MECHANISMS OF USE-DEPENDENT BLOCK OF SODIUM CHANNELS IN EXCITABLE MEMBRANES BY LOCAL ANESTHETICS

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ABSTRACT Many local anesthetics promote reduction in sodium current during repetitive stimulation of excitable membranes. Use-, frequency-, and voltage-dependent responses describe patterns of peak I_{Na} when pulse width, pulse frequency, and pulse amplitude are varied. Such responses can be viewed as reflecting voltage-sensitive shifts in equilibrium between conducting, unblocked channels and nonconducting, blocked channels. The modulated-receptor hypothesis postulates shifts in equilibrium as the result of a variable-affinity receptor and modified inactivation gate kinetics in drug-complexed channels. An alternative view considers drug blocking in the absence of these two features. We propose that drug binds to a constant-affinity channel receptor where receptor access is regulated by the channel gates. Specifically, we view channel binding sites as guarded by the channel gate conformation, so that unlike receptors where ligands have continuous access, blocking agent access is variable during the course of an action potential. During the course of an action potential, the m and h gates change conformation in response to transmembrane potential. Conducting channels with both gates open leave the binding site unguarded and thus accessible to drug, whereas nonconducting channels, with gates in the closed conformation, act to restrict drug access to unbound receptors and possibly to trap drug in drug-complexed channels. We develop analytical expressions characterizing guarded receptors as "apparently" variable-affinity binding sites and predicting shifts in "apparent" channel inactivation in the hyperpolarizing direction. These results were confirmed with computer simulations. Furthermore, these results are in quantitative agreement with recent investigations of lidocaine binding in cardiac sodium channels.

INTRODUCTION

Local anesthetics block neuronal impulse transmission by inhibition of the sodium current. Tasaki (1953) showed that block of impulse transmission in toad sciatic nerve, treated with the anesthetic urethane, depended on stimulation rate; responses to high-frequency stimulation were blocked more readily than responses to low-frequency stimulation. This enhanced blocking potency during rapid stimulation has now been confirmed with a wide variety of local anesthetics (Matthews and Rushworth, 1958; Trubatch, 1972; Strichartz, 1973; Courtney, 1974, 1975; Khodorov et al., 1976; Hille, 1977; Courtney et al., 1978). Courtney (1975) termed this effect of the pattern of stimulation on the action of anesthetics "use-dependent block." A similar use dependence of the local anesthetic class of antiarrhythmic drugs has been observed in cardiac muscle (Weidmann, 1955; Johnson and McKinnon, 1957; Heistracher, 1971; Tritthart et al., 1971; Courtney, 1979; Hondeghem and Katzung, 1977; Colatsky, 1982; Grant et al., 1982; Weld et al., 1982). Use-dependent block is thought to be a particularly important mechanism of antiarrhythmic drug action. In general, action potentials occurring at physiologic rates are conducted normally; high-frequency discharges are strongly suppressed (Hondeghem and Katzung, 1977). A framework that explains use-dependent block is potentially important in understanding the mechanism of action and structure activity relationships of these agents in nerve and heart muscle.

The voltage clamp technique has been applied extensively in nerve and to a lesser extent in cardiac muscle to delineate the features of use-dependent block. Several hypotheses based on the interaction of the drugs with receptor site(s) have been proposed (Strichartz, 1973; Courtney, 1975; Khodorov et al., 1976; Hille, 1977; Hondeghem and Katzung, 1977). These hypotheses have provided a basis for understanding use dependence and pH effects on local anesthetic channel blockade.

The modulated-receptor hypothesis, proposed by Hille (1977) and independently by Hondeghem and Katzung (1977), is based on a variable-affinity channel binding site and a requisite modification of inactivation gate kinetics in drug-complexed channel. Hille (1978) describes the modulated-receptor hypothesis in terms of stronger binding of drug to inactive channels. On the basis of observed shifts in the inactivation curve derived from nerve preparations exposed to local anesthetic, Hille (1978) proposed the existence of modifications of one or both of the inactivation

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gate rate coefficients (α_h^* and β_h^*) in drug-complexed channels. A detailed balance among blocked and unblocked states at equilibrium requires that changes in the gating coefficients be coupled to a requisite change in binding affinity of the channel receptors.

One might question whether these two features are required to describe observations acquired during investigation of drug-channel interactions. Although Hille (1978) stated that these effects "must be included in any model of inactivation in the presence of drug," no data have been published that eliminate other, potentially simpler, blocking descriptions. One alternative, for instance, is to consider apparent shifts in channel inactivation and receptor affinity as the result of gated regulation of the diffusion path between the unbound drug pool and the channel binding site. Since gate behavior is voltage sensitive, the population of accessible receptors would appear voltage dependent, as would the resultant channel blockade. This sort of indirect, voltage-sensitive channel blockade could produce apparent shifts in inactivation curves as a result of blockade acquired during the various phases of the stimulus protocol. When there is a voltage-dependent net reduction in conducting channels over and above that caused by gate-mediated channel inactivation, an apparent inactivation shift in the hyperpolarizing direction will be observed.

Along this line of reasoning, we propose a guarded-receptor hypothesis where the channel gates are viewed as restricting drug access to and from a constant-affinity channel binding site. During the course of an action potential, the gates change conformation. Conducting channels with both activation and inactivation gates in the open conformation provide a postulated unrestricted access of hydrophilic drug to the binding site. For nonconducting channels, the channel gates are proposed to guard the binding site from blocking agents such as local anesthetics. (For drugs using the hydrophobic or membrane path to the channel receptor, the guarding may be limited to the activation gate.) On binding drug to a receptor, the m and/or h gate may become immobilized. We define gate immobilization as the inability of the gating protein to change from an open to a closed conformation. Such a hindrance might come from local interactions between the channel blocking agent and the gating mechanism.

On the basis of this viewpoint, we develop analytical results indicating "apparent" variations in inactivation and receptor affinity with membrane potential. We find that the major features of the modulated-receptor hypothesis (variable-affinity receptor and modified h gate kinetics in drug-complexed channels) can be viewed not as requisite channel features, but rather as "apparent" features arising as a natural consequence of channel gates restricting ligand access to a fixed-affinity receptor. Simulations of several stimulus protocols illustrating patterns of peak I_{Na} consistent with observations are described. In addition, we have computed h_∞ curves in the presence of local anesthetic and found apparent shifts in $V(h = \frac{1}{2})$. We have simulated dose-response experiments and have found apparent variations with potential of a fixed dissociation constant. Finally, we discuss the points of departure of the present model from earlier models of use-dependent block.

MODEL

In the presence of local anesthetics, variations of peak I_{Na} during repetitive stimulation suggest voltage-sensitive shifts in equilibrium between unblocked and blocked channels. Furthermore, development of block, which is dependent on the availability of open, conducting channels, suggests that the channel gates limit the access of drug to the channel binding site. We consider the kinetics of channel-blocking agents to be composed of two processes: one representing the coupling of drugs to a binding site, and the other representing the effect of channel gate conformations on drug access to the binding site.

Development of the model proceeds in three stages. We first consider the interaction of drug with the binding site as a first-order transition between unblocked and blocked channels. We next modify the rate coefficients, incorporating the energy requirements necessary to move charged drug from its primary pool to the binding site. Finally, we incorporate the effect of channel gate conformation on binding site access.

