be viewed continuously during a single excitatory stimulus and where equilibrium conditions could be achieved. Not so, though, with the class of n-aminopyridines

studied by Yeh *et al.* (1976). These agents appear to block during the rest interval and unblock during the excitatory interval, thereby masking the dynamics of the blocking process. A sort of "reverse" use-dependence was shown where increasing the stimulus rate resulted in blockade recovery, as shown in Fig. 5 of Yeh *et al.* (1976). With these data, we estimated the exponential uptake rates and steady state fractions of blocked channels as shown in Table 1 for 4-aminopyridine.

TABLE 1

Blockade parameter estimates

Stimulus interval (ms)	λ (pulse^{-1})	M_{ss}	Predicted λ	Predicted M_{ss}
5000	1·761	0·893	1·783	0·889
2000	1·200	0·844	1·123	0·847
1000	0·867	0·816	0·903	0·817
500	0·782	0·791	0·793	0·795
400	0·748	0·788	0·771	0·789
200	0·738	0·786	0·727	0·778

Figure 4 shows the original data and the best fit exponential derived from a nonlinear least squares procedure (Marquardt, 1963). Figure 5 shows the agreement between observed uptake rates and the predicted linear function of the stimulus interval.

Estimating the potassium channel rate constants, we must modify the method used for sodium and calcium channels. Recently (Starmer & Grant, 1985), we found that for sodium channels the interval between the time blockade was measured and the end of the depolarizing stimulus could be ignored. Because the K channel does not inactivate, significant block can occur during this interval so that the measurement interval must be considered separately along with the remainder of the depolarizing interval. Therefore, we define the excitation interval as composed of

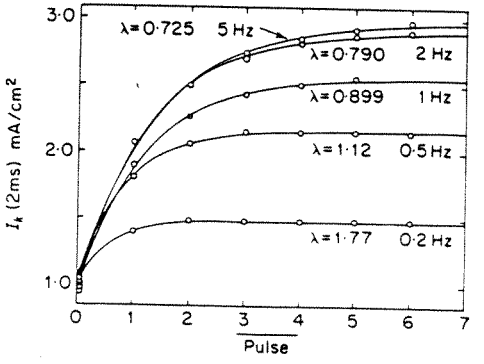

FIG. 4. Use-dependent recovery of potassium channels from aminopyridine block (from Fig. 5 of Yeh *et al.*, 1976). The solid lines represent the least squares fit of single exponentials for stimulus rates of , 0·5, 1, 2, and 5 Hz.

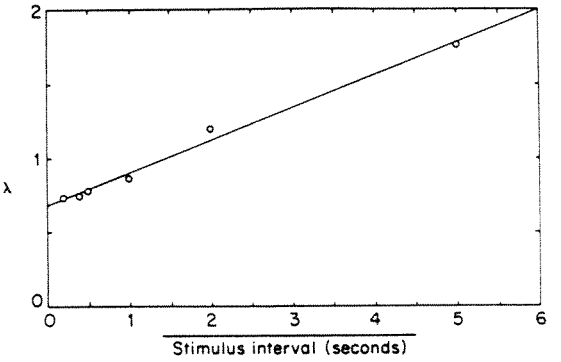

FIG. 5. Uptake rates of aminopyridine in potassium channels. Each uptake rate was determined by a least squares fit to the observed uptake curves in Fig. 4. The observed stimulus interval is predicted by equation (12).

two sub-intervals: one from the onset of depolarization to the time of current measurement (t_m), and one from t_m to the end of depolarization (t_d). Thus, we use three intervals (t_d, t_m, t_r) instead of two (t_e and t_r), where $t_e = t_m + t_d$. The stimulus protocol of Yeh *et al.* (1976) was based on $t_m = 2$ ms, $t_d = 6$ ms, and t_r as the recovery interval. The resultant measured steady state blockade (M) for this three-state process, as derived from equation (24), is described by

$$M_{ss} = [E_\infty[(1-e^{-\lambda_e t_m}) + (1-e^{-\lambda_e t_d})\, e^{-(\lambda_r t_r + \lambda_e t_m)}] + R_\infty(1-e^{-\lambda_r t_r})\, e^{-\lambda_e t_m}]/(1-e^{-\lambda}) \quad (51)$$

where $\lambda = \lambda_e(t_m + t_d) + \lambda_r t_R$, which relates measured steady state block, (M_{ss}), to state equilibria as modified by functions of the intervals t_m, t_d, and t_r and the two state-dependent rates, λ_e and λ_r. From Fig. 5, the least squares estimates of the uptake slope and intercept were found to be $0{\cdot}22\ s^{-1}$ and 0·685, respectively. Since the depolarizing interval was 8 ms, the resulting time constants were $\tau_e = 0{\cdot}01168$ s and $\tau_r = 4{\cdot}54$ s. Fitting equation (51) using these time constants and the values of stimulus interval, λ and M_{ss} from Table 1, yielded least squares estimates of $E_\infty = 0{\cdot}766$ and $R_\infty = 0{\cdot}949$. From these values, we estimated $k = 66\ M^{-1}\ ms^{-1}$, $l(0) = 7{\cdot}61 \times 10^{-3}\ ms^{-1}$, $n^4(-80) = 3{\cdot}19 \times 10^{-3}$, and $\delta = 0{\cdot}24$, where δ is the fraction of the membrane potential seen by the binding site. These estimates were based on assuming all K channels were conducting at +100 mvolts ($n^4(100) = 1$). The overall blockade model assumed both guarding and trapping by the four H-H n gates; i.e.

$$U + D \underset{n^4 l e^{\delta V_m F/RT}}{\overset{n^4 k}{\rightleftharpoons}} B$$

where V_m is the membrane voltage. With these values, the predicted uptake rate, steady state block and use-dependent block were computed. The predicted uptake rates and steady state block values are shown in Table 1, while Fig. 6 compares predicted and observed values of use-dependent block.

