THEORETICAL CHARACTERIZATION OF ION CHANNEL BLOCKADE

Competitive Binding to Periodically Accessible Receptors

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ABSTRACT Competitive ligand binding to periodically activated or accessible receptors is influenced by the interaction between ligand binding kinetics and the interval of time the binding site is accessible. This interaction produces a paradoxical reduction in bound receptors under certain conditions. A mathematical description of multi-ligand binding to a single binding site is presented for both the continuously and transiently accessible cases. The theoretical results predict paradoxical "agonism" and are consistent with the results of studies of lidocaine and bupivacaine binding to cardiac sodium channels.

INTRODUCTION

As part of our investigation of ion channel blockade, we recently developed a macroscopic model that describes use-dependent blockade for sodium channel antagonists (Starmer et al., 1984, 1986; Starmer and Grant, 1985; Starmer, 1986; Starmer and Courtney, 1986) where binding sites are considered to be transiently accessible as determined by one or more allosteric conformations of the channel protein. A periodic stimulus that switches between two voltages is viewed as switching the channel population between two apparent states where each "state" is a unique mixture of channel conformations, some with accessible sites and the remainder with inaccessible sites. Binding to accessible sites is assumed to follow a pseudo first-order process. The theoretical description of blockade under such "switched" conditions predicts the apparent uptake rate to be a linear function of the mixture-dependent uptake rates and the steady state blockade to be a linear function of the mixture-dependent equilibria. These results provide the basis for a simple procedure for estimating rate constants (Starmer et al., 1987). With these new tools, it has been possible to quantitatively assess drug channel binding in nonequilibrium settings using pulse train stimulation.

Studies of two agents competing for a single binding site by Rimmel et al. (1978), Schmidtmayer and Ulbricht (1980), and Clarkson and Hondeghem (1985*a*) indicated that under certain conditions the fraction of channels blocked by the mixture is less than the fraction of channels blocked by the more potent (higher affinity) agent when used by itself. Because this is the reverse of that predicted by equilibrium models, I have labeled the effect as "paradoxical agonism." A theoretical description that accurately identifies the conditions for the paradox would be of considerable use, for instance, as a clinical tool for reversing toxic effects of an antiarrhythmic agent. Often, charged and neutral moieties of a tertiary agent such as quinidine are both active blockers. For these drugs, the same model would aid in understanding the interaction of protons with drug bound channels. Here I extend our characterization of single agent use-dependent blockade to the case of two agents competing for the same transiently accessible binding site with and without proton exchange between bound moieties. Conditions for the paradox are identified. The results are then tested with data derived from studies of bupivacaine and lidocaine obtained by Clarkson and Hondeghem (1985*a*).

THEORETICAL STUDIES

Single Agent Blockade

To fix ideas, I first review single agent binding to a periodically accessible binding site. Consider a stimulus protocol that switches the channel between two mixtures of accessible and inaccessible conformations: an excited mixture dominated by accessible sites and a resting mixture dominated by inaccessible sites, with apparent blocking and unblocking rates of k_e, l_e, k_r, and l_r. With pulse train stimulation, let t_e be the excited time interval, and t_r be the resting time interval, where each repeated interval of the train is of duration $t_e + t_r$. When t_e or t_r are exponentially distributed, the intervals reflect the mean conformation dwell times (see Appendix). During each interval, we

assume a pseudo first-order process (drug concentration, D, remains unchanged), such that the fraction of blocked channels during an interval when the mixture of channels forms a pseudo "state," c, for an interval t_c is

$$b(t_c) = b_\infty + (b_0 - b_\infty)e^{-\lambda_c t_c} \quad (1)$$

where

$$b_\infty = \left(1 + \frac{l_c}{k_c D}\right)^{-1} \lambda_c = (k_c D + l_c). \quad (2)$$

When the time constants for equilibration between inaccessible and accessible conformations are small relative to the dwell time in the accessible state, then with pulse train stimulation, the blockade at the end of the nth excited interval, E_n, and rest interval, R_n, is described by the recurrence sequence

$$E_n = R_n e^{-\lambda_e t_e} + E_\infty(1 - e^{-\lambda_e t_e}) \quad (3)$$

$$R_{n+1} = E_n e^{-\lambda_r t_r} + R_\infty(1 - e^{-\lambda_r t_r}). \quad (4)$$

Given the initial blockade, R_0, the solution for the sequence of rest interval blockade values, R_n, is

$$R_n = R_{ss} + (R_0 - R_{ss})e^{-n\lambda} \quad (5)$$

where

$$\lambda = \lambda_e t_e + \lambda_r t_r \quad (6)$$

and

$$R_{ss} = E_\infty + \gamma(R_\infty - E_\infty) \quad (7)$$

where

$$\lambda = \frac{1 - e^{-\lambda_r t_r}}{1 - e^{-\lambda}} \quad (8)$$

$$E_\infty = \left(1 + \frac{l_e}{k_e D}\right)^{-1}; \quad \lambda_e = k_e D + l_e \quad (9)$$

$$R_\infty = \left(1 + \frac{l_r}{k_r D}\right)^{-1}; \quad \lambda_r = k_r D + l_r. \quad (10)$$