From the resulting "limited access" or "guarded receptor" hypothesis, we investigate equilibrium relationships for the fraction of drug-complexed channels and the effect of block on channel inactivation. From the equilibrium block, we show that the receptor affinity appears to vary with transmembrane potential, and that channel inactivation appears shifted in the hyperpolarizing direction.

Binding Site Kinetics

For each conformation of channel, let the interaction of a blocking agent, D, with an unblocked channel binding site, U, be represented by the first order process

$$U + D \underset{r_o}{\overset{k_o}{\rightleftharpoons}} B, \tag{1}$$

where B represents drug-complexed channels, and k_o and r_o are the forward and reverse rate coefficients for drug binding. For a specific channel conformation, the fraction of drug-complexed channels, b, at any given time, will thus be given by the solution of:

$$\frac{db}{dt} = k_o[D](1 - b) - r_o b. \tag{2}$$

In the presence of a membrane charge, the binding kinetics are modified for charged drugs because of the energy requirements related to movement of drug across a potential difference. Let V_{pool} represent the potential of the source pool of drug and let V_{rec} represent the potential of the channel drug receptor. Then the additional energy

required to move a drug of charge z from its pool to the receptor is $z(V_{pool} - V_{rec})$. (Some investigators have represented the receptor potential as a fraction, δ, of the membrane potential, i.e., $V_{rec} = \delta V_m$.) The addition of the charge movement effect modifies the forward and reverse rate coefficients such that

$$k_o = k e^{z\epsilon(V_{pool} - V_{rec})F/RT}; \quad (3)$$

$$r_o = r e^{z(1-\epsilon)(V_{rec} - V_{pool})F/RT}, \quad (4)$$

where ϵ represents the partition of the charge effect between the forward and reverse reactions. For $\epsilon = 1$, all the charge effect resides in the binding reaction, whereas for $\epsilon = 0$, all the charge effect resides in the unbinding reaction. The resulting description of unrestricted binding of a charged drug to a binding site in the vicinity of a charged membrane is then

$$\frac{db}{dt} = k\,[D]\,(1 - b)e^{z\epsilon(V_{pool} - V_{rec})F/RT} - rbe^{-z(1-\epsilon)(V_{pool} - V_{rec})F/RT}. \quad (5)$$

Channel Gate Kinetics

A number of channel gating models, ranging in complexity from the independent gating model of Hodgkin and Huxley (1952*b*) to the multistate coupled model of Moore and Cox (1976), have been proposed. Overall blocking behavior will, to a varying extent, be influenced by the gating model selected for incorporation into the overall description of channel block. We have chosen initially to use the Hodgkin-Huxley (H-H) independent gate model for several reasons. First of all, the H-H model leads to closed-form expressions of equilibrium block, apparent receptor affinity, and apparent channel inactivation that aid in visualization of the complex behavior of guarded-receptor binding. In addition, these expressions can be compared with results of numerical simulations based on more complex models, and the sensitivity of channel block to the underlying gating model can be assessed. Moreover, equilibrium block may be insensitive to the underlying gate model. For instance, one can show the algebraic equivalence of equilibrium block defined by the H-H independent model and a simple, single state, coupled model (closed ↔ open ↔ blocked).

Hodgkin and Huxley (1952*b*) viewed the sodium channel as a conducting channel whose conductivity is controlled by two gates, m and h, in series. Assuming first-order transitions between the closed conformation, C, and the open conformation, O, as

$$C_m \underset{\beta_m}{\overset{\alpha_m}{\rightleftharpoons}} O_m$$

$$C_h \underset{\beta_h}{\overset{\alpha_h}{\rightleftharpoons}} O_h$$

and assuming three m particles per channel, four channel conformations result. The fraction of channels in each conformation is given in Table I, where m^3 is the fraction of channels with 3 m particles in the open conformation, and h as the fraction of channels with an h particle in the open conformation.

Further suppose that for each of the above four gate configurations there is a configuration-independent equilibrium dissociation constant, K_d, which reflects differing association (k_R, k_A, k_{I_1}, k_{I_2}) and dissociation (r_R, r_A, r_{I_1}, r_{I_2}) rate coefficients that characterize the transitions between unblocked and blocked channels. Therefore, although the forward and reverse rate coefficients may differ for different configurations, the dissociation constants, K_d, for each process are equal to each other. The distribution of the population of channels between drug-free and complexed states will therefore reflect the time-varying sum of the kinetic relationships describing each of the four gate configurations within the channel.

Fig. 1 illustrates the time-dependent fraction of channels (solid lines) in each of the four gate states during the time course of a membrane action potential (dashed lines) as computed using the parameters for the H-H equations (Hodgkin and Huxley, 1952*b*). The open channel fraction, m^3h, represents that fraction of channels where the diffusion path from the drug pool to the binding site is least restricted. For this simulated patch, there is a change of four orders of magnitude in the fraction of open channels for the membrane at the resting voltage and at the peak voltage of the action potential.

Combining the binding kinetics as described by Eq. 5 with the independent gating model leads to a general expression describing the overall kinetics for a guarded binding site. For s_u different conformational states of unblocked channels, the compound block can be described by

$$\frac{db}{dt} = \sum_{s=1}^{s_u} f_s k_s\,[D]\,(1 - b)\,e^{z\epsilon(V_{pool} - V_{rec})F/RT} - \sum_{s=1}^{s_u} g_s r_s b e^{-z(1-\epsilon)(V_{pool} - V_{rec})F/RT}, \quad (6)$$

where for the sth channel conformation, $f_s(1 - b)$ is the fraction of unblocked channels, $g_s b$ is the fraction of blocked channels, and k_s and r_s are the forward and reverse rate coefficients.

TABLE I
FRACTIONAL DISTRIBUTION OF CHANNEL STATES

m^3	h	State	Fraction of Channels
closed	open	rest (R)	$(1 - m^3)\,h$
open	open	active (A)	m^3h
open	closed	inactive$_1$ (I_1)	$m^3\,(1 - h)$
closed	closed	inactive$_2$ (I_2)	$(1 - m^3)\,(1 - h)$

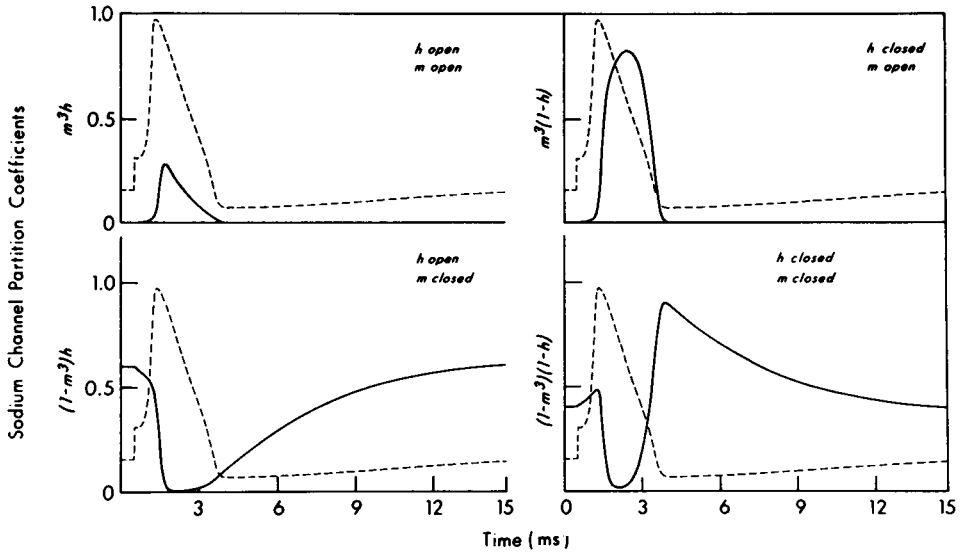


FIGURE 1 The time-varying fraction of each conformation of channel (solid lines) during the course of a single action potential (dashed lines). The upper left panel shows the most favorable channel configuration for drug interaction (h open, m^3 open). This open channel conformation enhances the binding of blocking agents for only a few milliseconds. The upper right panel represents the h closed, m^3 open configuration, which dominates the channel mixture during the active phase of the action potential. This conformation may enhance the action of blocking agents that are lipid soluble and thus able to bypass the h gate. The lower panels show the m^3 closed, h open, or h closed conformations. These two conformations dominate the channel mixture during the resting and recovery phases of the action potential and represent the most restricted access path from the blocking agent pool to the binding site.