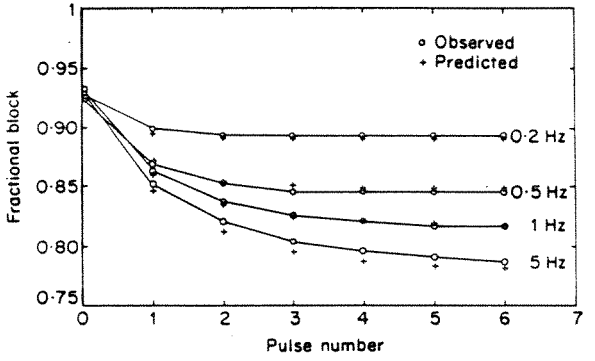

FIG. 6. Observed and predicted blockade by aminopyridine in potassium channels. From the frequency-dependent uptake rates and steady state values of block, parameters of the blockade process were estimated as $k = 66\ M^{-1}\ ms^{-1}$, $l(0) = 7{\cdot}61 \times 10^{-3}\ ms^{-1}$, $\delta = 0{\cdot}24$ and $n^4 = 3{\cdot}19 \times 10^{-3}$. With these values, values of use-dependent blockade were computed using equations (12), (51), and (15).

Discussion

Following Strichartz (1973), a number of models of sodium channel blockade have been proposed (Courtney, 1975; Hille, 1977; Hondeghem & Katzung, 1977; Yeh, 1979; Starmer *et al.*, 1984) that sought to describe the interaction between channel blocking agents and ion channels. Because these models described the blocking behavior in a channel capable of inactivation, it was necessary to include some description of the electrical sensitivity of the channel gating process. The empirical nature of the gating model added complexity to the blocking model and inhibited the development of closed form analytical descriptions of channel blockade, comparable to the theoretical description of ungated hormone–receptor binding. Furthermore, with the exception of the non-inactivating potassium channel, it was not possible to observe first hand the time course of blockade. Only by repetitive stimulation could one assess the degree of channel blockade. This protocol created a sort of "uncertainty" principle in that introducing a test pulse to assess the fraction of blocked channels sets the stage for additional channels to become blocked during the interval prior to the peak of the ion channel current. Thus, the more frequent the test pulses, the greater the "measurement"-induced blockade (and measurement error), while less frequent test pulses minimize the cumulative error.

Decomposing the stimulus pulse into regions of homogeneous conditions (by assuming conformation transition time is negligible), assuming all binding sites remain in the same conformation during a conditioning interval, and noting that the terminal blockade of an interval is the initial blockade of the succeeding interval, we have been able to derive closed form expressions that describe use-dependent blockade. These new and novel results allow one to visualize for the first time the complex interactions among drug concentration, membrane potential, drug charge, gate conformation, and pulse timing. These results provide a theoretical base for many observations including:

(1) Exponential uptake during pulse train stimulation (Kohlhardt & Seifert, 1983; Gintant *et al.*, 1983; Grant *et al.*, 1982).

(2) Linear relationship between uptake rate, drug dose and stimulus interval (Gintant *et al.*, 1983).

(3) Shift of measured channel inactivation in the hyperpolarizing direction (Courtney, 1975; Hondeghem & Katzung, 1977; Uehara & Hume, 1985).

(4) Voltage sensitive shifts in dose-response relations (Bean *et al.*, 1983; Bean, 1984).

Furthermore, this model admits to a straightforward procedure for parameter estimation. From the theoretical results, one can derive several tests of consistency between the observations and the predictions.

When analyzing binding of hormone to a single class of continuously accessible receptors, one has access to both the time course of hormone binding and the final equilibrium value achieved. Since equilibrium is dependent on the ratio of l to kD and the uptake rate is determined by their sum, it is feasible to estimate the two rate constants. We have found a parallel analysis strategy for periodically accessible binding sites. From the "sampled" or "apparent" uptake rate, λ, the individual state specific uptake rates (reciprocal time constants) can be estimated according to equation (26) (assuming the stimulus protocol is capable of varying $m-1$ of the m possible state intervals). Similarly, from the steady state value of blockade, the state specific equilibria can be estimated according to equation (24). Combining the uptake values and equilibrium values for each state readily yields the state specific rate constants.

Carrying the "state" model to a more molecular level requires one to define the relationship between binding affinities and channel conformations. We have suggested that channel gate control of the diffusion paths between drug pool and binding site might be adequate to provide this bridge for some agents. Undoubtedly there are other bridges and even refinement of the channel gate scheme.

Finally, this theoretical base provides an important tool for combining observations based on different measures of channel blockade. For instance, radioligand binding studies provide measures of equilibrium dissociation constants that can now be compared with estimates based on channel currents. Note that in equation (13) as t_e becomes large, E_{ss} becomes equal to the equilibrium block, E_∞. Thus, estimates of k_D derived from E_{ss} can be directly related to the results of equilibrium ligand binding studies.

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