Note that the envelope of blockade $(R_0, R_1, R_2 \ldots R_n)$ follows an exponential course with an apparent rate that is a linear function of the "excited" and "resting" rates. The rate is called an apparent rate since blockade in many cases can only be monitored from "sampled" data in contrast to continuous monitoring of the ligand binding process. Note also that the steady value of block, R_{ss}, is a linear function of the equilibria E_∞ and R_∞ associated with the excited and rest mixtures of accessible and inaccessible sites.

Competitive Binding to a Continuously Accessible Site

Consider two binding processes where agents compete continuously for the same site as in

$$\begin{array}{ccc} U + D_A \underset{\ell_A}{\overset{k_A}{\rightleftharpoons}} B_A \\ \qquad\qquad k_p H^+ \Big\| \ell_p \\ U + D_B \underset{\ell_B}{\overset{k_B}{\rightleftharpoons}} B_B \end{array}$$

Scheme I

where ${}_UD_A$ and D_B are the two blocking agents, and k_pH^+ and l_p represent rates of a possible conversion between B_A and B_B (for instance, a proton exchange process where D_A and D_B are neutral and charged moieties of the same drug). The fractions of blocked channels b_A and b_B are described by

$$\frac{db_A}{dt} = -(k_A D_A + l_A + k_p H^+)b_A - (k_A D_A - l_p)b_B + k_A D_A \quad (11)$$

$$\frac{db_B}{dt} = -(k_B D_B - k_p H^+)b_A - (k_B D_B + l_B + l_p)b_B + k_B D_B. \quad (12)$$

For notational convenience, let x be b_A and y be b_B. Then,

$$\frac{dx}{dt} = a_1 x + b_1 y + C_x \quad (13)$$

$$\frac{dy}{dt} = a_2 x + b_2 y + C_y. \quad (14)$$

The solution to this system is given by

$$\begin{Bmatrix} x(t) \\ y(t) \end{Bmatrix} = \{\phi\} \begin{Bmatrix} x(0) \\ y(0) \end{Bmatrix} + \begin{Bmatrix} X_\infty \\ Y_\infty \end{Bmatrix} \quad (15)$$

where

$$\phi = \begin{Bmatrix} A_{11} A_{12} \\ A_{21} A_{22} \end{Bmatrix}$$

$$A_{11} = \frac{(\lambda_2 - a_1)e^{\lambda_1 t} + (a_1 - \lambda_1)e^{\lambda_2 t}}{\lambda_2 - \lambda_1} \quad A_{12} = \frac{b_1(e^{\lambda_2 t} - e^{\lambda_1 t})}{\lambda_2 - \lambda_1} \quad (16)$$

$$A_{21} = \frac{a_2(e^{\lambda_2 t} - e^{\lambda_1 t})}{\lambda_2 - \lambda_1} \quad A_{22} = \frac{(\lambda_1 - b_2)e^{\lambda_1 t} + (b_2 - \lambda_1)e^{\lambda_2 t}}{\lambda_2 - \lambda_1} \quad (17)$$

$$X_\infty = \frac{(\lambda_1 x_\infty + C_x)e^{\lambda_2 t} - (\lambda_2 x_\infty + C_x)e^{\lambda_1 t}}{\lambda_2 - \lambda_1} + x_\infty \quad (18)$$

$$Y_\infty = \frac{(\lambda_1 y_\infty + C_y)e^{\lambda_2 t} - (\lambda_2 y_\infty + C_y)e^{\lambda_1 t}}{\lambda_2 - \lambda_1} + y_\infty \quad (19)$$

$$x_\infty = \frac{b_1 C_y - b_2 C_x}{a_1 b_2 - a_2 b_1}, \; y_\infty = \frac{a_2 C_x - a_1 C_y}{a_1 b_2 - a_2 b_1} \quad (20)$$

$$\lambda_1, \lambda_2 = \frac{a_1 + b_2 \pm \sqrt{(a_1 + b_2)^2 - 4(a_1 b_2 - a_2 b_1)}}{2}. \quad (21)$$

