

A Quantitative Study of Modulated Receptor and Allosteric Effector Models of Drug Action

Lisa A. Irvine, M. Saleet Jafri, and Raimond L. Winslow

Department of Biomedical Engineering and
Center for Computational Medicine and Biology
The Johns Hopkins University School of Medicine
Baltimore, MD 21205

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Correspondence to:

Raimond L. Winslow
The Johns Hopkins University School of Medicine
Department of Biomedical Engineering
411 Traylor Building, 720 Rutland Avenue
Baltimore, MD 21205
Phone: (410) 502-5090
Fax: (410) 614-0166
E-mail: rwinslow@bme.jhu.edu

ABSTRACT

Several models have been proposed to explain block of cardiac sodium channels by class I antiarrhythmic drugs. In this study, the ability of two of these models, a modulated receptor (MR) model developed by Hondeghem and Katzung (Hondeghem and Katzung, 1977) and an allosteric effector (AE) model developed by Balser et al (Balser, et al., 1996), to quantitatively reproduce a wide range of drug effects is tested. The Hondeghem-Katzung MR model reproduces use-dependence, onset of block, recovery from block, and dose-response data. However, model parameters that reproduce these data, do not yield reasonable drug affinities for each state, do not preserve microscopic reversibility, and do not reproduce changes in the charge-voltage curve due to drug binding. The AE model reproduces the dose-response curve and drug-induced shift of the steady-state inactivation function measured experimentally, but cannot reproduce time-dependent drug effects. Adding a single drug bound state to the AE model improves its ability to reproduce time-dependent drug effects, which suggests that explicit drug bound states are essential for modeling lidocaine's action. However, the inability of the AE and Hondeghem-Katzung MR models to comprehensively reproduce a wide range of drug effects suggests that neither model fully captures the mechanism of drug action.

INTRODUCTION

Several hypotheses have been formulated to explain block of cardiac sodium channels by class I antiarrhythmic drugs. They are the guarded receptor (GR) (Starmer, et al., 1984; Starmer and Grant, 1985; Starmer and Courtney, 1986; Starmer, 1987), modulated receptor (MR) (Hille, 1977; Hondeghem and Katzung, 1977), and allosteric effector (AE) (Balser, et al., 1996) hypotheses. The GR hypothesis states that the affinity of a receptor for drug is constant, while access to the receptor varies with channel state (Starmer, et al., 1984; Starmer and Grant, 1985; Starmer and Courtney, 1986; Starmer, 1987). Once drug binds, the channel remains in a non-conducting state until drug unbinds. Drug binding need not alter gating kinetics (Starmer and Courtney, 1986).

In contrast, the MR hypothesis states that the affinity of a receptor for drug changes with channel state, while access to the receptor remains constant. It is assumed that drug can bind to any channel state, but does so with different affinities for each state and each drug (Hille, 1977; Hondeghem and Katzung, 1977). By permitting state-dependent binding, gating of drug bound channels must be modified in order to preserve energy balances. Therefore, the MR hypothesis requires that drug bound channels gate with altered kinetics.

More recently, it has been proposed that lidocaine acts as an allosteric effector (Balser, et al., 1996). Under this hypothesis, drug binding is assumed to reduce the Gibbs free energy for the inactivated channel conformation, thereby stabilizing it and rendering the channel unavailable to conduct. Since the presence of drug induces channels to inactivate more readily and to remain inactivated for an extended period of time (Balser,

et al., 1996), the AE hypothesis requires that drug bound channels gate with altered kinetics.

Recent experiments support the hypothesis that drug bound channels gate with altered kinetics. Hanck and colleagues found that QX-222, a quaternary derivative of lidocaine, dramatically reduces the slope of the charge-voltage relationship, from which they conclude that gating of drug bound channels is less voltage dependent than gating of non-drug bound channels (Hanck, et al., 1994). Since the MR and AE hypotheses require drug bound channels to gate with altered kinetics, they are both supported by these experimental data.

Quantitative models have been formulated from the MR (Hille, 1977; Hondeghem and Katzung, 1977), GR (Starmer, et al., 1984; Starmer and Grant, 1985; Starmer and Courtney, 1986; Starmer, 1987) and AE (Balsler, et al., 1996) hypotheses, but no extensive analysis of the ability of these models to simultaneously reproduce a wide range of drug effects has been performed. In this study, models of lidocaine's action on the cardiac sodium channel based on both the MR and AE hypotheses are analyzed. The GR hypothesis is not included because it does not require drug bound channels to gate with altered kinetics. Model rate constants are determined by simultaneously fitting four data sets describing lidocaine's effects. In this manner, the ability of each model to comprehensively reproduce a wide range of drug effects is tested. The results provide additional insight into the mechanism of lidocaine's action and provide a basis for the design of improved antiarrhythmic drug models.

