

**MODELS OF THE CARDIAC SODIUM CHANNEL
AND THE ACTION OF LIDOCAINE**

by

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ABSTRACT

A Markov model of the cardiac sodium channel is presented. The model is similar to the sodium channel model developed by Kuo and Bean with the following modifications: a) an additional open state is added; b) open-inactivated transitions are made voltage dependent; and c) channel rate constants are exponential functions of enthalpy, entropy, and voltage and have explicit temperature dependence. Model parameters are determined using a simulated annealing algorithm to minimize the error between model responses and experimental data. The model reproduces a wide range of whole-cell and single channel experimental data over a temperature range of 10°C to 25°C.

The cardiac sodium channel model is used to study antiarrhythmic drug action. The ability of two models of drug action, a modulated receptor model and an allosteric effector model, to quantitatively reproduce a wide range of drug effects is tested. The modulated receptor model can reproduce use-dependence, onset of block, recovery from block, and the dose-response curve. Model parameters that reproduce these data, however, do not yield reasonable drug affinities for each state, do not preserve microscopic reversibility, and do not reproduce the changes in the charge-voltage curve. The allosteric effector model can reproduce the dose-response curve and the drug-induced shift of the steady-state inactivation curve, but none of the time-dependent drug effects. The inability of both models to simultaneously reproduce a

wide range of drug effects suggests that neither model fully reproduces the mechanism of drug action.

A new model of lidocaine's action is formulated based on the idea that lidocaine causes channels to switch from their normal gating mode to another mode, in which channels gate, but fail to open. Transitions between modes occur with rate constants equal to the drug binding and unbinding rates. The mode-switching model reproduces use-dependence, onset of block, recovery from block, the dose-response curve, the steady-state inactivation curve, and the charge-voltage curve, has reasonable drug affinities for each state, and preserves microscopic reversibility.

Both the sodium channel model and the model of lidocaine's action represent significant improvements to previous models of the channel and drug action, respectively.

(Primary advisor: Dr. Rai Winslow; Reader: Dr. Bill Agnew; Defense committee: Drs. Paul Bennett, Paul Fuchs, Les Tung, and David Yue.)

Dedication

To God the Father, Maker of Heaven and Earth, Giver of all Good Gifts:

Thank you for making the impossible possible, for moving barriers with Your love, and for shining Your light of hope in the dark world of academia.

To my Mom:

Thank you for believing in me even when I didn't believe in myself and for teaching me that I could do anything I wanted to do.

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Thanks for all the nights you helped me with my math homework past both of our bedtimes. (I think it finally paid off!)

To my husband, David:

Thanks for the shoulder on which to cry, the words of encouragement, and the never-ending attempts to make me smile and laugh.

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CHAPTER 1:
INTRODUCTION

Sodium Channel Models

What causes the heart to beat and muscles to twitch? What aids the absorption of nutrients and the elimination of wastes? What allows the brain to recognize sights and sounds? None of these functions are possible without ionic channels. Ionic channels are proteins that span the cell membrane forming an aqueous pore through which ions pass. They are found in every cell and tissue in the body in abundant variety. Some are selective and conduct only certain ions, while others are relatively non-specific. Among the most abundant and important ionic channels are those that are specific for sodium. Sodium channels provide the means by which signals are transmitted in nerves and by which the heart's contraction is coordinated. They have been studied since the 1940s and much is known about them.

Hodgkin and Huxley were the first to study sodium channels (Hodgkin and Huxley, 1952a-c). They proposed that sodium channels were either in a resting, activated, or inactivated state depending upon the membrane potential. They postulated that activation, the process by which channels rapidly open from a resting state during depolarization, and inactivation, the process by which channels slowly close during a prolonged depolarization, were controlled by separate gating particles (Hodgkin and Huxley, 1952d). Based on experimental data, they postulated that three activation particles (m) and one inactivation particle (h) controlled gating of the channel. Each particle was assumed to undergo transitions between permissive and non-permissive forms with rates satisfying the differential equations:

$$\frac{dm}{dt} = \alpha_m(1-m) - \beta_m m \quad (1.1)$$

$$\frac{dh}{dt} = \alpha_h(1-h) - \beta_h h \quad (1.2)$$

where α_m and β_m were the rate constants governing transitions to the permissive state and α_h and β_h were the rate constants governing transitions to the non-permissive state. The probability that all of the gating particles were in the permissive form was m^3h . Sodium current (I_{Na}) was then described by the following equation:

$$I_{Na} = m^3hG_{Na}(V - E_{Na}) \quad (1.3)$$

where G_{Na} was the sodium conductance, V was the membrane potential, and E_{Na} was the reversal potential for sodium. Using these equations for the sodium current and similar equations for the potassium and leak currents, Hodgkin and Huxley were able to mathematically reproduce the action potentials observed in squid axon.

Since Hodgkin and Huxley first developed their model of the sodium channel, Hodgkin-Huxley type models have been formulated for many channels in a variety of tissues. Of particular importance to the work described here, several Hodgkin-Huxley type models have been formulated for cardiac sodium channels. They include the Beeler-Reuter model (Beeler and Reuter, 1977), the Ebihara-Johnson model (Ebihara and Johnson, 1980), used in the Luo-Rudy ventricular cell model (Luo and Rudy, 1991), and the DiFrancesco-Noble model (DiFrancesco and Noble, 1985). Each of these models has the same form as Hodgkin and Huxley's model but has a different description for α_m , β_m , α_h , and β_h . The differences in these rate constants

can be attributed to variations in the experimental preparations upon which the models are based, including different species from which the myocardium was obtained and different temperatures or means of correcting for temperature. In spite of these differences, the models all capture the fundamental behavior of the cardiac sodium channel and are widely used in the interpretation of experimental results.

With the development of techniques to record from single sodium channels, more information about the mechanism of channel function has been acquired and a desire to have more biophysically-detailed channel models has emerged. While traditional Hodgkin-Huxley models can capture the ensemble-average behavior of sodium channels, they may not correctly reproduce the underlying channel kinetics (Aldrich, et al., 1983). In addition, these models may not adequately reproduce behaviors that are critically state-dependent. For example, we have shown that drug effects can be very sensitive to details of the drug model. In studies of drug action on spiral waves, simple models, such as a time-independent decrease in conductance, produce a very different effect than do use-dependent models (Irvine and Winslow, 1996). Similarly, Liu and Rasmusson have shown that while two different inactivation models may produce similar ionic currents, they can predict dramatically different drug effects (Liu and Rasmusson, 1997).

The goal of this work is to develop a quantitative explanation of lidocaine's effect on the cardiac sodium channel. If the details of the underlying sodium channel model are critical to meeting this goal, then a biophysically-detailed kinetic model of the cardiac sodium channel is needed.