Competitive Binding to a Periodically Accessible Site

As shown above, binding of two agents to a single site follows a bi-exponential time course, so that with repetitive simulation the time course is piecewise bi-exponential where the initial condition for the current interval is the final condition from the previous interval. Let E and R be vectors of excited and recovery blockade. Further, let ϕ_e be the matrix of excited coefficients, $A_{e,ij}$, and ϕ_r be the matrix of recovery coefficients, $A_{r,ij}$, and let the equilibrium vectors be described by

$$B_{e,\infty} = \begin{Bmatrix} X_{e,\infty} \\ Y_{e,\infty} \end{Bmatrix}, B_{r,\infty} = \begin{Bmatrix} X_{r,\infty} \\ Y_{r,\infty} \end{Bmatrix},$$

then

$$E_n = \phi_e R_n + B_{e,\infty} \tag{22}$$

$$R_{n+1} = \phi_r E_n + B_{r,\infty}. \tag{23}$$

By substitution, the description of block acquired during an interval can be written in terms of block acquired during the same interval of the preceding stimulus, i.e.,

$$E_n = \phi_e \phi_r E_{n-1} + \phi_e B_{r,\infty} + B_{e,\infty} \tag{24}$$

$$R_{n+1} = \phi_r \phi_e R_n + \phi_r B_{e,\infty} + B_{r,\infty}. \tag{25}$$

Rewriting the recovery block equations as:

$$\begin{Bmatrix} R_{x_{n+1}} \\ R_{y_{n+1}} \end{Bmatrix} = \begin{bmatrix} \alpha_1 & \beta_1 \\ \alpha_2 & \beta_2 \end{bmatrix} \begin{Bmatrix} R_{x_n} \\ R_{y_n} \end{Bmatrix} + \begin{Bmatrix} \gamma_1 \\ \gamma_2 \end{Bmatrix}. \tag{26}$$

The solution is described by

$$\begin{Bmatrix} R_{x_n} \\ R_{y_n} \end{Bmatrix} = \begin{bmatrix} C_{11} & C_{12} \\ C_{21} & C_{22} \end{bmatrix} \begin{Bmatrix} \lambda_1^n \\ \lambda_2^n \end{Bmatrix} + \begin{Bmatrix} R_{x_\infty} \\ R_{y_\infty} \end{Bmatrix} \tag{27}$$

where

$$C_{11} = \frac{\lambda_2 \Delta_{R_x} - \alpha_1 R_{x_0} - \beta_1 R_{y_0} - \gamma_1 + R_{x_\infty}}{\lambda_2 - \lambda_1} \tag{28}$$

$$C_{12} = \frac{\alpha_1 R_{x_0} + \beta_1 R_{y_0} + \gamma_1 - R_{x_\infty} - \Delta_{R_x} \lambda_1}{\lambda_2 - \lambda_1} \tag{29}$$

$$C_{21} = \frac{\lambda_2 \Delta_{R_y} - \alpha_2 R_{x_0} - \beta_2 R_{y_0} - \gamma_2 + R_{y_\infty}}{\lambda_2 - \lambda_1} \tag{30}$$

$$C_{22} = \frac{\alpha_2 R_{x_0} + \beta_2 R_{y_0} - \gamma_2 - R_{y_\infty} - \Delta_{R_y} \lambda_1}{\lambda_2 - \lambda_1} \tag{31}$$

$$\Delta_{R_x} = R_{x_0} - R_{x_\infty} \tag{32}$$

$$\Delta_{R_y} = R_{y_0} - R_{y_\infty}. \tag{33}$$

The steady state block resulting from a long pulse train is described by:

$$R_{x_\infty} = \frac{\gamma_1(1 - \beta_2) + \gamma_2 \beta_1}{(1 - \alpha_1)(1 - 1\beta_2) - \alpha_2 \beta_1} \tag{34}$$

$$R_{y_\infty} = \frac{\gamma_2(1 - \alpha_1) + \gamma_1 \alpha_2}{(1 - \alpha_1)(1 - \beta_2) - \alpha_2 \beta_1}, \tag{35}$$

while the rates associated with the transient phase of block development are described by:

$$\lambda_1, \lambda_2 = \frac{(\alpha_1 + \beta_2) \pm \sqrt{(\alpha_1 + \beta_2)^2 - 4(\alpha_1 \beta_2 - \alpha_2 \beta_1)}}{2}. \tag{36}$$

The two apparent uptake rates for the two drugs competing for a single site are expressed as $-ln(\lambda_1)$ and $-ln(\lambda_2)$.

For many situations, there is the possibility of more than two states (resting, open, and inactivated). The method for generating the recurrence relations (Eqs. 22 and 23) readily generalizes for more than two states.

Analytical Methods

Clarkson and Hondeghem (1985*a*, *b*) studied bupivacaine and lidocaine binding under conditions of pulse train stimulation (to characterize frequency-dependent binding) and continuous excitation (to characterize the time dependent nature of binding when the membrane potential is held constant). Therefore, it is feasible to estimate rate constants from both protocols and compare the results.