METHODS

The Hondeghem-Katzung Modulated Receptor Model

The MR hypothesis is tested using the Hondeghem-Katzung MR model (Hondeghem and Katzung, 1977). This model is shown in Fig. 1. The top row of states are the resting, activated, and inactivated states in which drug is not bound (R, A, and I respectively). The lower row of states are the corresponding drug bound states (RD, AD, and ID). In this model, it is assumed that drug can interact with any of the channel states, that the drug binding and unbinding rate constants are unique for each drug, and that drug bound channels do not conduct (Hondeghem and Katzung, 1977). Hodgkin-Huxley type equations, with the modification that drug bound channels have their voltage dependence of inactivation shifted to more negative potentials, govern state transitions within each row. The Hodgkin-Huxley equations for the sodium current are taken from the DiFrancesco-Noble ventricular cell model (DiFrancesco and Noble, 1985). These equations apply at a temperature of 37°C, but the experimental data describing lidocaine's effects are at 17°C. To revise these equations for a temperature of 17°C, experimental data show that the steady-state activation and inactivation curves should be shifted to more negative potentials and the time constants of activation and inactivation should be increased (Colatsky, 1980; Murray, et al., 1990). Shifts in the steady-state activation and inactivation curves, time constants of activation and inactivation, and maximal sodium conductance are determined by fitting model responses to ionic currents measured experimentally in isolated human ventricular myocytes at 17°C (provided by Wasserstrom, similar to (Sakakibara, et al., 1993)). The resulting modified equations are as follows:

$$g_m = \frac{0.2(V - 48.8)}{1 - \exp[-0.1(V - 48.8)]} \quad (1)$$

$$g_m = 8 \exp[0.056(V - 73.8)] \quad (2)$$

$$g_h = 0.02 \exp[0.125(V - 88.6)] \quad (3)$$

$$g_h = \frac{2}{1 + 320 \exp[0.1(V - 88.6)]} \quad (4)$$

$$g_m = \frac{1.2}{g_m + g_m} \quad (5)$$

$$g_h = \frac{3.9}{g_h + g_h} \quad (6)$$

The maximal sodium conductance, G_{Na} , is 0.085 μ S. Figure 2A shows representative traces of the model-derived ionic current in comparison to experimental data for clamp voltages of +50 mV, +30 mV, and +10 mV. Figure 2B compares the current-voltage relationships of the model and experimental data. Peak current values as well as the time course of activation and inactivation are well fit by the model throughout the voltage range tested. Fitting both the model and experimental current-voltage curves using a modified Boltzmann function yields a conductance, slope factor, and half-maximal voltage of 0.017 mV^{-1} , +6.7 mV, and +41 mV for the experimental data and 0.017 mV^{-1} , +5.9 mV, and +40.6 mV for the model, respectively. Although the model has a slightly steeper slope, the conductance and half-maximal voltages are similar.

Allosteric Effector Model

The AE hypothesis is tested using the Balser model (Balser, et al., 1996). This model assumes that binding and unbinding of drug occur faster than channel state

transitions. Therefore, drug bound states are not explicitly included in this model. Rather, the assumed action of drug is to increase the rate constants from the closed and open states into the closed-inactivated and inactivated states and to decrease the rate constants governing the reverse transitions.

The AE model of Balser et al has as its basis a Markov model of the skeletal muscle sodium channel. In order to facilitate comparison with the Hondeghem-Katzung MR model, the underlying Markov model should be that of a cardiac sodium channel. Therefore, our implementation of the AE model, shown in Fig. 3, has as its basis a thirteen-state model of the cardiac sodium channel developed previously (Irvine, et al., 1999). The top row of states corresponds to zero to four voltage sensors being activated (C_0 through C_4) plus an additional conformational change required for opening ($C_4 \rightarrow O_1$ and $C_4 \rightarrow O_2$). The bottom row of states corresponds to the inactivation particle blocking the pore at each position of the voltage sensors. All transitions are voltage dependent except for those between the closed and closed-inactivated states, which are voltage independent. Rate constants are of the form from Eyring rate theory (Hille, 1992):

$$k_{ij} = \frac{kT}{h} \exp\left(\frac{\Delta S_{ij}^\ddagger}{R}\right) \exp\left(-\frac{\Delta H_{ij}^\ddagger + z_{ij} FV}{RT}\right) \quad (7)$$

where k is the Boltzmann constant, T is the absolute temperature, h is the Planck constant, R is the gas constant, F is Faraday's constant, ΔH_{ij}^\ddagger is the change in enthalpy, ΔS_{ij}^\ddagger is the change in entropy, z_{ij} is the effective valence (ie., the charge moved times the fractional distance the charge is moved through the membrane), and V is the membrane potential in volts. The model's rate constants are formulated so that microscopic reversibility is

preserved. Drug effect is incorporated into the model by modifying the transition rates between the top and bottom rows (Balser, et al., 1996):

$$D_n = C_n(1 - A_{on}[\textit{lidocaine}]) \quad (8)$$

$$D_f = C_f(1 - A_{off}[\textit{lidocaine}]) \quad (9)$$

$$DO_n = O_n(1 - A_{on}[\textit{lidocaine}]) \quad (10)$$

$$DO_f = O_f(1 - A_{off}[\textit{lidocaine}]) \quad (11)$$

where C_n , C_f , O_n , and O_f are the rate constants from the original model (Irvine, et al., 1998) and A_{on} and A_{off} are the scale factors that render the dose-dependent changes in the activation-inactivation coupling (Balser, et al., 1996).

Determination of Model Parameters

Parameters of the MR and AE models are determined by fitting a variety of experimental data sets describing lidocaine's action. For the MR model, differential equations for the Hodgkin-Huxley variables can be written in matrix notation as

$$\frac{d\mathbf{H}(t)}{dt} = \mathbf{W}\mathbf{H}(t) + \mathbf{B} \quad (12)$$

where $\mathbf{H}(t)$ is a vector containing the Hodgkin-Huxley variables m , h , and h' (the inactivation gating variable for drug bound channels), \mathbf{W} is the transition matrix, and \mathbf{B} is a vector of constants. The experimental protocols for each data set can be divided into time segments during which membrane potential is held constant. Therefore, during each of these time segments, \mathbf{W} is time-independent and the differential equations have an analytic solution. This solution is computed using linear algebra subroutines running on a Silicon Graphics computer. For the AE model, the probability of occupying any

particular channel state can be written as a set of differential equations of the form of Eq. 12 with \mathbf{B} equal to zero. Therefore, these equations also have an analytic solution.