Detailed kinetic models that describe channel gating as a Markov process have been developed to replace the traditional Hodgkin-Huxley models. Markov process theory states that the future evolution of channel state is dependent only on the current channel state and not on the path by which the channel reaches that state (Hille, 1992). Comprehensive Markov models exist for neuronal sodium channels (Patlak, 1991; Vandenberg and Bezanilla, 1991), but only partial models have been formulated for cardiac sodium channels (Benndorf, 1988; Berman, et al., 1989; Scanley, et al., 1990). Each of these cardiac sodium channel models lacks rate constants with explicit voltage and temperature dependence. In addition, they treat inactivation as an absorbing state, so that once a channel inactivates, there is no pathway by which it can recover. Thus, with these models, channel behavior can be simulated in response to only a single voltage clamp stimulus. This restriction is unacceptable for studying antiarrhythmic drug action because it prevents the simulation of drug effects in response to repeated stimuli; that is, the use-dependent property of these drugs, thought to be a main determinant of their efficacy, would be eliminated from such models. The neuronal models, which include recovery from inactivation, cannot be used directly due to the numerous differences in channel kinetics between neuronal and cardiac tissue (Kirsch and Brown, 1989; Kuo and Bean, 1994; Hanck and Sheets, 1995; Fozzard and Hanck, 1996). Therefore, a comprehensive Markov model of the cardiac sodium channel must be formulated.

Chapter 2 describes a model of the cardiac sodium channel based on Kuo and Bean's model for sodium channels in CA1 hippocampal neurons (Kuo and Bean,

1994). Like the neuronal sodium channel models of Vandenberg and Bezanilla (1991) and Patlak (1991), a wide variety of experimental data is used to determine the model's rate constants, including gating currents and single channel open times. The model reproduces all of the data that Hodgkin-Huxley models reproduce, including recovery from inactivation. It can also predict measures of single channel activity such as first latency, probability of a null sweep, and probability of reopening. In addition, unlike Hodgkin-Huxley models, which are usually formulated for a single temperature, the cardiac sodium channel Markov model functions well over a temperature range of 10°C to 25°C. Thus, the cardiac sodium channel Markov model reported here improves on previous Hodgkin-Huxley and Markov models of this channel and can be used as the basis for modeling antiarrhythmic drug action.

Hypotheses of Antiarrhythmic Drug Action

Antiarrhythmic drugs are one of the most widely prescribed classes of pharmaceuticals today, yet much remains unknown about their mechanisms of action. Generally, these drugs prevent the initiation or continued propagation of arrhythmias by blocking certain ionic channels necessary for the excitation or relaxation of cardiac myocytes. It is thought that these drugs do not simply occlude the channel pore, but rather, alter the gating properties of the channel such that transitions to the open state are less likely (Hanck, et al., 1994). The most researched drug-receptor interaction in the heart is the block of cardiac sodium channels by class I antiarrhythmic drugs such as lidocaine. Although experimental evidence exists to provide clues as to what

gating properties are being modified, no complete quantitative explanation of class I drug effects on cardiac sodium channels has emerged.

Over the last two decades, four distinct hypotheses have been proposed to explain drug action. These hypotheses are: a) Pore Block; b) Guarded Receptor (GR) (Starmer, et al., 1984; Starmer and Grant, 1985; Starmer and Courtney, 1986; Starmer, 1987); c) Modulated Receptor (MR) (Hille, 1977; Hondeghem and Katzung, 1977; Hondeghem and Katzung, 1980); and d) Allosteric Effector (AE) (Balsler, et al., 1996). In a pore block model, drug has continual access to its receptor site and the affinity of the receptor for drug is independent of the channel's state. Therefore, drug binding is modeled as simply a decrease in conductance. Such a model is useful for predicting drug effects which are independent of how a channel gates in response to a given stimulus. Actions of class I antiarrhythmic drugs, however, are dependent upon a channel's response to a particular stimulus as evidenced by the observation that drug effect is greater at faster rates of stimulation (use-dependence). Thus, pore block models cannot be used as a general framework for quantitative modeling of these drug-receptor interactions.

Both the GR and MR hypotheses can account for use-dependence, but the hypotheses differ in their assumptions about the mechanisms and consequences of drug binding. The GR hypothesis states that the affinity of a receptor for drug is constant, while access to the receptor varies with channel state. Once drug binds, the channel remains in a non-conducting state until drug unbinds from the receptor (Starmer, et al., 1984; Starmer and Grant, 1985; Starmer and Courtney, 1986;

Starmer, 1987). The GR hypothesis is unclear as to whether drug binding alters gating kinetics. In 1984, Starmer suggested that drug binding could cause immobilization of the *m* or *h* gate (Starmer, et al., 1984). In 1986, though, he stated that “no modified gate kinetics are postulated in drug-complexed channels” (Starmer and Courtney, 1986).

In contrast, the MR hypothesis states that the affinity of a receptor for drug changes as the channel changes state, while access to the receptor is constant. Drug can bind to any of three channel states (resting, activated, or inactivated), 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.

There is an extensive literature describing experiments directed at testing the validity of the GR and MR hypotheses, but no study has proven conclusively that one hypothesis is correct. Nevertheless, recent findings provide additional arguments against the GR hypothesis. Several studies have used channels with fast inactivation removed by enzymatic digestion or with selective mutations of three amino acids in the III-IV interdomain (West, et al., 1992) in order to estimate the drug binding affinity of the open state. These studies estimate the open state binding affinity to be at least 400 μ M for lidocaine (Bennett, et al., 1995a; Balsler, et al., 1996) and

QX-314, a permanently positively charged form of lidocaine (Gingrich, et al., 1993). These estimates are an order of magnitude greater than estimates of binding affinity to the inactivated state (Bean, et al., 1983; Matsubara, et al., 1987; Kodama, et al., 1990; Bennett, et al., 1995a; Nuss, et al., 1995). Thus, it appears that the affinity of the receptor for drug depends upon the channel's state, as suggested by the MR hypothesis. This finding, however, must be interpreted with caution, as it has been proposed that the inactivation particle traps drug in the channel (Starmer, et al., 1984). This scenario would result in a much lower apparent affinity for the open state when inactivation is removed. Additional support for the MR hypothesis is provided by Hanck and colleagues. They studied gating currents of sodium channels in canine cardiac Purkinje cells in the presence of an excess of QX-222, a quaternary derivative of lidocaine. They found QX-222 dramatically reduced the slope of the charge-voltage relationship and concluded that gating of drug bound channels was less voltage dependent than gating of non-drug bound channels (Hanck, et al., 1994). This result supports the MR hypothesis by providing evidence that drug bound channels have altered gating kinetics.

Recently, a fourth hypothesis of drug action has been proposed – the AE hypothesis (Balser, et al., 1996). Like the MR hypothesis, this hypothesis requires drug binding to alter gating kinetics. However, unlike models based on the MR hypothesis, models based on the AE hypothesis have no explicit drug bound states. Drug is assumed to decrease the Gibbs free energy for the inactivated channel

conformation. This change causes channels to inactivate more readily and to remain inactivated for an extended period of time.