When binding takes place under a constant potential, V_c, then blockade follows a time course $b(t) = c_\infty + (b_0 - c_\infty) \exp(-\lambda_c t)$. The rate constants can be directly estimated from the exponential rate (λ_c) and the equilibrium (c_∞) by

$$k_c D = \lambda_c c_\infty \tag{37}$$

$$l_c = \lambda_c (1 - c_\infty). \tag{38}$$

When binding takes place with repetitive stimulation, blockade follows a pulse course $b_n = b_{ss} + (b_0 - b_{ss}) \exp(-n\lambda)$. The rate constants can be estimated from the frequency dependent exponential rates and steady states, since $\lambda = \lambda_e t_e + \lambda_r t_r$ and $b_{ss} = e_\infty + \gamma(r_\infty - e_\infty)$. The individual rate constants are then estimated by Eqs. 37 and 38 for the excited mixture and rest mixture.

RESULTS

Clarkson and Hondeghem (1985*b*) measured both uptake as a function of depolarizing interval (their Fig. 6) and recovery as a function of rest interval (their Fig. 7) for lidocaine and bupivacaine. From the 3.5-μM bupivacaine uptake data they found the equilibrium block to be 0.83 and the uptake rate to be 1.60 s^{-1}. Using Eqs. 37 and 38, I estimated $k_e = 379\ M^{-1}ms^{-1}$ and $l_e = 2.72 \times 10^{-4} ms^{-1}$. The recovery rate (their Fig. 7) at the resting potential, λ_r, was estimated as 0.66 s^{-1} with an equilibrium of 0.08. From these, $k_r = 7.54\ M^{-1}ms^{-1}$ and $l_r = 6.07 \times 10^{-4} ms^{-1}$. For 21.5 μM lidocaine, the uptake rate and equilibrium were 6.29 s^{-1} and 0.59, yielding rate constants of $k_e = 173\ M^{-1}ms^{-1}$ and $l_e = 2.57 \times 10^{-3} ms^{-1}$. Lidocaine recovery rate was reported as 6.49 s^{-1} and I estimated the equilibrium as 0.01. These values yielded a $k_r = 3.02\ M^{-1}ms^{-1}$

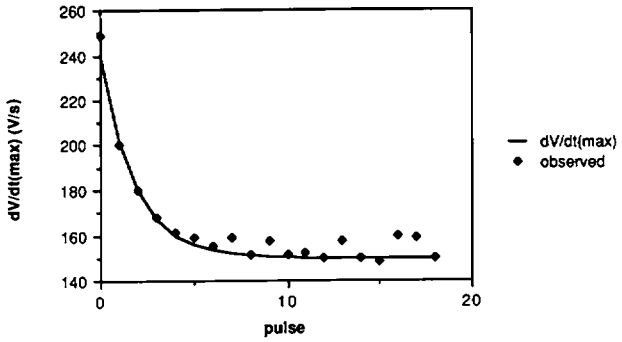


FIGURE 1 Observed and predicted reduction in dV/dt_{max}, resulting from 3.5 μM bupivacaine. Using rate constants derived from Figs. 6 and 7 of Clarkson and Hondeghem (1985*b*), apparent binding and unbinding rates were estimated by using Eqs. 37 and 38. With the resulting values (k_r = 7.54 M^{-1} ms^{-1}, l_r = 6.07 × 10^{-4} ms^{-1}, k_e = 379 M^{-1} ms^{-1}, l_e = 2.72 × 10^{-4} ms^{-1}), use-dependent block was computed using Eqs. 6–15 (*solid line*). The excitation interval was 0.180 s and the recovery interval was 0.420 s.

and l_r = 6.32 × $10^{-3}ms^{-1}$. These values were used to compute use-dependent blockade using a 180-ms excitation interval and a 420-ms recovery interval (Fig. 1). There is good agreement between the observed values of Clarkson and Hondeghem and the solid line representing the predicted time course.

Alternatively, bupivacaine rates were estimated from frequency-dependent reductions in dV/dt_{max} in the presence of 3.5 μM bupivacaine (Clarkson and Hondeghem, 1985*b*). First the exponential rates and steady states were estimated by nonlinear least squares (Fig. 2). Resultant uptake rates were plotted against the recovery interval, t_r (Fig. 3 *A*). The intercept (0.528) represents $\lambda_e t_e$, while the slope (0.478) represents λ_r. The activation interval, t_e, was 0.18 s, so that λ_e = 2.93 s^{-1}. From these values, γ was computed according to Eq. 8. Steady state block was plotted against γ in order to estimate R_∞ and E_∞ (0.044 and