Three different data sets at a lidocaine concentration of 200 μM (Furukawa, et al., 1995), as well as the dose-response curve (Jia, et al., 1993) are used to test the models. The first data set describes use-dependence. Use-dependent block is induced by applying a train of 3 ms depolarizing pulses from -140 mV to -20 mV at a rate of 5 Hz (Furukawa, et al., 1995). The second data set describes the rate of block onset due to a -20 mV conditioning pulse of varying duration. The extent of block is measured using a 30 ms test pulse to -20 mV after a 500 ms recovery interval at -140 mV (Furukawa, et al., 1995). The third data set describes the rate of recovery from block. Block is induced by applying a train of 10 ms depolarizing pulses from -140 mV to -20 mV at a rate of 30 Hz. The extent of recovery is then measured using a 30 ms test pulse to -20 mV after holding at -140 mV for various intervals (Furukawa, et al., 1995). The dose-response curve is defined as the fraction of unblocked sodium current elicited by the twentieth pulse of a 2 Hz train as a function of drug concentration. Pulses are from -140 mV to -20 mV for 100 ms (Jia, et al., 1993). Together these data sets reflect drug binding (onset data), drug unbinding (recovery data), the balance between binding and unbinding (use-dependence data), and concentration-dependence (dose-response data).

The drug binding (k_R , k_A , k_I) and unbinding rates (l_R , l_A , l_I) and the shift of the steady-state inactivation curve (V) of the Hondeghem-Katzung MR model are parameters to be determined by fitting model responses to experimental data. The scaling factors A_{on} and A_{off} of the AE model are determined in the same manner. Parameters are determined using a simulated annealing algorithm (Corana, et al., 1987). This algorithm

minimizes a cost function, which is the weighted sum of the least-squared errors between model responses and experimental data, by randomly searching the parameter space and incrementally decreasing the search radius. Whereas many minimization algorithms accept only downhill moves and tend to converge on local minima, the simulated annealing algorithm accepts uphill moves as well and thus, is more likely to find the global minimum. Uphill moves are accepted based on the Metropolis criterion, a probabilistic function determined from the difference between new and old errors and the annealing temperature. The annealing temperature controls the rate of convergence by influencing what uphill moves are accepted and by limiting the search radius. In order to reach a minimum, as the algorithm converges on a solution, the annealing temperature is decreased by 5% per 50N function evaluations, where N is the number of parameters to be determined. The algorithm is terminated when there is no more than 0.1% change in error since the last temperature reduction.

RESULTS

Parameters of the Hondeghem-Katzung MR model reproducing individual experimental data sets were determined first. The results are shown in Fig. 4. For each of the four data sets, parameters can be found for which the model response (?) fits the experimental data (O) well. Although parameters can be found which yield good fits to each individual data set, the resulting rate constants cannot be used to predict any data sets not included in the fitting process. For example, Fig. 5 shows that the rate constants yielding good fits to the use-dependence data alone do not accurately predict the onset, recovery, and dose-response data. These results suggest that accurate fitting of each

experimental data set is dependent on different combinations of model parameters. For example, accurate fitting of use-dependence data depends critically on the ratio of drug binding and unbinding rates, and is much less dependent on the absolute value of each of these rates. In contrast, fitting of the onset data depends critically on the absolute value of the binding rates. Therefore, in order to develop a well-constrained model that reproduces a range of drug effects, model parameters must be determined by fitting more than one experimental data set.

Parameters of the Hondeghem-Katzung MR model were also obtained by fitting all four experimental data sets simultaneously. The resulting parameters produce model responses (⊗) that fit all four data sets (O) reasonably well (Fig. 4). However, the drug-induced shift of the model's steady-state inactivation curve (V) is 41.5 mV - much larger than the 10.9 mV shift measured experimentally at the same lidocaine concentration (Furukawa, et al., 1995). The affinities of the resting, activated, and inactivated states are 529 μ M, 9.8 μ M, and 32.6 μ M, respectively. The resting and inactivated state affinities are similar to those reported by Bean (440 μ M and 10 μ M, respectively) (Bean, et al., 1983). However, the activated state affinity is much higher than experimental estimates, which find it to be less than that of the inactivated state (Bean, et al., 1983; Kodama, et al., 1990; Bennett, et al., 1995). The affinity of the activated state has been estimated using the rate of block development at various depolarized potentials and is calculated only relative to that of the inactivated state (Bean, et al., 1983; Kodama, et al., 1990). Additional estimates of the activated state affinity can be obtained by disabling inactivation (Bennett, et al., 1995), but this method does not guarantee that in disabling inactivation one is not also changing the affinity. Using this

method, Bennett estimated the affinity of the activated state to be 600 μ M. Regardless of the method used to calculate the activated state affinity, it should be significantly less than that of the inactivated state. This is not the case in the Hondeghem-Katzung MR model.