By assuming the receptor affinity is independent of channel conformation, the expression for equilibrium block can be simplified considerably. At a constant holding potential, the partition of channels among the different conformations reaches a steady state, and the equilibrium block can be written as

$$b(\infty) = \left[1 + \frac{WK_d}{D} e^{-z(V_{pool} - V_{rec})F/RT}\right]^{-1}, \quad (7)$$

where $K_d = r_s/k_s$ and W is a weighting factor determined by the fraction of channels in each conformation, f_s and g_s.

Measurement of Block

As yet, there is no error-free technique available for assessing the fraction of drug-complexed channels. The time-varying amplitude of sodium current provides an accurate measure of aggregate channel conductivity as determined by channel gating and blocking effects of drugs, but only provides an approximate measure of block immediately prior to the stimulus. Since many blocking agents bind to open channels (Strichartz, 1973; Yeh, 1979), the process of stimulating the membrane in order to measure I_{Na} will change the fractional block present just prior to the stimulus by an amount dependent on the binding kinetics. Therefore, peak I_{Na} will be biased depending on the experimental setting. It is interesting, then, to explore the postulated modification of I_{Na} by drug-complexed channels, assuming no bias in the measurement, in order to gain insight into the proposed binding mechanism.

Here we assume that drug-complexed channels do not conduct, so the effect of blocked channels is to reduce $\bar{g}_{Na}$ as

$$I_{Na} = \bar{g}_{Na}(1 - b)m^3h(V - V_{Na}). \quad (8)$$

On the basis of this definition of I_{Na}, we can evaluate the effect of drug-complexed channels on estimates of apparent channel inactivation, h^*.

Using the two-pulse protocol of Hodgkin and Huxley (1952*a*), the channel inactivation, h, measured in the absence of drug, is determined by the ratio of peak I_{Na} after a selected conditioning potential to peak I_{Na} after a reference hyperpolarizing potential. In the presence of a channel-blocking agent in equilibrium with the sodium channels, an apparent inactivation parameter, h^*, can be written as

$$h^*(V_{cond}) = \frac{I_{Na_{max}}[V_{cond}, b(\infty, V_{cond})]}{I_{Na_{max}}[V_{ref}, b(\infty, V_{ref})]}, \quad (9)$$

where V_{cond} is the conditioning potential, V_{ref} is the reference potential, and $b(\infty, V)$ is the equilibrium value of block at a potential, V. Assuming m gate kinetics to be much faster than h gate kinetics and using Eq. 8 as the definition of I_{Na}, then

$$h^*(V_{cond}) = \frac{h(V_{cond})[1 - b(\infty, V_{cond})]}{[1 - b(\infty, V_{ref})]}. \quad (10)$$

Since $1 - b(\infty, V_{ref})$ is a scale factor and $b(\infty, V_{ref})$ is frequently negligible, we can approximate h^* as

$$h^*(V_{cond}) \approx h(V_{cond})[1 - b(\infty, V_{cond})]. \quad (11)$$

Thus, as a first approximation, the apparent inactivation is equal to the true inactivation reduced by the fraction of unblocked channels. It should be noted that this effect is independent of drug charge.

SPECIAL CASES

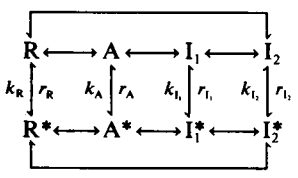
Sodium current response patterns to repetitive stimulation in the presence of different local anesthetics are not uniform. Pancuronium, for instance, reduces sodium current during depolarizing stimuli and immobilizes both activation and inactivation charge (Yeh, 1982), whereas the lidocaine derivative QX314 shows use dependence with repetitive stimuli but only immobilizes the activation charge (Cahalan and Almers, 1979). Hille (1977) has suggested that hydrophobic and hydrophilic drugs may access the channel binding sites using different diffusion paths. For instance, one might view both m and h gates as guarding receptor access of a hydrophilic drug, but only the m gate as guarding receptor access of a hydrophobic drug. These features affect the kinetic description of drug binding, which is reflected in the state transition diagram. In the following special cases, we will adapt the general model to some interesting cases.

Each different transition diagram has a different blocking equation associated with it. For each case, we will describe the steady state fractional block, the apparent dissociation coefficient, K_d^*, and the apparent inactivation parameter, h^*. For convenience, we assume $V_r = V_{pool} - V_{rec}$ for each example.

Detailed Balance Restriction

Although there are eight forward and reverse rate coefficients in the unrestricted model, they are not all independent. We have assumed that h gate kinetics are unaffected in drug-complexed channels so that the equilibrium dissociation coefficients (r_s/k_s) for each channel conformation are equal. Thus, the eight free variables are reduced to five. These five free variables include a forward and reverse rate coefficient and three proportionality factors. With gate immobilization, the number of free rate constants is reduced further, as shown in the following special cases.

Case 1: No Gate Immobilization. The resulting eight-state transition diagram is



For this case, assume $k_R = w_R k_A$, $k_{I_1} = w_{I_1} k_A$, $k_{I_2} = w_{I_2} k_A$, $r_R = w_R r_A$, $r_{I_1} = w_{I_1} r_A$ and $r_{I_2} = w_{I_2} r_A$. Then

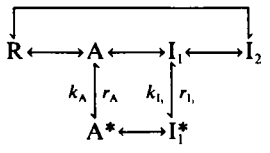
$$b(\infty) = \left(1 + \frac{r_A e^{-zV_mF/RT}}{k_A[D]}\right)^{-1}; \quad (12)$$

$$K_d^* = \frac{r_A e^{-zV_mF/RT}}{k_A}; \quad (13)$$

$$h^* = \frac{h}{\left(1 + \frac{k_A[D]\, e^{zV_mF/RT}}{r_A}\right)}. \quad (14)$$

The apparent dissociation constant, K_d^*, is a function of drug charge z and is equivalent to the standard dissociation constant, K_d. Therefore, one would expect a shift in equilibrium for charged drugs but not for neutral drugs. Moreover, neutral drugs will not produce a shift in apparent inactivation, h^*, but charged drugs will.