Of the four proposed hypotheses of drug action, thus only two, the MR hypothesis and the AE hypothesis, require drug binding to alter channel gating. However, neither hypothesis has undergone a quantitative analysis to test the extent to which each can reproduce drug effects. Chapter 3 describes one such analysis in which models based on each of these hypotheses are required to simultaneously reproduce a wide range of drug effects. The Hondeghem-Katzung model, based on the MR hypothesis, can reproduce use-dependence, onset of block, recovery from block, and the dose-response curve. This model, though, does not preserve energy balances and does not yield reasonable drug affinities for each state. The Balser model, based on the AE hypothesis, can reproduce the dose-response curve and the drug-induced shift of the steady-state inactivation curve, but none of the other drug effects. It is concluded that neither model fully reproduces the mechanism of drug action, but improved models can be built based upon the MR hypothesis.

Mode-Switching Models of Drug Action

Even though the MR hypothesis is supported by experimental data, difficulties exist in translating this hypothesis into a quantitative model of drug action. The assertion that drug may bind to channels in a state-dependent fashion, while of clear importance, is such a vague and general principle that it provides virtually no detailed guide in the drug modeling process. Consequently, previous models formulated from

the MR hypothesis (the Hondeghem-Katzung model) have sought to simulate only the net macroscopic changes due to drug binding, rather than the mechanisms responsible for these changes.

Here the mechanism of drug action is proposed to be that of mode-switching. Many different channels in a variety of tissues exhibit mode-switching behavior. Experimentally, a single channel is said to exhibit different gating modes when the following criteria are satisfied (Nilius, 1988):

- (1) distinct patterns of channel openings and closings (referred to as gating schemes) coexist within the same recording;
- (2) transitions between these different gating schemes are slow; that is, rate constants governing transitions between modes are much smaller than those governing transitions within a mode;
- (3) chemical or physical agents exist which favor a gating scheme different from the normal scheme.

As an example, cardiac sodium channels are thought to have at least two gating modes: a fast-inactivating mode (the normal gating mode) and a slow-inactivating, bursting mode (Patlak and Oritz, 1985; Kohlhardt, et al., 1987; Nilius, et al., 1987; Nilius, 1988). In single channel patches from isolated guinea pig ventricular cells, sweeps exhibit one of two different gating patterns representative of either fast or slow inactivation. Sweeps with a single gating pattern are clustered in time suggesting that transitions between fast and slow inactivation are much slower than rate constants governing channel activation and inactivation (Nilius, 1988; Bohle and

Benndorf, 1995). Channels switch from the fast-inactivating mode to the slow-inactivating mode when their membrane patches are excised (Nilius, 1988).

Although mode-switching has not been proposed previously to explain the effects of sodium channel blockers, it has been used to explain a variety of agent-induced changes in gating kinetics including: a) calcium-mediated inactivation of the calcium current (Imredy and Yue, 1994); b) the action of dihydropyridine calcium agonists and antagonists on L-type calcium channels (Hess, et al., 1984); and c) the effect of luteinizing hormone-releasing hormone on N-type calcium channels (Boland and Bean, 1993). Imredy and Yue studied calcium-sensitive inactivation of L-type calcium channels in rat ventricular myocytes. They found the first latency distribution was different with calcium, instead of barium, as the charge carrier. In addition, with calcium as the charge carrier, decay of the conditional open probability was enhanced. They attributed these changes in gating kinetics to a calcium-induced shift to mode Ca, in which closed states were more stable (Imredy and Yue, 1994).

Hess and colleagues used mode-switching to explain the gating behavior of L-type calcium channels in the presence of Bay K 8644 and nitrendipine. They found that in the presence of a maximally effective dose of drug, a channel could exhibit the same gating behavior as when no drug was applied. They therefore concluded any ensemble behavior observed in the presence of drug resulted from a combination of normal gating and drug-induced gating. They also showed drug binding did not cause a new gating mode, but simply increased the probability of occupying an already existing mode (Hess, et al., 1984).

Finally, Boland and Bean studied the modulation of N-type calcium channels in bullfrog sympathetic neurons by luteinizing hormone-releasing hormone (LHRH). Their hypothesis was that LHRH activates G-proteins, which alter gating kinetics by binding to the channel. They justified the existence of an alternative gating mode favored by LHRH by citing changes in activation and deactivation kinetics. Additionally, they showed LHRH had little or no effect when applied to channels that were activated, but had a large effect when applied to resting or inactivated channels. Therefore, the ability of LHRH to cause a shift in gating mode was dependent upon channel state (Boland and Bean, 1993).

In a similar manner as the above mode-switching stimuli alter gating kinetics, lidocaine also alters gating kinetics as suggested by four independent studies. First, Nilius and colleagues studied lidocaine block of single cardiac sodium channels and observed a reduction in peak ensemble averaged current. They attributed this decrease in current to an increased number of null sweeps and a shortened mean open time. They also found lidocaine prevented bursting (Nilius, et al., 1987). Therefore, lidocaine significantly decreased the probability of occupying the slow-inactivating mode and, as evidenced by the increase in null sweeps, increased the probability of occupying a “non-conducting” mode. Second, in an attempt to measure lidocaine’s effect on gating more directly, Hanck and coworkers studied gating currents of sodium channels in canine cardiac Purkinje cells in the absence and presence of QX-222, a quaternary derivative of lidocaine. They found the charge-voltage relationship of drug bound channels was shifted to more negative potentials, had a more shallow

slope, and had a lower maximum charge than that of non-drug bound channels. They concluded a reduction in slope implied gating of drug bound channels was less voltage dependent than gating of non-drug bound channels (Hanck, et al., 1994). Third, a study of human cardiac sodium channels by Nuss and colleagues provided additional support for drug-induced changes in gating kinetics. Using a standard two-pulse protocol for examining inactivation, they found lidocaine not only slowed the rate of recovery from inactivation, but also greatly prolonged the initial delay associated with deactivation before recovery occurred (Nuss, et al., 1995). Finally, Bennett and coworkers found 25 μM lidocaine blocked recombinant human cardiac sodium channels with a time constant of 589 ms. This time constant is roughly two orders of magnitude larger than that of any process associated with normal gating (Bennett, et al., 1995a). Therefore, lidocaine alters channel gating and this change in kinetics occurs at a slower rate than normal state transitions. Both of these observations support a mode-switching mechanism of action for lidocaine.

Chapter 4 describes a mode-switching model of lidocaine's action. Lidocaine induces sodium channels to switch from the fast-inactivating mode to another mode (the drug mode), distinct from the slow-inactivating mode, in which the channel gates, but fails to open. The model is based on the sodium channel Markov model described in Chapter 2. The drug mode is an additional set of eight states with profoundly altered kinetics that can be entered only in the presence of drug. The model reproduces all of the data that is reproduced by the Hondeghem-Katzung model. In addition, it preserves energy balances, yields reasonable drug affinities for

each state, produces the correct shift of the steady-state inactivation curve, and predicts the experimentally measured changes in the charge-voltage curve. The mode-switching model thus improves on previous models of class I antiarrhythmic drug action and can be used as a tool to study drug action in various diseases.