Three possible explanations exist for the discrepancies between experimental data and the model's activated state affinity and shift of the steady-state inactivation function. First, despite use of methods to prevent it, the minimization algorithm could have converged to a local minimum. Such convergence can typically be avoided by changing the initial parameter values used in the fitting process. However, even when different starting points were chosen, the simulated annealing algorithm could not find a set of parameters having the correct activated state affinity. A second possibility is that the activation and shift parameters are not well constrained by the experimental data sets. For example, drug binding and unbinding rate constants cannot be measured directly for the activated state because occupancy of this state is very brief. However, any choice of data sets is subject to this limitation. Additionally, the shift of the steady-state inactivation function may not be well constrained because the experimental data sets used in the fitting process did not directly measure this shift. To test this hypothesis, the shift of the drug bound steady-state inactivation function was fixed at ≈ 10 mV and the drug binding and unbinding rate constants were determined using all four data sets simultaneously. The resulting parameters provide as good a fit of all four data sets as when the shift of the drug bound steady-state inactivation function is not held constant (data not shown). The resulting affinities of the resting, activated, and inactivated states are 547 μ M, 6.2 nM, and 27.2 μ M, respectively. Once again, while the resting and

inactivated state affinities are similar to those measured experimentally (Bean, et al., 1983), the affinity of the activated state is much too large (Bean, et al., 1983; Kodama, et al., 1990; Bennett, et al., 1995). Therefore, while it is possible to find a set of parameters that reproduce all four data sets as well as the shift of the steady-state inactivation function, the activated state affinity still differs significantly from the experimentally measured value. Since neither of these explanations seems adequate to account for the discrepancy in the activated state affinity, a third possible explanation is that the mechanism by which the Hondeghem-Katzung MR model reproduces drug effects is not correct.

The Hondeghem-Katzung MR model employed so far does not satisfy microscopic reversibility. Microscopic reversibility is derived from the law of conservation of energy and states that the product of rate constants when traversing a loop in one direction must be equal to the product of rate constants when traversing the same loop in the opposite direction (Hille, 1992). All chemical reactions, including drug binding and unbinding, must satisfy microscopic reversibility. To examine microscopic reversibility in the Hondeghem-Katzung MR model, the underlying Hodgkin-Huxley sodium current model was expanded to an eight-state Markov model (Fig. 6A) (Chay, 1991) and eight corresponding drug bound states were added (Fig. 6B). To maintain the spirit of the Hondeghem-Katzung MR model, all resting (R_1 , R_2 , and R_3) and inactivated (I_1 , I_2 , I_3 , I_4) states were assumed to have the same drug binding (k_R and k_I) and unbinding (l_R and l_I) (Fig. 6C) rates. Also, drug bound channels had their voltage dependence of inactivation shifted to more negative potentials (denoted by $\phi_{h'}$ and $\phi_{h''}$ in Fig. 6B).

In the resulting model, there are three loops that do not automatically satisfy microscopic reversibility (Fig. 6C): the resting-activated drug bound loop, the activated-inactivated drug bound loop, and the resting-inactivated drug bound loop. The equations to ensure microscopic reversibility for each of these loops, respectively, are as follows:

$$\frac{k_A}{l_A} \gamma \frac{k_R}{l_R} \quad (13)$$

$$\frac{k_A l_I}{k_I l_A} \gamma \frac{?_{h'} ?_h}{?_h ?_{h'}} \quad (14)$$

$$\frac{k_R l_I}{k_I l_R} \gamma \frac{?_{h'} ?_h}{?_h ?_{h'}} \quad (15)$$

These equations are not independent; satisfying only two equations guarantees that all three are satisfied. Twelve different pairs of rate constants can be constrained by solving these equations; this number can be reduced by examining the voltage dependence of Eqs. 14 and 15. These equations require k_R and k_A to increase with voltage and k_I to decrease with voltage. Experiments show that activated state block increases as voltage increases (Strichartz, 1973; Courtney, 1975). Therefore, the voltage dependence of k_A imposed by microscopic reversibility is consistent with experimental data. Similar experimental data exist for inactivated state block (Furukawa, et al., 1995). However, Eqs. 14 and 15 predict that k_I decreases with voltage, which is contrary to the experimental data. The pairs of rate constants constraining k_I can thus be eliminated as possible solutions. A similar argument applies to the unbinding rates and therefore, the pairs of rate constants constraining l_I can also be eliminated. This line of reasoning results in four pairs of rate constants that can be constrained by Eqs. 13-15 to satisfy microscopic reversibility.

The ability of the expanded Hondeghem-Katzung MR model to reproduce experimental data was tested using each of these four pairs of rate constants to satisfy microscopic reversibility. First, microscopic reversibility was used to constrain k_R and k_A . From Eqs. 14 and 15, the following equations were obtained for k_R and k_A :

$$k_R = \frac{k_I l_R}{l_I} \frac{h'}{h} \quad (16)$$

$$k_A = \frac{k_I l_A}{l_I} \frac{h'}{h} \quad (17)$$

The remaining rates of drug binding and unbinding (k_I , l_I , l_R , l_A) and the shift of the steady-state inactivation curve were then determined such that the model reproduced each data set individually. Once again, for each of the four data sets, parameters can be found for which the model response (—) fit the experimental data (O) (Fig. 7). Parameters of the Hondeghem-Katzung MR model that reproduce all four data sets simultaneously were then determined. This set of parameters provides a reasonable fit of the model response (—) to all four data sets (O) (Fig. 7). The resulting shift of the steady-state inactivation curve is -38.5 mV. The resulting affinities of the resting, activated, and inactivated states are 539 μ M (at -140 mV), 15 μ M (at -20 mV), and 93 nM, respectively. Even though the model satisfies microscopic reversibility and reproduces all four data sets simultaneously, the affinities of the activated and inactivated states and the shift of the steady-state inactivation curve all differ significantly from their experimentally measured values (Bean, et al., 1983; Kodama, et al., 1990; Bennett, et al., 1995; Furukawa, et al., 1995). An attempt was made to better match these values to experimental data by setting the shift of the steady-state inactivation curve to -10 mV and refitting the remaining

parameters. However, when the shift of the steady-state inactivation curve was held constant, the model failed to fit all four data sets well (data not shown).