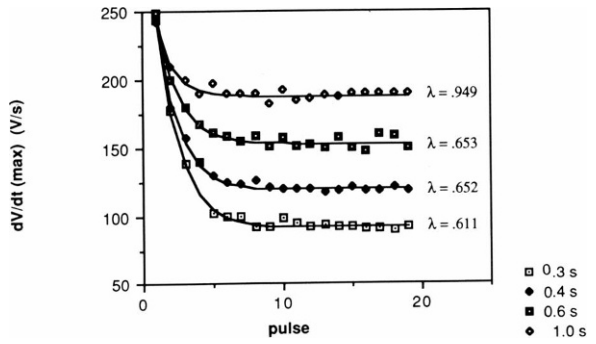
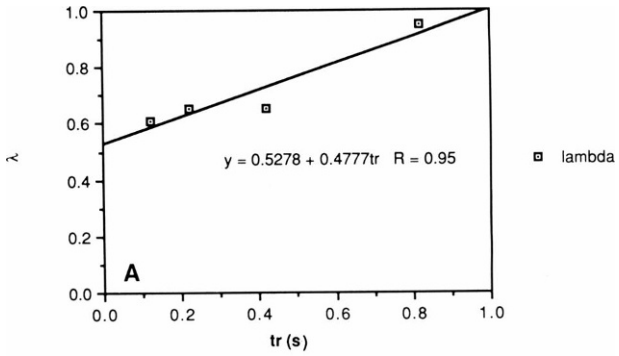


FIGURE 2 Frequency-dependent reduction in dV/dt_{max} resulting from 3.5 μM bupivacaine. Pulse train stimulation was used to induce frequency-dependent blockade with a constant excitation interval of 0.18 s. Recovery intervals were 0.12, 0.22, 0.42, and 0.82 s. Associated uptake rates were 0.611, 0.652, 0.653, and 0.949. The associated values for steady state block were 0.632, 0.520, 0.388, and 0.248. Increasing the recovery interval resulted in reducing the steady state blockade. Each set of points was fit to an exponential in order to estimate the rate and steady state values.



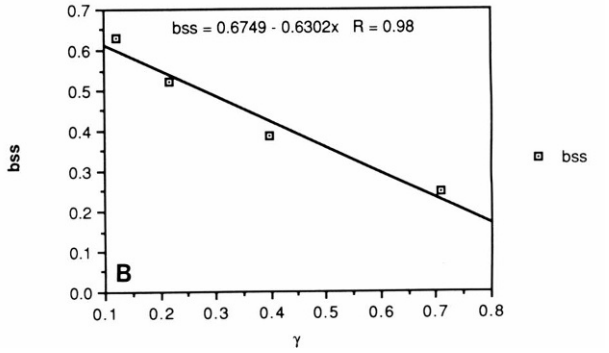


FIGURE 3 (*A*) Apparent 3.5 μM bupivacaine uptake rate as a function of recovery interval. The theoretical model requires that $\lambda = \lambda_e t_e + \lambda_r t_r$. Thus, λ should vary linearly with t_r. In addition, the slope and intercept should be positive. There is good agreement between observed values and the theoretical prediction. (*B*) Steady state bupivacaine block as a function of the stimulus parameter, γ. The theoretical model requires that $b_{ss} - e_\infty + \gamma(r_\infty - e_\infty)$, so that b_{ss} is a linear function of γ. Further, the slope should be less than the intercept. The observed values are in good agreement with the theoretical predictions.

0.675) (Fig. 3 *B*). Both relations were linear, consistent with the theoretical model as described by Eqs. 6 and 7. These values were then used to estimate the apparent binding and unbinding rates according to eqs. 37 and 38, yielding k_e = 565 M^{-1} ms^{-1}, l_e = 9.53 × 10^{-4} ms^{-1}, k_r = 6.08 $M^{-1}ms^{-1}$, and l_r = 4.56 × 10^{-4} ms^{-1}. These values, estimated from pulse train data, are in substantial agreement with those estimated from constant voltage conditions. Estimating the recovery time constant λ_r^{-1} from these values yields 2.1 s, in close agreement with the 1.56 s value observed by Clarkson and Hondeghem (1985*b*) under non–use-dependent conditions.

With the constant condition estimates of the rates, I next computed use-dependent blockade for 43-μM lidocaine alone, 3.5-μM bupivacaine alone, and a mixture of the two agents (Fig. 4). For a 0.4-s stimulus interval, the lidocaine values reach a steady state within 1 pulse, while bupivacaine reaches a steady state within 10 pulses. The mixture reaches a steady state within 3–4 pulses.

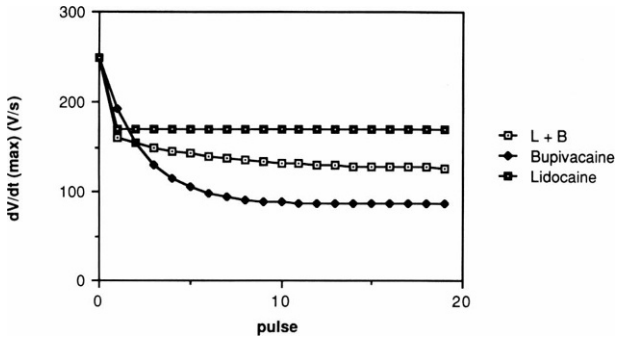


FIGURE 4 Predicted reduction in dV/dt_{max} resulting from 43 μM lidocaine, 3.5 μM bupivacaine, and a mixture of these two concentrations. Note that the mixture curve reaches a steady state value greater than that due to bupivacaine alone and the mixture steady state is achieved sooner.