CHAPTER 2:
CARDIAC SODIUM CHANNEL MODEL

INTRODUCTION

For many years Hodgkin-Huxley models (Hodgkin and Huxley, 1952a,b) have been the standard for describing ionic current kinetics. However, with the development of better recording techniques, new data have shown that these models have significant limitations. First, many single channel behaviors such as mean open time and first latency cannot be described using traditional Hodgkin-Huxley models. These behaviors can be estimated by expanding the Hodgkin-Huxley models to have multiple resting and inactivated states, but it is controversial as to how well these expanded models can predict single channel experimental data (Horn and Vandenberg, 1984; Chay, 1991). Second, while a Hodgkin-Huxley model can reproduce ionic currents, it does not necessarily correctly reproduce the underlying single channel kinetics. For example, Aldrich and coworkers found that for neuroblastoma sodium channels, activation has very slow components, while inactivation is fast (Aldrich, et al., 1983). This finding contradicts Hodgkin's and Huxley's assumption that activation is rapid and inactivation is slow (Hodgkin and Huxley, 1952a,b). Even though Hodgkin-Huxley models can reproduce this current, they do not correctly reproduce the underlying channel kinetics. Although single channel recordings of cardiac sodium channels indicate that activation is rapid relative to inactivation (Yue, et al., 1989), it is questionable whether Hodgkin-Huxley models are sufficient for reproducing behaviors that may be critically state-dependent, such as how ionic channels interact with drugs and toxins (Irvine and Winslow, 1996; Liu and Rasmusson, 1997). In addition, since much more is now

known about the structure of the sodium channel (Noda, et al., 1984; Noda, et al., 1986; Guy, 1988), it is desirable to incorporate this information into a description of channel function. Thus, future channel models should be biophysically-detailed kinetic models, consistent with current generalizations of channel structure, capable of reproducing single channel behavior.

For the cardiac sodium channel, models that describe channel gating as a Markov process (Benndorf, 1988; Berman, et al., 1989; Scanley, et al., 1990) are a step in this direction. Existing models for the cardiac sodium channel are, however, incomplete in that they describe only certain features of channel behavior. Specifically, each of these models lacks rate constants with explicit voltage and temperature dependence. In addition, these models treat inactivation as an absorbing state, so that once a channel inactivates, there is no pathway by which it can recover. Thus, they can only be used to simulate certain channel behaviors in response to a single voltage clamp stimulus. They do not reproduce channel activity to the same extent as Hodgkin-Huxley models and therefore have not been as widely used.

More comprehensive Markov models exist for sodium channels of the squid giant axon (Patlak, 1991; Vandenberg and Bezanilla, 1991). Vandenberg and Bezanilla and Patlak have been able to develop these models by using a wide variety of both whole cell and single channel data simultaneously. Unfortunately, because of the many differences in channel kinetics between cardiac and neuronal tissue (Kirsch and Brown, 1989; Kuo and Bean, 1994; Hanck and Sheets, 1995; Fozzard and Hanck, 1996), these models cannot be used directly to model cardiac sodium channels.

Nevertheless, the neuronal models and the techniques used to develop them are a starting point from which to develop a more complete model of the cardiac sodium channel.

Although Markov models exist from which cardiac sodium channel Markov models can be developed for a single temperature, no models exist that can reproduce ensemble-average and single channel behaviors for a range of temperatures. The models of Vandenberg and Patlak have a temperature dependent rate constant coefficient and a temperature dependent voltage term (Patlak, 1991; Vandenberg and Bezanilla, 1991). Changing the temperature in these terms, however, does not yield the correct channel activity at multiple temperatures. Each term in the model's rate constants needs to have its own temperature dependence or its own Q_{10} factor (Kohlhardt, 1990). Temperature dependent closed-closed and closed-open transitions have been incorporated into a partial model of neuronal sodium channels by formulating the rate constants as exponential functions of enthalpy and entropy (Correa, et al., 1992). The same rate constant formulation can be used in a model of cardiac sodium channels to reproduce ensemble-average and single channel behaviors for a range of temperatures.

The goal of this chapter is to use neuronal models as a framework for developing a Markov model of the cardiac sodium channel. The model should exhibit correct macroscopic and single channel behavior, including recovery from inactivation, for a voltage range of -150 mV to 20 mV and a temperature range of

10°C to 25°C. Such a model would improve on existing Hodgkin-Huxley and Markov models significantly and may yield more insight into the molecular basis of channel function. In addition, such a model could be used as the basis for studies of antiarrhythmic drug action.

MODEL

The cardiac sodium channel Markov model is patterned after that by Kuo and Bean for sodium channels in CA1 hippocampal neurons (Kuo and Bean, 1994). This model is chosen as a starting point because it is consistent with current generalizations of channel structure, but uses symmetry and cooperative movement of the voltage sensors to reduce the number of free parameters. As shown in Fig. 2.1, the channel can occupy any of 13 states. 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. As in Kuo and Bean's model, the affinity of the inactivation particle binding site is hypothesized to increase by a scaling factor (a) as the channel activates and to decrease by the same factor as the channel deactivates. Closed-closed and closed-open transitions (horizontal transitions) are voltage dependent and closed-inactivated transitions (vertical transitions) are voltage independent (Kuo and Bean, 1994).

Figure 2.1: State diagram for the cardiac sodium channel Markov model. 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 and temperature dependent except for those governing transitions between closed and closed-inactivated states, which are only temperature-dependent.

In order to represent the cardiac sodium channel more accurately, two modifications are made to the Kuo and Bean model. The first modification is that an additional open state (O_2), with the same conductance as the first, is added. Transitions between the two open states are voltage independent. The addition of a second open state provides another pathway by which the channel can open ($C_4 \rightarrow O_2$) and improves the fit to the decay of the ionic currents. Two arguments can be made for the existence of more than one open state. First, although single channel open time distributions are generally well fit by a single exponential (Patlak and Oritz, 1985; Berman, et al., 1989; Scanley, et al., 1990), they can also be fit well by multiple exponentials, particularly in the presence of toxins (Kunze, et al., 1985; Nagy, 1987; Schreibmayer and Jeglitsch, 1992; Correa and Bezanilla, 1994). Second, sodium channels from many tissues, including cardiac tissue, produce tail currents with two exponential components (Goldman and Hahn, 1978; Dubois and Schneider, 1982; Hanck and Sheets, 1995; Elinder and Arhem, 1997). Elinder and Arhem suggest that this biexponential decay can only be produced by two open states connected by different pathways to a common closed state (Elinder and Arhem, 1997).