Similar results were obtained with each of the other three pairs of rate constants constrained by microscopic reversibility. For each pair, a set of parameters can be found that provide a good fit of all four data sets simultaneously (data not shown). These sets of rate constants yield approximately the same affinities of the resting, activated, and inactivated states as when k_R and k_A are constrained by microscopic reversibility. Also, for each pair, setting the shift of the steady-state inactivation curve to ≈ 10 mV results in a model that does not fit all four data sets simultaneously (data not shown). Therefore, since microscopic reversibility must be satisfied for all chemical reactions, the inability of the Hondeghem-Katzung MR model to simultaneously satisfy microscopic reversibility and have reasonable drug affinities for each state suggests that the model does not correctly reproduce the mechanism of drug action.

Similar analyses were performed using the AE model. As for the Hondeghem-Katzung MR model, parameters of the AE model that reproduce each data set individually were determined first. The results are striking and are summarized in Fig. 8. Only parameters that reproduce the dose-response curve can be found. These parameters are $A_{on}=8542 \text{ M}^{-1}$ and $A_{off}=1.0256 \text{ M}^{-1}$. Using these parameter values, the steady-state inactivation function at a lidocaine concentration of $200 \text{ }\mu\text{M}$ was computed and compared to that in the absence of drug. Figure 9A shows that the AE model produces a negative shift of the steady-state inactivation curve. Curves in the presence and absence of lidocaine were both fit using a Boltzmann function. The half-maximal voltage and slope factor values are ≈ 104.2 mV and ≈ 12.5 mV for no drug and ≈ 116.5 mV and ≈ 14

mV for the AE model. Therefore, the model predicts that lidocaine shifts the steady-state inactivation curve by -12.3 mV, which agrees well with the experimental data (Furukawa, et al., 1995). The same parameters and lidocaine concentration were also used to compute the charge-voltage curve. Gating current was calculated according to the formula (Vandenberg and Bezanilla, 1991): (Vandenberg and Bezanilla, 1991):

$$I_g = \sum_{jk} ne(z_{jk} - z_{kj})[P_j \alpha_{jk} - P_k \alpha_{kj}] \quad (18)$$

where n is the number of channels, e is the elementary charge unit, z is the effective valence, P_j is the probability of occupying state j , and α_{jk} is the rate constant for the transition from state j to state k . Gating charge was found by integrating the gating current. Figure 9B shows the charge-voltage curves in the presence and absence of lidocaine. The AE model reduces the maximum charge slightly ($Q_{\max} = 0.958$) and significantly reduces the slope of the curve without changing its half-maximal voltage. Both curves were fit using a Boltzmann function. In the absence of drug, the half-maximal voltage and slope factor are 75 mV and 19 mV, respectively. For the AE model, the corresponding values are 75.3 mV and 25.4 mV, respectively. Hanck and coworkers found that QX-222, a permanently positively charged form of lidocaine, reduces the maximum charge and shifts the charge-voltage curve to more negative potentials (Hanck, et al., 1994). Thus, the AE model, while able to reproduce the dose-response curve and shift of the steady-state inactivation function, is unable to reproduce changes in the charge-voltage curve due to lidocaine.

The AE model, with A_{on} and A_{off} determined by fitting each data set independently, fails to reproduce use-dependence, onset of block, and recovery from block data. As shown in Fig. 8, the best fit of the model to experimental data produces

the same amount of current for each pulse or at each time for both the use-dependence and onset protocols. For the recovery protocol, the AE model produces a small increase in current during the first 100 ms, but then maintains this current for all longer recovery intervals. The AE model thus appears able to reproduce steady-state drug effects (ie., the dose-response curve and the shift of the steady-state inactivation curve), but unable to reproduce drug effects that evolve over time.

Drug effects in the AE model are incorporated by scaling transition rate constants by the drug concentration. The resulting lack of drug bound states is equivalent to assuming that drug binding and unbinding are significantly faster than channel state transitions. It was hypothesized that the AE model cannot reproduce time-dependent drug effects due to this lack of explicit drug bound states. As illustrated in Fig. 1, models with drug bound states have two separate pools of channels – those that are not drug bound and those that are drug bound. In the presence of lidocaine, a fraction of available channels enter the drug bound states and become non-conducting. Exit from these states (ie. drug unbinding) occurs more slowly than recovery from inactivation. With each depolarization, additional channels bind drug. Since unbinding is slow, these channels tend to remain drug bound and thus are unavailable to conduct for many pulses. The number of channels available to produce the sodium current therefore decreases with each pulse. More channels become drug bound until the rate of drug binding equals the rate of drug unbinding. The fraction of drug bound channels is then constant and each pulse produces the same amount of sodium current.

In order to reproduce time-dependent drug effects, channels must accumulate in a non-conducting state. In the AE model, channels do not accumulate in the inactivated

states even though drug binding increases the rate of inactivation and decreases the rate of recovery from inactivation. At the end of a depolarizing pulse, all of the channels are in an inactivated state. With repolarization, some channels recover to the closed states. The next pulse causes the channels in the closed states to inactivate or to open and then inactivate. Therefore, at the end of the second pulse, all of the channels are again in an inactivated state. The same number of channels recover to the closed states upon repolarization and are available to conduct with the next pulse. Thus, for each depolarization, the same number of channels are available to produce the sodium current and no time-dependent drug effects are produced.