Case 2: m Gate Immobilization. m gate immobilization requires that the open-to-closed rate coefficient for the m gate in drug-complexed channels, β_m, is significantly reduced. For illustrative purposes, assume that $\beta_m = 0$ for drug-complexed channels. The resulting six-state transition diagram with three free rate coefficients is



Let $k_{I_1} = w_{I_1} k_A$ and $r_{I_1} = w_{I_1} r_A$. Then

$$\frac{db}{dt} = [k_A m^3 h + k_{I_1} m^3 (1 - h)]\,(1 - b)\,[D]\, e^{zV_mF/RT} - [r_A h + r_{I_1}(1 - h)]\, b e^{-z(1-\epsilon)V_mF/RT}; \quad (15)$$

$$b(\infty) = \left(1 + \frac{r_A}{k_A m^3 [D]} e^{-zV_mF/RT}\right)^{-1}; \quad (16)$$

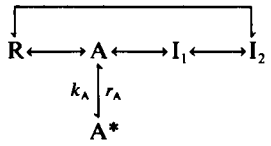
$$K_d^* = \frac{r_A}{k_A m^3} e^{-zV_mF/RT}; \quad (17)$$

$$h^* = \frac{h}{\left(1 + \frac{k_A m^3 [D]\, e^{zV_mF/RT}}{r_A}\right)}. \quad (18)$$

Here, the apparent dissociation constant varies with both the membrane potential and the fraction of channels with their m^3 gates open. We would then expect equilibrium shifts, even for neutral drugs, with changes in the transmembrane potential. It is interesting to note also that for hyperpolarizing potentials where m^3 is 0, there is no binding, and the apparent K_d, K_d^*, is infinite. As the

transmembrane potential becomes progressively less negative, K_d^* becomes smaller, and when $m^3 = 1$, it becomes solely a function of the drug charge and membrane potential. For this case, the apparent inactivation is shifted in the hyperpolarizing direction, regardless of drug charge. With the same rate constants, anionic drugs will produce the largest shift, with neutral and cationic drugs producing progressively smaller shifts. (Kendig et al. [1979] found inactivation shifts for cationic, neutral, and anionic drugs. Without kinetic parameters, comparison of the degree of shift is ambiguous.)

Case 3: m and h Gates Immobilized. Again, for illustrative purposes, we assume for drug-complexed channels that β_m and β_h are zero. The only remaining drug-complexed state is A*. The resulting five-state transition diagram with two free rate coefficients is



Then

$$\frac{db}{dt} = k_A m^3 h\,(1 - b)\,[D]\,e^{z\epsilon V_m F/RT} - r_A b\,e^{-z(1-\epsilon)V_m F/RT}; \quad (19)$$

$$b(\infty) = \left(1 + \frac{r_A}{k_A m^3 h [D]}\,e^{-zV_m F/RT}\right)^{-1}; \quad (20)$$

$$K_d^* = \frac{r_A}{k_A m^3 h}\,e^{-zV_m F/RT}; \quad (21)$$

$$h^* = \frac{h}{\left(1 + \dfrac{k_A m^3 h [D]\,e^{zV_m F/RT}}{r_A}\right)}. \quad (22)$$

For this interesting case, the apparent dissociation constant is parabolic in form as a result of the dependence on the activation and inactivation gates. In addition, K_d^* is dependent on the interaction between drug charge and membrane potential. Independent of drug charge, the apparent inactivation will be shifted in the hyperpolarizing direction.

DETAILED EXAMPLE WITH QX314

We start by using Eq. 6, the most general statement of the blocking relationship, and simplify it on the basis of experimentally derived observations. Cahalan and Almers (1979) found evidence of m gate immobilization with the lidocaine derivative QX314. Immobilization of the m gates upon binding of drug to the channel receptor has the effect of reducing the set of blocked channels from four to two, represented by A* and I_1^*. The resultant six-state model is equivalent to that described by Yeh (1982) for QX314 (see case 2). With a six-state model (four unblocked states and two blocked states), the number of free rate coefficients is reduced to three. We assume that the majority of the charge effect is related to drug dissociation as shown for 9AA by Cahalan (1978) and for pancuronium by Yeh (1982). Therefore, we let ϵ be equal to 0. For six compounds (etidocaine, tetracaine, *N*-methylstrychnine, 9AA, QX222, and QX314), Cahalan (1978) found the fractional electrical distance between the membrane interior and receptor to vary between 0.83 and 0.90, which indicates a receptor site electrically near the exterior surface. We consider QX314 as accessing the receptor via the hydrophilic path where both m and h gates guard the receptor. Let the potential of the intracellular drug pool equal the membrane potential, V_m, and assume the receptor is located electrically near the surface of the membrane, so that V_{rec} can be approximated by 0 mV. The resultant simplified model with m gate immobilization as shown for case 2 is

$$\frac{db}{dt} = [k_A m^3 h + k_{I_1} m^3 (1 - h)]\,(1 - b)\,[D] - [r_A h + r_{I_1}(1 - h)]\,b e^{-zV_m F/RT}. \quad (23)$$

COMPUTATIONAL MODEL

The set of equations required to fully describe the model consists of two components: the gating model and the drug binding model. We have used both the Hodgkin and Huxley (1952*b*) model of sodium channel gating in squid axon and the Ebihara and Johnson (1980) model of sodium channel gating in cardiac muscle. Both models use the same first-order gating model, but the rate coefficients differ. The rate coefficients for an x gate, α_x and β_x, describing the H-H gating model, have been adjusted for a resting membrane potential of −60 mV. For drug binding, we use a first-order model of drug-receptor interaction. The set of equations describing the gates, drug blocking, and sodium current is:

$$\frac{dm}{dt} = \alpha_m(1 - m) - \beta_m m; \quad (24)$$

$$\frac{dh}{dt} = \alpha_h(1 - h) - \beta_h h; \quad (25)$$

$$\frac{db}{dt} = [k_A m^3 h + k_{I_1} m^3 (1 - h)]\,(1 - b)\,[D] - [r_A h + r_{I_1}(1 - h)]\,b e^{-zV_m F/RT}; \quad (26)$$

$$I_{Na} = \bar{g}_{Na}\,(1 - b)\,m^3 h (V_m - V_{Na}). \quad (27)$$

Initial conditions were computed from

$$m(\infty) = \frac{\alpha_m}{\alpha_m + \beta_m}; \quad (28)$$

$$h(\infty) = \frac{\alpha_h}{\alpha_h + \beta_h}; \tag{29}$$

$$b(\infty) = \left(1 + \frac{r_A}{k_A m^3 [D]} e^{-zV_m F/RT}\right)^{-1}. \tag{30}$$

For the squid channels, $\bar{g}_{Na} = 120$ mS and $V_{Na} = 55$ mV. For the cardiac channels, $\bar{g}_{Na} = 23$ mS and $V_{Na} = 33.4$ mV. These relationships describe drug-channel interaction for a single active drug moiety.

Several active moieties of drug, either multiple drugs or charged and neutral forms of the same compound, can be modeled as separate entities. The total fractional block, b, is then computed from the sum of the block due to each species. For charged and neutral elements, external pH and drug-specific pK_a can be used to compute concentrations of the charged and neutral fractions, as suggested by Schwarz et al. (1977). Drug concentrations on either side of the membrane are considered in equilibrium. With improved models of diffusion across the membrane, these approximations can be made more realistic.

All computations were based on a voltage clamp driving function. Durations and amplitudes of a particular pulse protocol were used to drive the integration of Eqs. 24–26 using the appropriate gating rate coefficients. An Adams-Moulton predictor-corrector integration technique (Conte, 1965) using a step size of $1/128$ ms was used to perform all simulations.