Increasing the concentration of ligand in single agent binding studies results in increasing fractions of bound sites in both the continuous-access and periodic-access models of binding and are expressed by:

$$b_\infty = \left(1 + \frac{l_c}{k_c D}\right)^{-1} \tag{39}$$

and

$$R_{ss} = \frac{R_\infty(1 - e^{-\lambda_r t_r}) + E_\infty(1 - e^{-\lambda_+ t_+})e^{-\lambda_r t_r}}{1 - e^{-\lambda}}. \tag{40}$$

In both cases, increases in D always lead to increases in b_∞ and R_{ss} independent of the stimulus intervals. With mixtures of certain channel blocking agents, Schmidtmayer and Ulbricht (1980) and Clarkson and Hondeghem (1985*b*) noted the reverse behavior. In both cases, under certain conditions, the amount of observed channel blockade associated with the mixture was less than that due to the high affinity agent alone. I next sought to evaluate the theoretical model developed above looking for a critical stimulus interval, above which paradoxical agonism was evident and below which the agonism disappeared.

The lidocaine unbinding rate (l_r) is about an order of magnitude greater than that of bupivacaine, resulting in only small amounts of accumulated use-dependent block. One would expect, on the basis of equilibrium studies, that mixtures of lidocaine and bupivacaine would result in a greater fraction of blocked channels than either agent alone. While this is correct for a "true" equilibrium, under pulse train stimulation, a true equilibrium is not attained; only a steady state where the fraction of channels blocked during the excited interval is equal to that unblocked during the rest interval. Paradoxical agonism exists when an antagonist with fast recovery kinetics competes with a high affinity antagonist agent with slower recovery. The longer the recovery interval (allowing near complete dissociation of the fast recovery agent), the greater the apparent paradox, as shown in Fig. 5. When the stimulus rate is increased to the point that lidocaine recovery is incomplete,

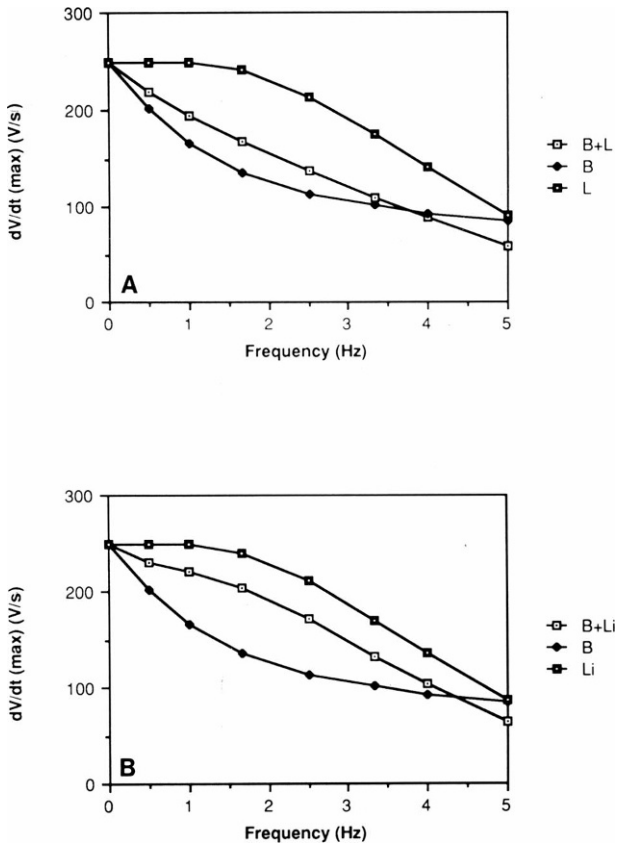


FIGURE 5 Predicted frequency-dependent steady state blockade resulting from 43 μM lidocaine, 3.5 μM bupivacaine, and a mixture of the two agents. (*A*) Competitive block: equivalent binding intervals. These curves were computed assuming that lidocaine (L) and bupivacaine (B) blocked only activated channels with a constant affinity. Note that the degree of blockade reduction of the mixture is less than that of panel *B*. (*B*) Competitive block: enhanced lidocaine rate during the open channel interval. These curves were computed assuming that lidocaine (Li) binds to open channels (access time = 1 ms) at a binding rate of 300 times the binding rate of that associated with the activated conformation. This increase in forward velocity results in a greater fraction of lidocaine-bound sites, that are then free to unbind during the recovery interval, resulting in less apparent blockade than that due to bupivacaine alone.

block in excess of that seen with bupivacaine alone is predicted. Under conditions of rapid excitation, the results approach those expected from studies of continuous ligand-receptor binding.