The hypothesis that the AE model fails to reproduce time-dependent drug effects because it lacks drug bound states was tested. Channels were allowed access to a single drug bound state from either the closed state C_4 , the open state O_1 , or the inactivated state I . Drug binding was assumed to be concentration-dependent and drug unbinding was assumed to be concentration-independent. The AE model formulation was retained for transitions between the closed and closed-inactivated states only, since a change in these transitions reproduced the shift in the steady-state inactivation curve.

The four model parameters were determined by fitting each data set individually. Results of adding a drug bound state from the open state O_1 are shown in Fig. 8 (2). By adding this single drug bound state, fits to the use-dependence and recovery from block data are improved significantly. The dose-response curve is fit just as well as with the original AE model. The fit to the onset data is improved slightly, although it is not optimal. Lack of optimality may result from choosing to connect the drug bound state only to the open state. Fitting the onset data requires that drug binding increase as the

voltage-clamp duration is increased. Drug binding cannot increase with increasing voltage-clamp duration with this configuration of drug bound states, because channels rapidly enter the inactivated state, from which they cannot bind drug.

Similar improvements in fits to all four data sets were obtained using a closed drug bound state (C₄D) or an inactivated drug bound state (ID). One possible explanation for these improved fits could be the addition of free parameters to the model. However, when free parameters were added to the model by allowing coupling between the open and inactivated states to have different dose-dependent scale factors (B_{on} and B_{off}) than those for coupling between the closed and closed-inactivated states (A_{on} and A_{off}), fits to the use-dependence, onset of block, and recovery from block data were not improved (data not shown). Therefore, the existence of additional free parameters is not by itself an adequate explanation for the improved fit of the data sets. The inability of the AE model to reproduce time-dependent drug effects appears to arise from its lack of drug bound states.

Although adding a single drug bound state to the AE model improves the fit to each experimental data set individually, this change alone does not produce an accurate drug model. Parameters of the AE model plus a single open drug bound state were determined by fitting all four data sets simultaneously. As shown in Fig. 10, the model parameters determined by fitting all four data sets simultaneously (\otimes) yield a much worse fit to each of the experimental data sets (O) than do the parameters determined by fitting each data set individually (?). Therefore, neither the AE model nor the AE model plus a single drug bound state can adequately reproduce the mechanism of lidocaine's action.

DISCUSSION

The Hondeghem-Katzung MR model is able to reproduce use-dependence, onset of block, recovery from block, and the dose-response curve simultaneously. However, the resulting parameters predict the affinity of the activated state to be much too large. This discrepancy between the experimental data and the model's activated state affinity suggests that the mechanism by which the Hondeghem-Katzung MR model reproduces lidocaine's effect is not correct. Further, as noted in the paper describing this model (Hondeghem and Katzung, 1977), it does not satisfy microscopic reversibility. In the construction of this model, Hondeghem and Katzung assumed that drug binding and unbinding are exceptions to microscopic reversibility. However, as previously argued, all chemical reactions, including drug binding and unbinding, must conserve energy and satisfy microscopic reversibility.

The Hondeghem-Katzung MR model can be required to satisfy microscopic reversibility. This requirement, however, leads to ambiguity because any of twelve different pairs of rate constants can be used to satisfy microscopic reversibility. The choice of rate constants matters since to satisfy microscopic reversibility, some rate constants must be made voltage dependent. While some pairs can be eliminated based on experimental data, there is little data to guide the choice of the remaining pairs. For example, in nerve, the rate of unbinding from the activated state (l_A) may be voltage dependent (Yeh and Narahashi, 1977; Cahalan, 1978). To satisfy microscopic reversibility, however, two rate constants must be voltage dependent. In addition to l_A , should one choose k_R or l_R ? Given that it is extremely difficult to measure k_A and l_A in

intact channels due to the brief time a channel occupies the open state, could k_A be voltage dependent instead of I_A ? Could they both be voltage dependent with different dependencies?

Using each rate constant pair in turn to satisfy microscopic reversibility and determining the other parameters by fitting experimental data does not resolve any of these issues. All four data sets can be fit well simultaneously using any of these pairs. However, in each case the affinities of the activated and inactivated states and the shift of the steady-state inactivation curve differ significantly from their experimental values. When the shift of the steady-state inactivation curve is set to its experimentally measured value, parameters can no longer be found which fit all the data. The inability of the Hondeghem-Katzung MR model to simultaneously satisfy microscopic reversibility, reproduce all four data sets, and reproduce the shift in the steady-state inactivation curve suggests a fundamental shortcoming in the model. We suggest that the flaw lies not in the MR hypothesis itself, but rather in the underlying cardiac sodium channel and the particular assumptions used to formulate Hondeghem and Katzung's model.

Measurements of gating currents also support the conclusion that the Hondeghem-Katzung MR model does not fully reproduce the mechanism of lidocaine's action. Hanck and coworkers measured gating currents in the presence and absence of QX-222, a permanently positively charged form of lidocaine (Hanck, et al., 1994). They integrated the currents to find the charge moved at each potential and plotted the charge versus voltage. They found that QX-222 reduced the maximum charge moved and shifted the charge-voltage curve to more negative potentials. They concluded that when drug was bound, the voltage sensors did not move as far as when drug was not bound. The

mechanism of lidocaine's action thus seems to include modulation of channel gating. Charge-voltage curves can be produced from Hodgkin-Huxley models if the assumption is made that the inactivation particle contributes little gating charge. The gating charge is then given by the following equation (Armstrong, 1981):

$$Q(V) = Nq_m m_\infty(V) \quad (19)$$

where N is the number of channels, q_m is the charge of the activation gates, and $m_\infty(V)$ is the steady-state activation curve. The Hondeghem-Katzung MR model assumes that drug binding does not affect activation and thus does not change $m_\infty(V)$ (Hondeghem and Katzung, 1977). Therefore, this model predicts that the charge-voltage curve should be unaltered in the presence of lidocaine. This prediction clearly contradicts the experimental data and thus supports the conclusion that the Hondeghem-Katzung MR model does not fully reproduce the mechanism of lidocaine's action.