SIMULATIONS

Cahalan (1978), in a study of quaternary lidocaine derivatives, found QX222, QX314, QX572, etidocaine, 9-aminoacridine, and procaine to exhibit varying degrees of use dependence and different recovery time constants in normal squid giant axon. From the recovery data in Fig. 2 of Cahalan (1978), we have estimated the values for k_A, and r_A, assuming that k_{I_1} and r_{I_1} are negligible. We assumed that the binding kinetics could be described by case 2, which leads to recovery kinetics consisting of an equilibrium component [$b(\infty)$] and a transient component as described by Eqs. 32 and 33. The value of resting block [$b(\infty)$] was estimated from the last data point. The values of k_A and r_A were then estimated from Eqs. 32 and 33, using a least-squares procedure. Table II indicates the values estimated from these data.

These values were used to simulate progressively more complex stimulus protocols. For illustrative purposes, we have chosen to use the QX314 rate constants. With QX314 values, the peak I_{Na} in response to a stimulus pulse train was simulated using the case 2 model, in order to demonstrate use dependence. The holding potential was then varied and the relationship between use dependence and holding potential was investigated. Results of these computations are presented below in the simulation results.

TABLE II
RATE CONSTANTS

Drug	k_A	r_A	K_d
	$M^{-1}ms^{-1}$	ms^{-1}	M
Etidocaine	9.262×10^3	1.974×10^{-5}	2.13×10^{-9}
QX222	1.250×10^3	5.540×10^{-6}	4.43×10^{-9}
QX314	4.119×10^3	5.534×10^{-6}	1.34×10^{-9}
QX572	1.720×10^4	2.702×10^{-6}	1.57×10^{-10}

Recent investigations of lidocaine binding with cardiac sodium channels by Bean et al. (1983) lead to the conclusion that lidocaine binds more tightly to depolarized, inactive channels than to hyperpolarized, resting channels. Our hypothesis suggests that these data are also consistent with a constant-affinity receptor whose access is regulated by gating. We simulated dose-response experiments using the case 2 model, following the Bean protocol in order to evaluate this alternative interpretation.

Using the cardiac gating model of Ebihara and Johnson (1980), coupled with Eqs. 24–27, we computed dose response curves at holding potentials of -120 and -44 mV. (Bean et al. used a potential of -65 mV, which corresponded to an $h < 1\%$ in their preparation. For the Ebihara-Johnson model, a voltage of -44 mV was used in order to achieve comparable values of h.) The computations were based on a single theoretical receptor K_d of 10^{-8} M evaluated at 0 mV based on the data provided in Bean et al. (1983). We further assumed that $V_{pool} = V_{membrane}$, $V_{rec} = 0$ mV, $z = +1$, and $T = 18$°C. Based on this value of K_d, the individual rate constants were set to $k_A = 2 \times 10^4\ M^{-1}\ ms^{-1}$, $r_A = 2 \times 10^{-4}\ ms^{-1}$, $k_{I_1} = 1\ M^{-1}\ ms^{-1}$, and $r_{I_1} = 10^{-8}\ ms^{-1}$.

ANALYTICAL RESULTS

Using case 2 to characterize QX314 binding at a drug concentration of 1 mM, the apparent receptor dissociation constant and the apparent channel inactivation vary with voltage, as shown in Figs. 2 and 3. Note in Fig. 2 that for hyperpolarizing potentials, K_d^* is quite large and asymptotically approaches infinity as V_m becomes more negative. As the membrane potential becomes more negative, the fraction of m gates in the closed conformation increases, with a resultant reduction in apparent receptor population.

Fig. 3 illustrates the effect of equilibrium block on the apparent channel inactivation, h^*. The rightmost curve is the control curve computed with $[D] = 0$. The curve labeled $z = 1$ is for 10^{-3} M QX314. The curves labeled $z = 0$ and $z = -1$ depict apparent inactivation for a neutral and anionic drug inducing m gate immobilization and having the same rate coefficients as QX314. These curves were computed from Eq. 18, which does not include the effect of channels blocked during a test pulse. The apparent inactivation curve for QX314 is shifted ~10 mV in the hyperpolarizing direction. Computing an apparent inactivation curve for neutral anionic drugs with equivalent rate

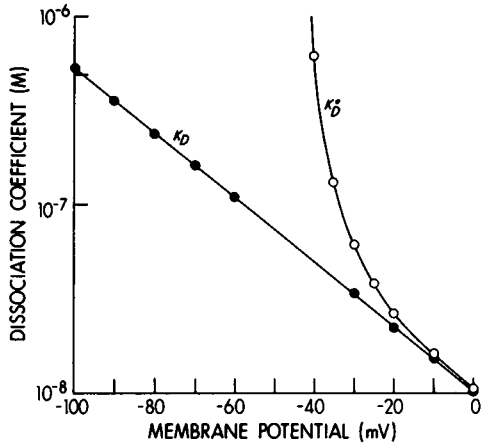


FIGURE 2 True and apparent dissociation functions. The linear relationship between potential and K_d shows the "true" binding site dissociation function for a positively charged binding agent. Including the effect of gated restriction to the binding site access, as described by case 2, results in a nonlinear relationship between the apparent K_d and the potential. As the potential becomes progressively more hyperpolarized, the equilibrium distribution of sodium channels shifts to the closed m gate conformation that guards the binding site from drug access and results in values of apparent K_d in excess of "true" K_d.

coefficients results in shifts in the hyperpolarizing direction of 15 and 20 mV.

SIMULATION RESULTS

The basis of transient modification of the peak I_{Na} in the presence of drug with depolarizing pulses requires a shift in

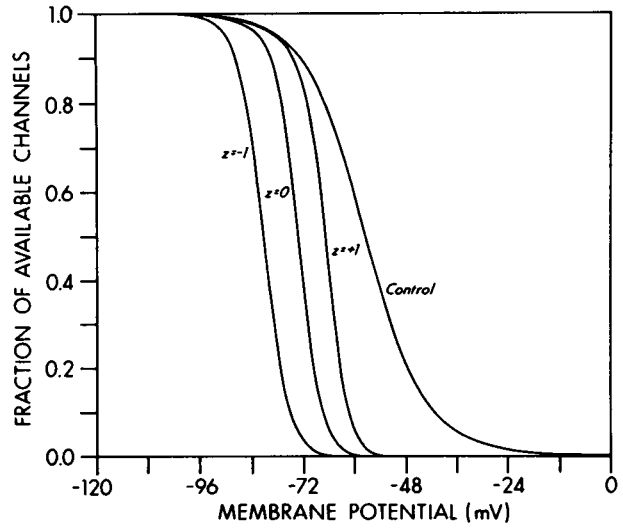


FIGURE 3 The theoretical effect of drug-complexed channels on apparent inactivation. The rightmost curve is a standard inactivation curve based on the Hodgkin-Huxley gating model (control). Based on a binding process described by case 2, the $z = 1$ curve reflects apparent inactivation in the presence of 1 mM QX314. Emphasizing the role of gating in contrast to drug charge, the $z = 0$ and $z = -1$ curves reflect apparent inactivation in the presence of hypothetical neutral and anionic drugs with QX314 rate constants. For both charged and neutral drugs, the shift in apparent inactivation is a result of voltage-sensitive equilibrium block determined by both transmembrane potential and gating effects.