The second modification of the Kuo and Bean model is that open-inactivated transitions are made voltage dependent. The change in the open-inactivated rate constants is supported by Yue, Lawrence, and colleagues' finding that a voltage dependent open-to-inactivated transition is necessary to produce the correct voltage dependence of channel reopenings and mean open times (Yue, et al., 1989; Lawrence,

et al., 1991). It is also supported by Sheets' and Hanck's measurement of a significant component of gating current due to this transition (Sheets and Hanck, 1995).

Rate constants are of the form from Eyring rate theory (Hille, 1992)

$$k = \frac{kT}{h} \exp\left(\frac{-\Delta H^\ddagger}{RT}\right) \exp\left(\frac{\Delta S^\ddagger}{R}\right) \exp\left(\frac{-zV}{RT}\right) \quad (2.1)$$

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^\ddagger is the change in enthalpy, ΔS^\ddagger is the change in entropy, z 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. By convention, along the top row, all transitions towards an open state have positive valences because they are favored by depolarization, while those away from an open state have negative valences because they are favored by repolarization. The same convention is used along the bottom row; transitions towards the inactivated state have positive valences, while those away from the inactivated state, have negative valences.

There are several loops in the model that must 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 clockwise must be equal to the product when traversing the same loop counterclockwise (Hille, 1992). For the closed-closed-inactivated loops, satisfying microscopic reversibility requires that the transitions among the closed-inactivated states be scaled by a , the same factor used to scale the transitions between rows. Microscopic reversibility is

preserved around the closed-open-inactivated loop by isolating the ΔH , ΔS , and z terms in the product and satisfying each term separately using the following equations:

$$\Delta H_{??} \Delta H_{\gamma} \Delta H_{on} \Delta H_{??} \Delta H_{cf} \Delta H_{\gamma} \Delta H_{cn} \Delta H_{of} \quad (2.2)$$

$$\Delta S_{??} \Delta S_{\gamma} \Delta S_{on} \Delta S_{??} \Delta S_{cf} \Delta S_{\gamma} \Delta S_{cn} \Delta S_{of} \quad (2.3)$$

$$z_{??} z_{\gamma} z_{on} z_{\gamma} z_{of} z_{??} \quad (2.4)$$

Similarly, microscopic reversibility is preserved around the closed-open-open loop using the following equations for ΔH_{γ} , ΔS_{γ} , and z_{γ} :

$$\Delta H_{\gamma} \Delta H_{\gamma} \Delta H_{\gamma} \Delta H_{\gamma} \Delta H_{\gamma} \Delta H_{\gamma} \quad (2.5)$$

$$\Delta S_{\gamma} \Delta S_{\gamma} \Delta S_{\gamma} \Delta S_{\gamma} \Delta S_{\gamma} \Delta S_{\gamma} \quad (2.6)$$

$$z_{\gamma} z_{\gamma} z_{\gamma} z_{\gamma} \quad (2.7)$$

METHODS

Model development

The probability of occupying any particular channel state is described mathematically by a set of ordinary differential equations, written in matrix notation as

$$\frac{d\mathbf{P}(t)}{dt} = \mathbf{W}\mathbf{P}(t), \quad (2.8)$$

where $\mathbf{P}(t)$ is a vector describing the probabilities of occupying each state and \mathbf{W} is the transition matrix. In general, \mathbf{W} will be a function of voltage and thus time. For

voltage-clamped conditions, however, \mathbf{W} is time-independent; thus Eq. 2.8 has the analytic solution

$$\mathbf{P}(t) = \exp(\mathbf{W}t)\mathbf{P}(0). \quad (2.9)$$

Equation 2.9 is solved on a Silicon Graphics computer using linear algebra subroutines from the Silicon Graphics mathematics library (complib.sgimath).

Parameters of the model are determined using a simulated annealing algorithm (Corana, et al., 1987). This algorithm minimizes the 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 the 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, the annealing temperature is decreased by 5% per 50N function evaluations, where N is the number of parameters to be determined, as the algorithm converges on a solution. The algorithm is terminated when there is no more than 0.1% change in error since the last temperature reduction.

In order to limit the number of free parameters to be determined during each minimization, the fitting procedure is done in parts. First, the enthalpy and entropy terms are collapsed into a single Gibbs free energy term and the Gibbs free energies and effective valences are determined for a temperature of 13°C. Then, holding the effective valences constant, the enthalpy and entropy terms are determined. The entropy terms are written in terms of the enthalpy and the Gibbs free energy (ΔG):

$$\frac{\Delta S_{\gamma}}{R} = \frac{\Delta H_{\gamma} - \Delta G_{\gamma}}{RT} \quad (2.10)$$

where T is the temperature, 286 K. Since the Gibbs free energies and valences are known from the previous minimization, substitution of Eq. 2.10 into Eq. 2.1 leaves only the enthalpies to be determined. The enthalpies are determined by fitting experimental data at 21°C using the simulated annealing algorithm.

As shown by Vandenberg and Bezanilla (1991) and Patlak (1991) in developing models of sodium channels in squid giant axon, a variety of experimental data sets are needed to fully determine the model parameters. In this study, the experimental data for 13°C include ionic currents (provided by Hanck and Sheets, similar to (Sheets, et al., 1996)), gating charge accumulation (Hanck and Sheets as above), the steady-state inactivation curve (Hanck and Sheets as above), the rate of tail current relaxation (Hanck and Sheets, 1995), the time course of recovery from inactivation (Sakakibara, et al., 1993), and single channel open times (Sheets and Hanck, 1995). The majority of data are taken from hH1 sodium channels or, where these data are unavailable, from canine sodium channels. Recovery data at 13°C are

unavailable, so data at 17°C are used to approximate the data at 13°C. This approximation is acceptable because the difference in recovery rate between 13°C and 17°C is probably similar to the variation in recovery rate among cells at a single temperature. Ionic currents are calculated as

$$I_{Na} = G_{Na} P_{open} (V - E_{Na}) \quad (2.11)$$

where I_{Na} is the sodium current, G_{Na} is the maximal channel conductance, P_{open} is the probability of occupying the open states ($O_1 + O_2$), V is the membrane potential, and E_{Na} is the reversal potential for sodium. G_{Na} is a function of temperature and thus is a parameter to be determined at both 13°C and 21°C. E_{Na} is dependent upon the experimental solutions, which are different for the data at 13°C and 21°C, and so is set accordingly at each temperature. Gating current is calculated according to the formula (Vandenberg and Bezanilla, 1991):

$$I_g = \sum_{jk} ne(z_{jk} - z_{kj}) [P_j \alpha_{jk} - P_k \alpha_{kj}] \quad (2.12)$$