The AE model reproduces the dose-response curve and the shift of the steady-state inactivation curve. However, it does not reproduce use-dependence, onset of block, or recovery from block. This shortcoming appears to result from a lack of explicit drug bound states. Drug bound states are needed so that when drug is bound, channels are unable to conduct for longer intervals than when drug is not bound. When even a single drug bound state is added to the AE model, the model is then capable of reproducing time-dependent drug effects.

The conclusion that drug bound states are needed to model lidocaine's action differs from the findings of Balsler et al (Balsler, et al., 1996). In their study, these investigators tested the ability of various models of lidocaine's action to account for tonic block dose-response curves. Responses were calculated using the AE model and two

different MR models developed from a Markov state model of skeletal muscle sodium channels. Tonic block dose-response curves were determined by measuring peak current produced on stepping from a holding potential of -120 mV to -20 mV for 20 ms and plotting this peak current as a function of lidocaine concentration. The dose-response curve calculated in this fashion differs from the use-dependent block dose-response curve used in the present study, since it depends on drug binding to the resting and inactivated states at hyperpolarized potentials rather than drug binding to the open and inactivated states at depolarized potentials. Indeed, the skeletal muscle sodium channel model described by Balser et al could not be used to calculate a use-dependent block dose-response curve, because this model has absorbing inactivated states.

Balser et al first examined an MR model in which four closed drug bound states and one open drug bound state were added to the sodium channel model. Affinity of the channel for lidocaine was assumed to increase with transitions towards the open state. This model was found to produce a dose-dependent reduction in current, but the percent reduction was not correct for all drug concentrations. Although the model rate constants for drug binding and unbinding to each of these states were not reported, examination of the results suggests that the underlying behavior of the model was correct, and that with adjustment of the rate constants, the model would perform as well as the AE model does. Thus, the conclusion that MR models with closed and open state block cannot reproduce the tonic block dose-response curve is not well supported.

The authors next analyzed a model in which four closed-inactivated drug bound states and one inactivated drug bound state were added to the sodium channel model. The model was tested using an infinite lidocaine concentration and drug bound states

were assumed to be absorbing. This model produced no reduction in peak current. The authors therefore concluded that MR models with inactivated state block cannot reproduce the tonic block dose-response curve. However, it is likely that this particular model failed because of deficiencies in the underlying representation of the sodium channel. Although not stated, it was assumed that at -120 mV all of the channels were in the first closed state. Since drug cannot bind from this state, no block develops at -120 mV. At -20 mV, the rate constants governing transitions to the open state are much larger than those governing transitions to the closed-inactivated states. Thus, very few channels inactivate and become blocked before opening. If channels are not blocked before they open, then there will be no reduction in peak current regardless of the lidocaine concentration. The current decays faster in the presence of lidocaine because once channels inactivate, they rapidly become blocked. Thus, the conclusion that MR models are incapable of reproducing the tonic block dose-response curve, and are thus more limited than the AE model, is not well supported.

An implicit assumption of the AE model is that drug binding and unbinding are significantly faster than the channel kinetics. This assumption produces a model in which there are no drug bound states and changes in drug concentration instantaneously alter certain transition rates, as described by Eqs. 8-11. This assumption, however, is not consistent with experimental data. Bennett and coworkers measured the rate at which lidocaine block develops (Bennett, et al., 1995). They applied conditioning pulses of varying duration to -50 mV, -30 mV, and -20 mV, stepped to -120 mV for 300 ms, and then applied a -20 mV test pulse. Block developed with a time constant of 589 ms independent of the pre-pulse potential. The onset of block was much slower than the

onset of fast inactivation, the time constants for which were between 1 ms and 20 ms. This finding argues against the AE model assumption that drug binding is faster than the channel kinetics. If drug binding is not faster than inactivation, then the kinetics of drug binding cannot be neglected in developing a model of drug action.

It is important to acknowledge that both the AE and Hondeghem-Katzung MR models have provided critically useful information for use in building new models of antiarrhythmic drug action. Although the Hondeghem-Katzung model has its shortcomings, the basic tenets of the MR hypothesis seem to be true. In order to reproduce use-dependence, onset of block, recovery from block, and the dose-response curve, there must be drug bound states and the rates of drug binding and unbinding must vary as the channel changes states. From the AE model, the idea that drug binding enhances the rate of inactivation and stabilizes the inactivated states also seems to be true. To this foundation, the constraints of microscopic reversibility and the idea that drug alters the charge-voltage relationship need to be added to more correctly model the mechanism of drug action. Such a model would further enhance understanding of the complex drug-channel interaction and would be an important step towards developing better antiarrhythmic agents.

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FIGURE LEGENDS

Figure 1: The Hondeghem-Katzung MR model (Hondeghem and Katzung, 1977). The top row contains the resting (R), activated (A), and inactivated (I) states in which drug is not bound. The lower row contains the corresponding states in which drug is bound. k and l are the rates of drug binding and unbinding, respectively. HH symbolizes that transitions between states are governed by Hodgkin-Huxley type equations. HH' symbolizes that transitions between drug bound states are governed by Hodgkin-Huxley type equations with the voltage dependence of inactivation shifted towards more negative potentials.