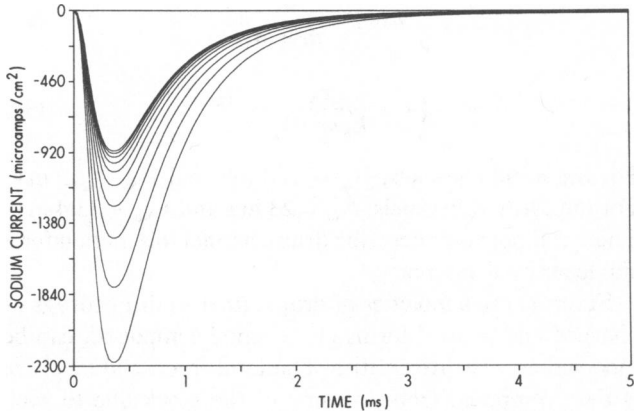


FIGURE 4 Progressive reduction of inward sodium current in the presence of 0.2 mM QX314 at a holding potential of −120 mV. A pulse train of 5-ms depolarizations to 0 mV at a frequency of 5/s was used. The largest curve is associated with the first depolarizing pulse. Each successive curve in I_{Na} is of reduced amplitude as a result of prior accumulated block, as well as block acquired during the given depolarizing pulse.

the equilibrium between blocked and unblocked channels. Such a shift can arise from voltage-dependent rate coefficients or from gate immobilization. One means for eliciting transient shifts in drug-channel equilibrium is to stimulate with repetitive voltage pulses. For drugs with a recovery time constant in excess of one-quarter of the interstimulus interval, a new equilibrium will be reached as evidenced by a reduced peak I_{Na}. Fig. 4 demonstrates the results of this shift using pulses at 5/s. The largest curve is associated with the first pulse and the smallest is associated with the last pulse.

Variation of the holding potential for the pulse train produces varying degrees of use dependence as shown in Fig. 5. The relative sodium current reflects peak I_{Na} for each pulse relative to the peak I_{Na} in the absence of drug. Initial block at time zero was computed from the equilibrium block (Eq. 30) at each holding potential. For a hyperpolarizing holding potential ($V_{hold} = -150$ mV, $h = 1$), there is a decline in sodium current during the pulse train. With more depolarized potentials (-120 mV $< V_{hold} < -70$ mV), there are larger reductions in sodium current during the train. More drug is bound during the period when a significant fraction of channels have their m^3 and h gates open than is lost during the recovery period between stimuli, with a net increase in channel-bound drug. Thus, a greater amount of use dependence is apparent. As the holding potential is further decreased ($V_{hold} = -60$ mV), there is a little further decline in sodium current. The parabolic relationship between membrane potential and the fraction of open channels is reflected in the amount of use dependence at each holding potential. For holding potentials sufficient for h to reach a value of 1, much of the drug incorporated during an action potential is released during the recovery period. With $h \leq 0.5$, very little additional drug is incorporated during channel activation. With $1 > h > 0.5$, there is

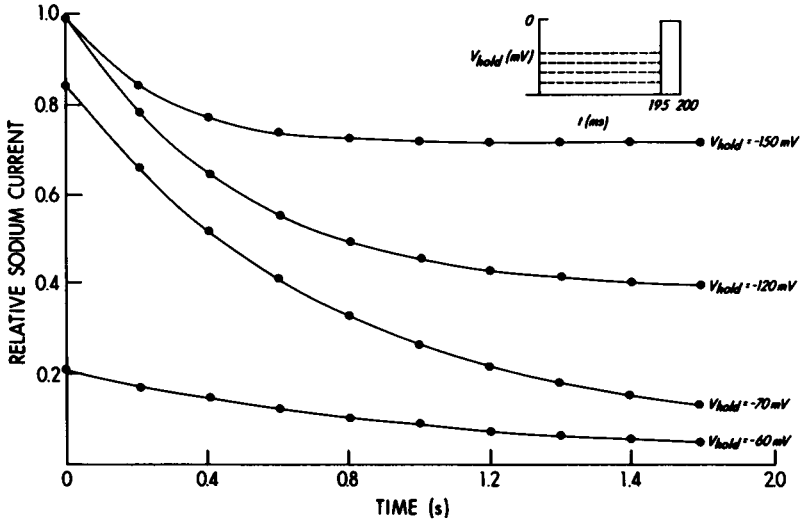


FIGURE 5 The effect of holding potential on the peak sodium current during repetitive stimulation in the presence of 0.2 mM QX314. Relative I_{Na} is defined as the ratio of peak I_{Na} associated with each data point to peak I_{Na} in the absence of drug. Initial block was computed from Eq. 30 and evaluated at each holding potential. At a frequency of 5/s with a holding period of 195 ms, a progressive decline in peak sodium current occurs for each holding potential. The amount of use dependence, however, varies with the holding potential. For a hyperpolarizing holding potential of −150 mV, a small attenuation of peak sodium current results because of the voltage-enhanced recovery of block during the interstimulus interval. For holding potentials between −120 and −70 mV, the progressive reduction in peak I_{Na} is much greater. For holding potentials more depolarizing than −60 mV, very little use dependence is apparent.

more drug binding than unbinding during each stimulus period until steady state is reached.

Simulation of the cardiac sodium channel-lidocaine dose-response curves produces results similar to those in Bean et al. (1983). From simulated measurements of I_{Na} for different concentrations of lidocaine, two curves were plotted (Fig. 6); one for $V_{hold} = -120$ mV and $V_{hold} = -44$ mV. The half-response points on each curve were then determined. With a fixed, "true" K_d of 10 nM, we found an apparent K_d^* of 350×10^{-6} M for $V_{hold} = -120$ mV and 9×10^{-6} M for $V_{hold} = -44$ mV, in very close agreement with Bean et al. Furthermore, an apparent inactivation curve was computed and found to be shifted in parallel by 6 mV in the hyperpolarizing direction, again in agreement with the observations in Bean et al.

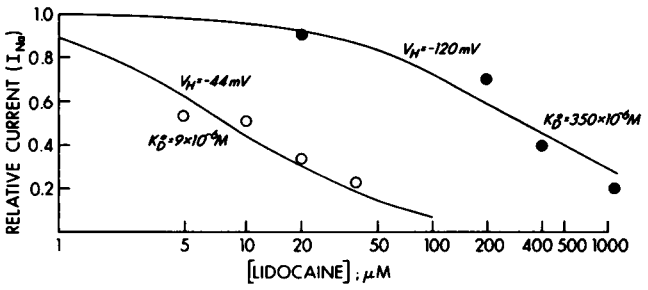


FIGURE 6 Predicted and observed dose-response curves for lidocaine in cardiac muscle. The empty circles and filled circles are data points from Bean et al. (1983) for measurements at holding potentials of −120 and −65 mV. A "true" dissociation constant of 10^{-8} M was derived from the −65 mV data. Using this dissociation constant, the two solid curves were computed for holding potentials of −120 and −44 mV (see text). The variation in dose-response as a function of holding potential is consistent with a fixed "true" K_d and a gating effect, as shown in Eq. 17.