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 is found by integrating the gating current. Ionic currents and gating charge are computed using the following protocol. The membrane potential is held at +150 mV and then stepped for 20 ms to a potential between +70 mV and 20 mV inclusive in 10 mV increments. To eliminate convergence problems introduced by experimental error for potentials greater than or

equal to -20 mV, gating charge accumulation curves are fit with a single exponential function and the curve fit values, instead of the experimental data, are used for the plateau portion of the gating charge accumulation curves. Tail currents are computed by stepping from -150 mV to 40 mV until the current reaches its maximal value (after 0.94 ms) and then stepping down to potentials between -150 mV and -90 mV inclusive in 10 mV increments for 5 ms. Recovery from inactivation is assessed using a double-pulse protocol. The membrane potential is held at -140 mV and then stepped to -20 mV for 1 s. The potential is then stepped down to either -100 mV or -140 mV for lengths of time varying from 5 ms to 600 ms. Current is then measured during a 4 ms step to 0 mV to assess the amount of recovery. Open time distributions are calculated for potentials between -90 and -10 mV inclusive in 10 mV increments using a simplified model in which all transitions out of the open states are to an absorbing non-open state. The simplified model and the equation for the open time distributions are shown in the APPENDIX. Each data set is weighted so that all sets have approximately the same influence on the cost function, and, so that the parameters determined by the algorithm are those parameters which best reproduce all of the channel kinetics. The weights for ionic current, gating charge, steady-state inactivation, tail current, recovery from inactivation, and open time cost function terms are 1 , 250 , 1 , 500 , 1000 , and $5,000$ respectively.

The experimental data for 21°C include ionic currents (Wang, et al., 1996a), gating charge accumulation (Josephson and Sperelakis, 1992), the steady-state

inactivation curve (Wang, et al., 1996a), the time course of recovery from inactivation (Wang, et al., 1996a), and single channel open times (Benndorf, 1988). To compute the ionic currents, the membrane potential is held at -120 mV and then stepped for 15 ms to a potential between -60 mV and 20 mV inclusive in 10 mV increments. The same protocol is used to compute gating charge accumulation except that the holding potential is -150 mV. From measurements of gating charge in squid giant axon, the maximum charge displaced at each potential does not vary with temperature (Jonas, 1989). Therefore, the model's computed maximum charge values for 13°C were used as the experimental charge values for 21°C . Recovery from inactivation is again measured using a double-pulse protocol. The holding potential is -120 mV and the test potential is -20 mV; recovery times are varied between 10 ms and 250 ms. Open time distributions are calculated for potentials between -70 and -20 mV inclusive in 10 mV increments. The weights for ionic current, gating charge, steady-state inactivation, recovery from inactivation, and open time cost function terms are 100 , 10 , 5000 , 500 , and 500 respectively.

Model testing

The single channel behavior of the model was tested, which required the state transitions to be determined using a stochastic approach (Clay and DeFelice, 1983). In this method, the length of time a channel stays in its current state (ie. its dwell time

denoted as T_j) is calculated according to the formula

$$T_j = -\ln(r) / \sum_{k=1}^x \gamma_{jk} \quad (2.13)$$

where r is a random number from the uniform distribution [0,1] and γ_{jk} is the transition rate from state j to state k . The sum is over the x pathways out of state j . At the end of the dwell time, the new state of the channel is determined by assigning random numbers to a portion of the interval [0,1] based on the probabilities of changing to neighboring states. These probabilities are equal to the rate constant for a particular transition divided by the sum of the rate constants for all possible transitions. For example, a channel in state C_1 can transit to C_2 or C_1I . The probability of changing to C_2 is $\gamma_{12} / (\gamma_{12} + \gamma_{1I})$ where γ_{12} is the rate constant for $C_1 \rightarrow C_2$ and γ_{1I} is the rate constant for $C_1 \rightarrow C_1I$. Once the new state is determined, another random number is used to calculate the dwell time in the new state. At an instantaneous voltage step, channels remain in their current state, but the dwell times are recalculated.

RESULTS

The model parameters determined to give the best total fit of model responses to experimental data for ionic currents, gating charge, steady-state inactivation, tail currents, recovery from inactivation, and open times are listed in Table 2.1. The rate constants governing the $O_1 \rightarrow C_4$ (deactivation) and the $O_1 \rightarrow I$ (inactivation) transitions

Table 2.1

Parameters	ΔH (J mol ⁻¹)	ΔS (J mol ⁻¹ K ⁻¹)	z
?	116,900	224.114	0
?	263,870	708.146	?0.9701
?	200.240	529.952	1.5703
?	127,970	229.205	?1.3266
O _n	62,385	39.295	0.6625
O _f	79,035	1.510	0
??	?99,967	?578.317	0
??	62,555	?130.639	?3.5596
?	79,183	70.078	0
?	123,020	225.175	0
?	150,333	338.915	1.5717
?	121,900	193.265	?1.3281
C _n	293,270	786.217	0
C _f	57,533	0.00711	0

Table 2.1: Markov model parameters determined by the simulated annealing minimization algorithm.

have been measured experimentally. Benndorf and Koopmann found the enthalpies, entropies, and effective valences to be 129 kJ mol^{-1} , $0.23 \text{ kJ mol}^{-1} \text{ K}^{-1}$, and 1.54 for deactivation and 79 kJ mol^{-1} , $0.10 \text{ kJ mol}^{-1} \text{ K}^{-1}$, and 0.68 for inactivation respectively (Benndorf and Koopmann, 1993). The model parameters are 128 kJ mol^{-1} , $0.229 \text{ kJ mol}^{-1} \text{ K}^{-1}$, and 1.33 for deactivation and 62 kJ mol^{-1} , $0.039 \text{ kJ mol}^{-1} \text{ K}^{-1}$, and 0.66 for inactivation respectively. The model parameters for deactivation are very similar to the experimental data, whereas the parameters for inactivation differ slightly from the experimental data. The Gibbs free energies for inactivation for the experimental data and the model parameters, however, are very similar. Therefore, the discrepancy in inactivation parameters probably results from the minimization algorithm not being able to discriminate between several pairs of enthalpy and entropy terms yielding the same Gibbs free energy.

To assess the sensitivity of the model parameters, each parameter is varied by $\pm 1\%$ of its value and the change in the cost function is computed. A change in the value of the Gibbs free energy produces a much larger change in the cost function than does a change in the value of the corresponding valence. Thus, the Gibbs free energies are much more sensitive to a change in their values than are the valences. This sensitivity difference can be attributed to the different importance Gibbs free energies and valences have in determining the rate constants. The Gibbs free energies are the larger of the two terms in the exponential function and therefore are mainly responsible for determining the rate constant. In contrast, the voltage dependent terms serve only to modify slightly the basic rates set by the Gibbs free energies.

Thus, by changing the Gibbs free energies, one can produce a much larger change in the rate constant and a much larger change in the cost function.

Changes in the enthalpy and entropy terms of ΔH , ΔS , and C_n produce the largest changes in the cost function. The total error increases by up to 10 times for a 1% change in these parameters. The large sensitivity of these parameters is probably due to their role in providing temperature dependence. Parameters ΔH and ΔS must have precise enthalpy and entropy terms in order to accurately describe the increased rate of channel activation and rate of recovery from inactivation with temperature. C_n requires precise enthalpy and entropy terms in order to describe the shift in the steady-state inactivation curve with temperature. At 13°C, a change in the enthalpy of ΔH also produces a large change in the cost function. However, at 21°C, the same change produces much less change in the cost function, because the probability of occupying the second open state is much lower at this temperature.