Figure 2: Sodium currents at 17°C produced with Eqs. 1-6. A. Comparison of voltage-clamped sodium current tracings for clamp voltages of -50 mV, -30 mV, and -10 mV for experimental data (---) (provided by Wasserstrom, similar to (Sakakibara, et al., 1993)) and the model (—). B. Normalized current-voltage curves for experimental data and the model. Curves are the best fits to a modified Boltzmann function. The conductance, slope factor, and half-maximal voltage are 0.017 mV^{-1} , -6.7 mV , and -41 mV for the experimental data and 0.017 mV^{-1} , -5.9 mV , and -40.6 mV for the model respectively.

Figure 3: The AE model (Balsler, et al., 1996) based on a thirteen-state model of the cardiac sodium channel (Irvine, et al., 1998). C_0 to C_4 are closed states, O_1 and O_2 are open states, C_0I to C_4I are closed-inactivated states, and I is the inactivated state. All rate constants are voltage dependent except for those governing transitions between closed

and closed-inactivated states. Transitions between the closed and closed-inactivated states and between the open and inactivated state are dependent on the drug concentration as described in Eqs. 8-11.

Figure 4: Comparison of experimental data (O) (Jia, et al., 1993, Furukawa, et al., 1995) and the Hondeghem-Katzung MR model responses with parameters determined by fitting each data set individually (?) and by fitting all four data sets simultaneously (⊗). A. Use-dependence. B. Onset of block. C. Recovery from block. D. Dose-response. The dose-response curves were fit using a sigmoidal curve of the form:

$$\frac{I}{I_{no\ drug}} = \frac{1}{1 + \frac{[lidocaine]}{IC_{50}}}$$

where the IC_{50} is the drug concentration at which the current is reduced by 50%. The IC_{50} values were 120.3 μ M for the experimental data, 121.5 μ M for parameters determined by fitting the dose-response data only, and 149.8 μ M for parameters determined by fitting all four data sets.

Figure 5: Comparison of experimental data (O) (Jia, et al., 1993, Furukawa, et al., 1995) and the Hondeghem-Katzung MR model responses with parameters determined by fitting the use-dependence data only (⊗). A. Use-dependence. B. Onset of block. C. Recovery from block. D. Dose-response. The IC_{50} values were 120.3 μ M for the experimental data and 484 μ M for parameters determined by fitting the use-dependence data only.

Figure 6: Expanded Hondeghem-Katzung MR model. A. Eight-state Markov model describing the non-drug bound states and their transitions. R_1 , R_2 , and R_3 are the resting states, A is the activated state, and I_1 , I_2 , I_3 , and I_4 are the inactivated states. The alphas and betas are described by Eqs. 1-4. B. Eight-state Markov model describing the drug bound states and their transitions. Activation rate constants are the same as in A. Inactivation rate constants have their voltage dependence shifted towards more negative potentials. C. Transitions between the non-drug bound and the drug bound states. k and l are the rates of drug binding and unbinding, respectively.

Figure 7: Comparison of experimental data (O) (Jia, et al., 1993, Furukawa, et al., 1995) and the expanded Hondeghem-Katzung MR model responses with microscopic reversibility satisfied by constraining k_R and k_A by Eqs. 16 and 17. Parameters were determined by fitting each data set individually (?) and by fitting all four data sets simultaneously (≈). A. Use-dependence. B. Onset of block. C. Recovery from block. D. Dose-response. The dose-response curves were fit using the equation in the legend of Fig. 4. The IC_{50} values were 120.3 μ M for the experimental data, 119.7 μ M for parameters determined by fitting the dose-response data only, and 154.3 μ M for parameters determined by fitting all four data sets.

Figure 8: Comparison of experimental data (O) (Jia, et al., 1993, Furukawa, et al., 1995) and model responses for the AE model (?) and for the AE model plus an open drug bound state (≈). Parameters were determined by fitting each data set individually. A. Use-dependence. B. Onset of block. C. Recovery from block. D. Dose-response. The

dose-response curves were fit using the equation in the legend of Fig. 4. The IC_{50} values were 120.3 μM for the experimental data, 122.4 μM for the AE model, and 120.6 μM for the AE model plus an open drug bound state.

Figure 9: A. Steady-state availability curves for no drug (---) and for drug action modeled with the AE model (—). Parameters of the AE model were $A_{\text{on}} = 8542 \text{ M}^{-1}$ and $A_{\text{off}} = 1.0256 \text{ M}^{-1}$. Curves were the best fits of a Boltzmann function. The slope factor and half-maximal potential were 12.5 mV and 104.2 mV for no drug and 14 mV and 116.5 mV for the AE model. B. Normalized gating charge-voltage curves for no drug (O) and for drug action modeled with the AE model (●). Parameters were the same as in A. Curves were the best fits of a Boltzmann function. The maximum charge, slope factor, and half-maximal potential were 1, 19 mV, and 75 mV for no drug and 0.958, 25.4 mV and 75.3 mV for the AE model.

Figure 10: Comparison of experimental data (O) (Jia, et al., 1993, Furukawa, et al., 1995) and model responses for the AE model plus an open drug bound state. Parameters were determined by fitting each data set individually (?) and by fitting all four data sets simultaneously (⊗). A. Use-dependence. B. Onset of block. C. Recovery from block. D. Dose-response. The dose-response curves were fit using the equation in the legend of Fig. 4. The IC_{50} values were 120.3 μM for the experimental data, 120.6 μM for parameters determined by fitting the dose-response data only, and 169.7 μM for parameters determined by fitting all four data sets.