DISCUSSION

Sodium channel conductivity in the presence of local anesthetics appears to be affected by the formation of drug-complexed, nonconducting channels with a resultant reduction in $\bar{g}_{Na}$. The fraction of drug-blocked channels reflects a balance between rates of binding and release of drug from the channels. This equilibrium varies with the "reactivity" of the channel-drug interaction, which we postulate as determined by the degree of restriction of drug access to the channel receptor and the drug charge. Indeed, Yeh (1979) suggests that the h gate limits the access of 9-aminoacridine to the channel binding site, and thus contributes to regulation of the drug-channel interaction. Similarly, Cahalan (1978) has found evidence that the sodium inactivation gating mechanism plays a direct role in producing use-dependent inhibition of QX222, QX314, strychnine, and 9AA. Thus, it seems clear from an experimental perspective that gate-controlled access to the channel binding site can produce use-dependent behavior. What remains unclear, though, is whether modified inactivation kinetics in drug-complexed channels are also necessary. So far, data supporting such a modification in kinetics have not been forthcoming.

The Hodgkin-Huxley formalism views a sodium channel as a gated conduit where two gates (m and h) operate in series to control ionic flow through the conduit. We view hydrophilic drug access to the site of binding as controlled by the same two gates. Thus, four channel configurations

of differing "reactivity" are possible, depending on the conformation (open/closed) of each gate. We postulate that the conformation with both gates open is the "most reactive" in terms of drug binding, while the conformation with both gates closed is the "least reactive." For hydrophobic drug access, we postulate receptor access as being controlled by only the activation or m gate.

Use and frequency dependence represent shifts in equilibrium between unblocked and blocked channels when the membrane is subjected to a fixed-frequency pulse train. The shifting fraction of blocked channels between the old equilibrium, as determined by the holding potential, and the new equilibrium, as determined by the depolarizing potential, is observable when the time constant of drug-receptor binding exceeds one-quarter of the interstimulus interval. These voltage-sensitive shifts in equilibrium can result from gate immobilization in drug-complexed channels or from interaction between membrane charge and drug charge.

Although equilibrium shifts caused by voltage-sensitive rate coefficients are well known, equilibrium shifts caused by gate immobilization in sodium channels by local anesthetics have not been previously proposed. The importance of the dependence of equilibrium between blocked and unblocked channels on gate immobilization is critical for visualizing the paradoxical behavior of neutral local anesthetics such as benzocaine.

Benzocaine is known to provoke large shifts in the apparent channel inactivation. Unlike charged drugs, this cannot be explained by an interaction between drug and membrane charge. However, benzocaine is known to immobilize the activation gates (Khodorov et al., 1979). As shown earlier, blocking agents that completely immobilize the m gates result in a reduction of blocked states to the two conformations A* and I_f^*. The impact of a reduction in the blocked states is a "rectification" effect on drug binding. In other words, while the blocking path is via the m^3h fraction of unblocked channels, the unblocking path is via a different fraction, h, of the blocked channels. The inequality of these two fractions of channels represents a sort of impedance difference for the overall diffusion path of drug to and from the receptor, since $m^3h < h$. This asymmetry of diffusional impedance forms the basis of the rectifier, which is readily derived from case 2. The unblocking rate constant is proportional to $[h + w_{I_f}(1 - h)]r_A$, while the blocking rate constant is proportional to $[h + w_{I_f}(1 - h)]m^3k_A$. The resulting ratio yields r_A/m^3k_A, where m^3 is the "rectifier" effect. Note that the apparent dissociation constant (Eq. 17) is an explicit function of not only membrane potential but also the fraction of channels with three m particles in the open conformation. The activation gate parameter effectively modulates the forward rate coefficient.

For a neutral drug such as benzocaine, there is no explicit dependence between block and membrane potential so that any shift in equilibrium block requires gate immobilization. This can be illustrated by reviewing the definition of channel inactivation as determined by I_{Na} measurements. For a neutral drug, $z = 0$, so that Eq. 18 becomes

$$h^*(V_{cond}) = \frac{h}{\left(1 + \frac{k_A m^3 [D]}{r_A}\right)}. \tag{31}$$

From this we see that for neutral drugs, the degree of shift between h and h^* is related to both the receptor affinity constant as well as the m gate properties. Clearly, for hyperpolarizing potentials where m is near 0, $h^* = h$, while for more depolarizing potentials where m is nonzero, $h^* < h$. For positively charged drugs such as lidocaine and the QX derivatives, the field effect serves to reduce the apparent affinity constant, as shown in Eq. 18, which results in less apparent reduction of h. Comparing the effects of a neutral and charged drug with the same rate constants at equilibrium, we expect h^* ($z = -1$) $< h^*$ (neutral) $< h^*$ ($z = +1$) $< h$.

Coupling the dissociation rates of drug-complexed channels with transmembrane potential, as originally suggested by Strichartz (1973), is important for explaining hyperpolarizing voltage-enhanced drug release of charged drugs. The incorporation of the voltage-dependent term, $r_A \exp(-zVF/RT)$, increases the rate of dissociation from drug-bound channels with increasing hyperpolarization. Yeh and Narahashi (1977) found a similar voltage dependence of unbinding in studies of pancuronium in squid giant axon. Our choice of a voltage-dependent factor rather than a shift in the $h_\infty - V$ curve (which implies a modification of gate properties, as suggested by Courtney, 1975), is a departure from previous models. Recently, Weld et al. (1982) failed to demonstrate such voltage dependence of inactivation in cardiac muscle and suggested, as an alternative, voltage-dependent binding constants.

The models of Courtney (1975), Khodorov et al. (1976), Hille (1977), and Hondeghem and Katzung (1977) have all been successful in accounting for use and frequency dependence, but have been based on a hypothetical coupling between drug-complexed channels and their inactivation gate kinetics. Furthermore, the modulated receptor model of Hille (1977) and Hondeghem and Katzung (1977) postulates a variable-affinity receptor whose properties change with channel state. If one assumes a variable receptor affinity, a detailed microscopic balance among the channel states requires a modification of inactivation gate kinetics. Our viewpoint differs significantly from that of these earlier investigators, primarily as a result of gating charge data that became available after their models were proposed. Specifically, we have found it unnecessary to require either a variable-affinity receptor or a modification of inactivation gate kinetics in drug-complexed channels. Reduction of the number of drug-complexed states for

those drugs that immobilize channel gate charges in conjunction with incorporation of the drug charge–membrane charge field effect appears adequate to describe current observations.

Recent data by Bean et al. (1983) provide a setting in which one can readily test some of the predictions of the guarded-receptor hypothesis. Studying the effects of lidocaine in cardiac muscle, dose-response curves were determined at two different holding potentials. Using analyses based on equilibrium conditions derived from the modulated receptor hypothesis, dissociation constants of 353×10^{-6} M at $V_{hold} = -125$ mV and 9.7×10^{-6} M at $V_{hold} = -65$ mV were found. This was interpreted as evidence that lidocaine binds much more tightly to inactivated channels than to resting channels. In addition, they inferred a shift in inactivation of drug-complexed channels as required by the principle of microscopic balance. It is not clear whether the K_d actually measured was a true receptor dissociation constant or an apparent K_d measuring some combined property of channel gating and receptor affinity.

Our hypothesis treats gating and binding as separate processes, so that an interaction between channel gating and receptor affinity should be derivable. From this theoretical relationship, we would expect predicted voltage-sensitive dose-response curves, equivalent to those in Bean et al. (1983), which would yield different apparent dissociation constants from a single true K_d. Inspection of Eq. 17 indeed shows both a voltage and gating effect on the theoretical steady state level of block. However, this relationship does not include any measurement bias introduced by drug uptake during the test pulse. Thus, we simulated the dose-response protocol used by Bean et al. in order to quantitatively assess the two competing hypotheses.