In contrast to the large sensitivity of ΔH , ΔS , C_n , and ΔG , a 1% change in the enthalpy and entropy terms of ΔH_f , ΔS_f , and O_f as well as in the entropy term of C_f , produces almost no change in the total error. Two different explanations account for this small sensitivity. First, the entropies of O_f and C_f are a much smaller fraction of their respective enthalpies than are the entropies of other parameters. Thus, based on their relative size alone, one would expect a 1% change in their values to have little influence on the total error. Second, a change in the enthalpy and entropy terms of ΔH_f , ΔS_f , and O_f most likely produces little affect on the error because there is not enough

information in the experimental data to adequately constrain two separate terms. This observation is in contrast to changing the Gibbs free energy term for these parameters, which does produce a measurable change in the total error. These results suggest that while the entropy and enthalpy terms of Q_1 , Q_2 , and O_f may not be well determined, the Gibbs free energy they define is. In other words, there may be several combinations of entropy and enthalpy terms that produce an appropriate Gibbs free energy for Q_1 , Q_2 , and O_f .

The parameter values can be used to assess the amount of charge moved with each transition in the model. The model parameters suggest that activation requires the movement of 6.8 charges and inactivation requires the movement of 0.66 charges. Estimates of the charge associated with activation usually range from 4 to 7 (Hodgkin and Huxley, 1952a; Oxford, 1981; Sheets and Hanck, 1995), although some researches have found that at least 12 charges are needed (Hirschberg, et al., 1995). Estimates of the charge associated with inactivation range from 0.75 to 1.9 charges (Horn, et al., 1984; Vandenberg and Horn, 1984; Yue, et al., 1989; Lawrence, et al., 1991; Sheets and Hanck, 1995;). Thus, the model's estimates of charges required for activation and inactivation are similar to values measured experimentally.

Most of the charge movement in the activation pathway is concentrated in the last transition ($C_4 \rightarrow O_1$ or $C_4 \rightarrow O_2$). This finding seemingly contradicts the hypothesis that the final transition in the activation pathway is voltage independent for all voltage-gated channels. However, these transitions in the model probably represent

several steps lumped together so that, in reality, the final step may really be voltage independent (Kuo and Bean, 1994). Furthermore, gating currents, which depend heavily on the voltage dependence of each transition, have only been used in developing a few models. In another sodium channel model developed using gating currents, the closed-to-open transition also has the greatest voltage dependence (Vandenberg and Bezanilla, 1991).

The model is able to reproduce a wide range of experimental data. Figure 2.2 shows representative traces of the model-derived ionic current in comparison to the experimental data at 13°C (provided by Hanck and Sheets, similar to (Sheets, et al., 1996)) and at 21°C (provided by Bennett, similar to (Wang, et al., 1996a)) for clamp voltages of -50 mV, -30 mV, -10 mV, and 10 mV. Although the peak current values deviate slightly from the experimental values, the time courses of activation and inactivation are generally well fit by the model at both temperatures. For potentials greater than -40 mV, the model-derived currents decay to zero within 50 ms, whereas the experimental currents do not. The model decays to zero faster than the experimental data at 13°C because the experimental data have both fast and slow components of inactivation, whereas the model has only fast inactivation.

Figure 2.3 compares model-derived current-voltage relationships at 13°C (Fig. 2.3A) and 21°C (Fig. 2.3C) with experimental data used in the fitting process. Figure 2.3B compares the current-voltage relation predicted at 17°C with experimental data (provided by Wasserstrom, similar to (Sakakibara, et al., 1993)). As shown by the

Figure 2.2: Comparison of voltage-clamped sodium current tracings for clamp voltages of -50 mV, -30 mV, -10 mV, and 10 mV for experimental data (---) and the model (?). *A.* At 13°C (experimental data provided by Hanck and Sheets similar to (Sheets, et al., 1996)). *B.* At 21°C (experimental data provided by Bennett similar to (Wang, et al., 1996a)).

Figure 2.3: Normalized current-voltage curves for experimental data and the model. Curves are the best fits to Eq. 2.14 in the text. See Table 2.2 for fitted parameters. *A.* At 13°C experimental data (O) and the model (⊖) (experimental data provided by Hanck and Sheets similar to (Sheets, et al., 1996)). *B.* At 17°C experimental data (⊖) and the model (⊗) (experimental data provided by Wasserstrom similar to (Sakakibara, et al., 1993)). *C.* At 21°C experimental data (⊖) and the model (⊖) (experimental data provided by Bennett similar to (Wang, et al., 1996a))

Table 2.2

	E_{Na} (mV)	G_{Na} (mV^{-1})	$V_{0.5}$ (mV)	slope factor (mV)
Experiment 13°C	44.675	0.0131	55.045	6.734
Model 13°C	44.675	0.0132	51.962	7.450
Experiment 17°C	38.020	0.0174	41.006	6.640
Model 17°C	38.020	0.0154	47.106	7.002
Experiment 21°C	55.000	0.0145	39.603	6.748
Model 21°C	55.000	0.0130	40.750	6.604

Table 2: Parameters of Eq. 2.14 that fit the current-voltage curves for the experimental data and the model at 13°C, 17°C, and 21°C.

current-voltage relationships, the model reproduces peak current well throughout the voltage and temperature range tested. At 13°C, the current magnitudes deviate most significantly from the corresponding experimental data over the voltage range -60 mV to -45 mV. In this range, the model produces less current than the experimental data. This reduction in current probably results from inactivation that is too fast, which reduces the number of channel reopenings. At 17°C, peak currents also differ over this voltage range. Considering, however, that data at this temperature were not fit and were obtained from a different experimental preparation than any of the other data, the model reproduces the data well. At 21°C, peak currents are well fit except at very depolarized potentials. Both the model and experimental current-voltage curves are fit using a modified Boltzmann function

$$I = \frac{G_{Na}(V - E_{Na})}{1 + \exp\left(\frac{V - V_{0.5}}{s}\right)} \quad (2.14)$$

where G_{Na} is the conductance, V is the voltage, E_{Na} is the reversal potential, $V_{0.5}$ is the potential at which the current is half-maximal, and s is the slope factor. The resulting parameters are listed in Table 2.2. The conductances and slope factors are similar with the model having a slightly shallower slope for 13°C and 17°C and a slightly steeper slope for 21°C. The model's half-maximal voltages differ from the corresponding experimental data by 3.1 mV, 6.1 mV, and 1.2 mV for 13°C, 17°C, and 21°C respectively. This difference is reflected in the slightly rightward shift of the model's current-voltage curve for 13°C and the slightly leftward shift of the

model's current-voltage curve for 17°C. The model shows a rightward shift of the half-maximal potential (11.2 mV per 8°C) as the temperature is increased. Rightward shifts in the half-maximal potentials of the steady-state activation (8 mV per 10°C) and inactivation curves (7 mV per 10°C) as the temperature is increased have been measured experimentally (Murray, et al., 1990). These shifts would produce a shift in the current-voltage relationship similar to that produced by the model.