For illustrative purposes, I initially assumed both agents blocked only activated channels (Fig. 5 *A*). The degree of paradox based on this assumption was small since the binding rates of the two agents are nearly the same. A more realistic assumption is that in addition to (neutral) blockade of activated channels, the charged moiety of lidocaine significantly interacts with open channels at a rate that exceeds that of bupivacaine. Under these conditions, lidocaine will occupy a larger fraction of sites in contrast to bupivacaine, resulting in a larger degree of paradox. Using the open channel rates 300 times larger than the activated rate, assuming a 1-ms mean open interval and extending the blockade model to three states, I

recomputed the steady-state frequency-dependent blockade patterns. Fig. 5 *B* illustrates the paradox as a function of stimulus rate and again shows that for high rates, as continuous access is asymptotically approached, the paradox disappears. Note that the incorporation of significant open channel lidocaine blockade decreases the fraction of binding sites available to the more toxic agent during the activated conformation interval, resulting in a larger "agonist" effect.

DISCUSSION

Earlier models of sodium channel blockade (Strichartz, 1973; Courtney, 1975; Hondeghem and Katzung, 1977; Yeh, 1982; Starmer et al., 1984) used the Hodgkin-Huxley gating variables to modulate binding and unbinding rates. These gating variables reflect the fraction of channels in a particular conformation. With periodic stimulation, these gating variables change in a manner precluding closed form solution of the pseudo first-order binding equation.

From single channel measurements, rapid transitions between allosteric conformations are observed, suggesting that the time-varying fraction of channels in a particular conformation can be replaced by a pulse. When conformation dwell times are exponentially distributed, the pulse duration should be set equal to the mean conformation dwell time. Using a pulse-gating function, the binding equation reduces to a sequence of equations, one for each interval of assumed constant conformation mixture. These equations are the familiar constant coefficient binding equations, with a solution described by Eq. 1. That this simplification is an adequate approximation is shown in the Appendix.

Based on these arguments, Starmer et al. (1987) showed that the blockade model of Armstrong (1966) could be written in terms of a simple gating-based guard function. Moreover, the trapping models of Strichartz (1973) and Starmer et al. (1986) could also be written in terms of simple guard and trap functions. These theoretical observations provided a basis for a general class of models of ion channel blockade that could be easily tailored to the particular drug and channel under investigation. Moreover, the simplification of the gate functions led to several theoretical predictions (Eqs. 6 and 7) between blocking rates, steady state values, and stimulus parameters. These tests provide a quick test of the consistency between observations and the model assumptions. When one or more of the underlying assumptions (e.g., rapid equilibration) are not met, one would expect the consistency tests of Eqs. 6 and 7 to fail.

Many studies of channel blockade involve two agents, possibly competing for the same binding site. Frequently the two agents are unrelated, but often they are the neutral and changed moieties of a tertiary agent such as quinidine. In addition, metabolites of a drug may be active and lead to competition. Therefore a model of competitive ion channel blockade would be helpful in not only identifying the conditions for paradoxical agonism, but also in identifying the pKa of a drug complexed channel as defined by the protonation and deprotonation rates, k_p and l_p.

A number of investigators have evaluated competitive binding between a number of agents. Specifically, Wagner and Ulbricht (1975, 1976), Rimmel et al, (1978), Schmidtmayer and Ulbricht (1980), Clarkson and Hondeghem (1985*a*,*b*), and Chapula (1985) studied the response of sodium channels to a variety of mixtures of ion channel blocking agents. Agents exhibiting use-dependence (lidocaine, benzocaine, quinidine, procaine, and bupivacaine), as well as non–use-dependent agents (tetrodotoxin [TTX] and saxitoxin [STX]), have been studied. In particular, Ulbricht and colleagues have used competition for the binding site as a tool for establishing the existence of one or more binding sites.

For mixtures of STX and TTX, Wagner and Ulbricht (1975) found blockade to be reversible and the time course of blockade to be consistent with a single common, continuously accessible, binding site. In particular, addition of STX to a preparation pretreated with TTX led to an overshoot in additional block. Such a response is expected for two agents continuously competing for the same binding site. In followup studies of a use-dependent agent (procaine) and a non–use-dependent agent (STX), both equilibrium behavior and kinetic behavior suggested one-to-one drug-receptor interaction and two separate, independent binding sites. In particular, no transient overshoot was observed when procaine was added to an STX pretreated preparation. Moreover, they found that the procaine-receptor binding process was most likely limited by diffusional access of the drug to its receptor.

In studies of two use-dependent agents, procaine and benzocaine, Rimmel et al. (1978) observed less mixture-related block at 1 Hz than with the slower agent (procaine) alone, while at 10 Hz there was more mixture-related block than that due to procaine alone. Using a model of first-order interaction between the two drugs and a single common receptor, and assuming rate constants to be an instantaneous function of voltage, Rimmel et al. (1978) were able to reproduce their observed paradox. When two independent binding sites were assumed, the paradox disappeared. In studies of lidocaine and benzocaine, Schmidtmayer and Ulbricht (1980) again observed a paradox at 1 Hz stimulus rate in both their computation of blockade as well as in a number of their experimental preparations.