As seen in Fig. 6, the resulting dose-response curves, based on simulations that included drug uptake during the test pulse, are in very close agreement with the data of Bean et al. The 40-fold change in the apparent K_d appears as the combined effects of gating, drug charge–membrane charge interaction, and measurement bias.

We find that many of the patterns of peak I_{Na} in the presence of local anesthetics can be described by a kinetic model of gate-mediated restricted access to a channel receptor. A use-dependent pattern where peak I_{Na} is progressively reduced by a train of depolarizing pulses is one example of a pattern predicted by our model, and is the result of a voltage-sensitive shift in equilibrium between blocked and unblocked channels.

During the normal transmembrane potential variation associated with an action potential, the fraction of channels in each conformation varies. For open channel binding, the time-varying population of channel partitions specifies that net drug binding occurs during the depolarizing phase of the action potential, while net release occurs during each interstimulus interval. After a number of stimuli at a fixed rate, a point is reached where the drug released during the interstimulus interval will equal the drug bound during depolarization. At this point, an apparent equilibrium between blocked and unblocked channels is reached. This apparent equilibrium depends on the relationship between the blocking time constant, the depolarization interval, and the interstimulus interval. The time constant of block is determined by the forward and reverse rate constants, fractional population of each channel partition, and transmembrane potential. Significant frequency-dependent I_{Na} response patterns appear only when the interstimulus interval is less than the four time constants of the drug-channel interaction.

The range of equilibrium block and time constants associated with changes from one condition to another is impressive when viewed as a result of gate behavior. For case 2, the level of "resting" or "tonic" block can be estimated from the equilibrium determined by Eq. 26. For a fixed holding potential, V_{hold}, and ignoring the initial transient change in m^3 and h, transient and steady state description of block can be written as

$$b(t) = b(\infty) - [b(\infty) - b(0)]e^{-(kD+r)t}, \tag{32}$$

where $k = k_A m^3 h + k_{I_1} m^3 (1 - h)$

$$r = [r_A h + r_{I_1}(1 - h)]\, e^{-zVF/RT}$$

and

$$b(\infty) = \frac{k_A m^3 [D]}{k_A m^3 [D] + r_a e^{-zVF/RT}}. \tag{33}$$

From this relationship, it is clear that the predicted steady state fraction of block is dependent on not only the concentration of drug and rate constants for dissociation and association but also on the steady state partition of sodium channels, as determined by $m(V_{hold})$ and $h(V_{hold})$ and the transmembrane potential. For very negative holding potentials, r becomes large relative to k such that $b(\infty)$ approaches zero. For holding potentials where $h > 0$, the predicted steady state level of block exhibits a dependence on the holding potential.

The time constant associated with achieving a steady state level of block is $(kD + r)^{-1}$ and is dependent on the holding potential. For a $[D]$ of 1 mM, the parameters for QX314 at $V_{hold} = -60$mV, $m = 0.05$, and $h = 0.6$ yield a time constant of 10.2 s, which is comparable to that found by Cahalan (1978). Such a long time constant is consistent with drug-related slow sodium inactivation noted by Khodorov et al. (1974) and Khodorov (1981). Immediately after a depolarizing potential, however, the time constant is altered radically. Immediately after shifting from a resting potential to $V_{hold} = 0$, where $m = 1$ and $h = 0.6$, the time constant is 0.4 ms, four orders of magnitude different from the "resting" time constant. This change is largely due to the change in m^3h between the two holding potentials. It is not surprising, then, that small changes in experimental protocol are capable of producing a broad spectrum of peak sodium current patterns where the factor controlling the pattern of I_{Na} response is the dynamic transition of sodium channels among the four configurations.

With estimated dissociation constants in the nanomolar region for lidocaine and the QX derivatives, can gate regulation of drug access to the receptor site provide a means for rationalizing such low K_d's with the doses required for an observable effect? Consider a stimulus rate of 1/s and a 1-ms depolarizing period during which the channel gates provide unimpeded drug access to the binding site. With continuous receptor access, one expects 50% bound channels at equilibrium for a drug concentration of K_d. For a transiently accessible receptor (1 ms in this example), a comparable effect requires a dose of 1,000 K_d, assuming very fast kinetics and negligible unbinding during the 999-ms "rest" or recovery period. In other words, to achieve the same blocking effect with a transiently accessible receptor seen with a continuously accessible receptor, the forward reaction velocity must be increased by increasing the drug concentration in order to compensate for the short period of receptor access. For net observable uptake, then, it is not surprising to find doses of 10^4 or 10^5 K_d required, depending on the relationship between binding kinetics and channel open time. These scaling factors are in agreement with values observed in the lidocaine interactions with cardiac muscle reported by Bean et al. (1983).

With several models of drug-channel interaction, what tests can be devised to differentiate between the alternatives? One immediate test is to determine the requisite level of binding process complexity necessary to account for experimentally derived observations. For instance, what experimentally derived observations require, as channel features, a variable-affinity receptor and modified inactivation gate kinetics in drug-complexed channels? On the other hand, can a binding process of reduced complexity, such as a guarded receptor, adequately account for these data?

In terms of new experiments, several studies are suggested from the guarded-receptor hypothesis. For instance, it would be interesting to study drugs that have comparable rate constants but differ in charge in order to test the relative shift in apparent inactivation, as shown in Fig. 3.

Another potentially discriminating test would be to evaluate the voltage sensitivity of recovery from block. For a drug with slow recovery kinetics, the modulated-receptor hypothesis suggests that with increasingly negative potentials, the block recovery time constant should stabilize at the potential where h^* (the apparent fraction of noninactivated drug-complexed channels) becomes 1. The guarded-receptor hypothesis suggests that for cationic drugs, accelerated recovery from block should continue for potentials beyond the point where $h^* = 1$.

The most revealing studies should be based on direct observation of gate behavior. Recently, Neher (1983) has shown that for low doses of QX222 interacting with acetylcholine (ACh)-activated channels, gate kinetics appear unmodified in drug-complexed channels, and drug blockade reflects a simple occlusion. Extending these procedures to electrically activated channels could directly address questions arising from postulated drug-gate interactions. Such studies may provide data suggesting a further decomposition of rate coefficients into more elemental effects than the simple field effect considered here.

Although we have developed the guarded-receptor hypothesis in the setting of the Na channel, it may be applicable to other settings where gated channels guard binding site access from a blocking agent. Candidates for extension of the model include the potassium channel and the calcium channel in cardiac muscle.

Finally, one cannot rule out, on the basis of our analyses, modified inactivation gate kinetics in drug-complexed channels as required by the modulated-receptor hypothesis. We have shifted the agent responsible for equilibrium behavior from the h gate, as suggested by Hille (1977), to the m gate. Recent channel gating data (Cahalan and Almers, 1979), as well as tail current data (Yeh, 1979), support this emphasis. For illustrative purposes, we have assumed the m gate is completely immobilized in a drug-complexed channel, though we see no reason to exclude partial immobilization. The strength of the guarded receptor hypothesis is that the number of free variables required to characterize the interaction between a gated ionic channel and a blocking agent is reduced.

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