More recently, Chapula (1985) and Clarkson and Hondeghem (1985*b*) have studied mixtures of use-dependent agents in cardiac preparations. With the very slow recovery from bupivacaine blockade, Clarkson and Hondeghem (1985*b*) showed a frequency-dependent paradox, with the paradox disappearing at the highest stimulus rate (4 Hz). This paradoxical interaction is easily visualized if one considers the recovery time constant of the lower affinity agent relative to the stimulus-induced recovery interval.

When the stimulus interval is greater than three or four time constants, then all channels blocked by the low affinity agent have time to become unblocked. The result is a lower fraction of blocked channels at the end of the recovery interval than would have resulted from use of the single, higher affinity agent. Thus, the degree of paradox is determined by the extent that the low affinity agent occupies sites that otherwise would be occupied by the high affinity agent.

In equilibrium binding studies where ligand has continuous access to binding sites, competition among antagonists leads to greater binding for a mixture of antagonists than for any individual antagonist. Such equilibrium conditions rarely exist for certain ion-channel blocking agents. Blockade of gated ion channels by many agents shows use-dependence, a progressive increase in the fraction of blocked channels associated with repetitive channel excitation that is consistent with transient access to the binding site. After many instances of excitation, a steady state level of blocked channels is achieved where blockade acquired during excitation is balanced by that lost during the rest interval. Such a steady state is dependent on the kinetics of binding and unbinding and thus mixtures of ligands with different rate constants exhibit behavior that is dependent on both the speed with which blocking and unblocking takes place relative to the excitation and recovery intervals as well as equilibrium properties.

This additional complexity, introduced by the kinetic effects on the steady state fraction of blocked channels, makes it difficult to predict drug effects when more than one agent or a tertiary agent is used. Under equilibrium conditions, blockade, b_∞, is described by a simple function of the equilibrium dissociation constants as

$$b_\infty = \left(1 + \frac{1}{\frac{k_A D_A}{l_A} + \frac{k_B D_B}{l_B}}\right) - 1. \qquad (41)$$

As either D_A or D_B are increased, b_∞ will also increase. With pulse train excitation, this relationship as modified to describe steady state blockade becomes dependent on the rates of binding and unbinding as well as the excitation and rest intervals, as described by Eqs. 34 and 35. These factors interact in such a way that predictions of steady state behavior require an accurate description of both the kinetics of the underlying binding process and the stimulus timing. With such a description, one can readily evaluate the interaction between stimulus parameters and kinetic rates.

To facilitate the investigation of these frequency- and kinetic-dependent effects, I have derived an analytic solution for competitive use-dependent blockade that aids in estimating the transient as well as steady state components. Moreover, the scheme generalizes easily in order to match the stimulus protocol. Using the model with parameters derived from data reported by Clarkson and Hondeghem (1985*a*), I have found agreement with their studies of mixtures (Clarkson and Hondeghem, 1985*a*) and have illustrated the theoretical trade-off between binding kinetics and stimulus intervals. This model of binding to transiently accessible sites facilitates exploration of use-dependent phenomena and complements Colquhoun's (1968) description of competitive ligand binding to continuously accessible sites.

APPENDIX

Stochastic Stimulus Intervals

I have developed the description of ion channel blockade in the setting of periodic excitation where the stimulus intervals have a fixed duration. Blockade is viewed as the result of collision between drug and the binding site during the time the binding site is accessible. This time, though, is not constant. Recent patch-clamp studies of single-channel events have shown that many times the channel-open and channel-closed intervals are exponentially distributed. Thus, the question arises as to what interval of time should be assumed for the accessible interval. Here I show that blockade acquired during a constant interval equal to the mean channel conformation dwell time approximates the expectation of block acquired during an exponentially distributed dwell time.

Consider an ensemble of channels with a fraction in a bindable conformation and the remaining in an unbindable conformation. With mixture specific binding and unbinding rates k and l and accessible interval, t_a, then the block acquired is

$$b(t_a) = b(\infty) + [(b(0) - b(\infty)]e^{-\lambda_d t_a}$$

where

$$\lambda_d = kD + l \text{ and } b(\infty) = (1 + l/kD)^{-1}.$$

When the conformation dwell interval is exponentially distributed with density $\lambda_c e^{-\lambda_c t}$, then the expected blockade is

$$\langle b \rangle = \int_0^\infty \{b(\infty) + [b(0) - b(\infty)]e^{-\lambda_d t}\}\lambda_c e^{-\lambda_c t} dt$$

$$= b(\infty) + [b(0) - b(\infty)]\frac{\lambda_c}{\lambda_c + \lambda_d}.$$

When $t_a = 1/\lambda_c$, then $e^{-\lambda_d/\lambda_c}$ can be approximated by $\lambda_c/(\lambda_c + \lambda_d)$, thus demonstrating the approximate equivalence of the two expressions.

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