Figures 2.4A and 2.4B show the time to peak current and the time constants of inactivation for 13°C, 17°C, and 21°C. The model's time to peak current is very similar to the corresponding experimental data for all the voltages and temperatures tested. As the temperature is increased, the time to peak current is reduced, as expected from data in the literature (Colatsky, 1980; Murray, et al., 1990). The time constants of inactivation are estimated by fitting an exponential function to the current decay. For potentials of ≥ 30 mV and greater, the model's ionic current decay is better fit with two exponentials. The experimental data also have a second exponential at these potentials. However, the time constant of this second exponential in the experimental data is too large to accurately determine with a voltage clamp of 35 ms. Therefore, in order to compare the model's time constants with those of the experimental data, a single exponential fit is used in Fig. 2.4B. At 13°C, the inactivation time constants predicted by the model are larger than those of the experimental data at both ends of the voltage range, while they are similar to those of the experimental data in the middle of the voltage range. The discrepancy in the

Figure 2.4: Temperature dependence of activation and inactivation at 13°C (experimental data (O), model (●)), at 17°C (experimental data (⊕), model (⊗)), and at 21°C (experimental data (⊙), model (⊚)). Experimental data from sources listed in Fig. 2.3. *A.* Time to peak current. *B.* Time constants of inactivation determined by fitting a single exponential to the current decay.

time constants at negative potentials is probably due to error in the fitting procedure as a result of having only two or three time constants worth of data. In addition, the model's time constants increase for very depolarized potentials, whereas the experimental data show that the time constants decrease monotonically as voltage is increased. This apparent discrepancy is an artifact of fitting the model's decay with a single exponential. As the potential is increased, the model's decay switches from that described by a large fast component and a small slow component to one described by a small fast component and a large slow component. As the slower component becomes larger, the time constant determined by fitting a single exponential to the decay increases. If just the fast or slow time constant from a biexponential fit of the model's decay is plotted, the time constants do decrease monotonically with increasing voltage. At 17°C and 21°C, the model's time constants of inactivation are similar to the corresponding experimental data. As the temperature is increased, the time constants become faster, as expected from data in the literature (Colatsky, 1980). Thus, the model reproduces well the activation and inactivation properties of the channel for a large voltage and temperature range.

The second data set reproduced by the model is gating charge movement in response to voltage-clamp stimuli. Figure 2.5A shows a comparison of the charge-voltage curves for the model and the experimental data (provided by Hanck and Sheets, similar to (Sheets, et al., 1996)) at 13°C. The magnitudes plotted here for the model are the maximum charge accumulated at 30 ms. Some error is associated with

Figure 2.5: Normalized gating charge-voltage curves. *A.* At 13°C for experimental data (O) (provided by Hanck and Sheets similar to (Sheets, et al., 1996)) and the model (●). Curves are the best fits to a Boltzmann function where the slope factor and half-maximal potential values are 15.7 mV and -63.3 mV for the experimental data and 14.0 mV and -70 mV for the model respectively. *B.* Model's charge-voltage curves at 13°C (●) and at 21°C (●). The slope factor and half-maximal potential values for 21°C are 19.6 mV and -63.9 mV respectively.

these values because charge is still being accumulated at a very slow rate at 30 ms; that is, in the model, the plateau portion of the charge accumulation curves is not completely flat, but rather has a small slope. The nonequilibrium movement of charge is most likely due to constraints imposed by microscopic reversibility on the closed-open-inactivated loop. Nevertheless, the magnitude of charge movement is well reproduced by the model over most of the voltage range tested. Fitting a Boltzmann function to the experimental data at 13°C yields slope factor and half-maximal potential values of 15.7 mV and -70 mV. However, the experimental data for -70 mV and -60 mV appear to deviate significantly from the remainder of the data. Exclusion of these two points results in a better fit (correlation coefficient 0.996 versus 0.984). The slope factor and half-maximal potential values for this fit are 15.8 mV and -70 mV. The model's half-maximal potential is identical to that of the experimental data (-70 mV) and its slope is slightly steeper (14 mV). Figure 2.5B compares the model's charge-voltage curves for 13°C and 21°C. At 21°C, maximum charge values are taken at 20 ms even though charge is still being accumulated at a very slow rate. Fitting a Boltzmann function to the 21°C curve yields slope factor and half-maximal potential values of 19.6 mV and -63.9 mV respectively. Thus, with increased temperature, the charge-voltage curve shifts rightward by 6.1 mV per 8°C. Although data on the temperature dependence of the charge-voltage curve for heart tissue have not been published, Hanck and coworkers have shown that the charge-voltage curve, although having a steeper slope, is similar to the peak sodium

conductance curve (Hanck, et al., 1990). Thus, one would expect the charge-voltage curve to be shifted rightward with increasing temperature since the peak sodium conductance curve is shifted rightward with temperature (Murray, et al., 1990). Therefore, the model can reproduce the charge-voltage relationship over a large range of voltages and temperatures.

The model can also approximate the rate of gating charge movement. Figure 2.6 shows the gating charge accumulation time constants as a function of voltage for the model and experimental data (provided by Hanck and Sheets, similar to (Sheets, et al., 1996)) at 13°C and for the model at 21°C. For potentials greater than or equal to -50 mV, gating charge accumulation is well fit by a single exponential. For potentials less than -50 mV, the model's gating charge accumulation curves exhibit an initial fast decay followed by a much slower return to zero. Since the gating charge accumulation is thus not well fit by a single exponential, these potentials were excluded from Fig. 2.6. Note that the experimental time constants plotted in Fig. 2.6 are those obtained from a single measurement of gating charge from one cell and are from a different cell than the ionic currents. Taking these facts into consideration, at 13°C, although the model has larger time constants at each potential, it approximates the voltage dependence of these time constants reasonably well. At 21°C, one would expect the time constants of gating charge accumulation to be much faster (Josephson and Sperelakis, 1992) and the model meets this expectation with approximately a 1 ms reduction in the time constants throughout the voltage range.

Figure 2.6: Time constants of gating charge accumulations (experimental data at 13°C (O), model data at 13°C (●), model data at 21°C (●)). Experimental data from source listed in Fig. 2.5.

A third data set reproduced by the model is steady-state availability. Figure 2.7A shows the steady-state availability curves for the model and the experimental data (provided by Hanck and Sheets) at 13°C. The model's curve is nearly identical to that of the experimental data as reflected in the slope factor and half-maximal potential values of the respective Boltzmann functions. For the experimental data, the slope factor and half-maximal potential values are -9.9 mV and -106.1 mV. For the model, the respective values are -10.6 mV and -107 mV. Figure 2.7B compares the steady-state availability curves at 13°C and 21°C. At 21°C, there is a noticeable rightward shift of the curve. Fitting a Boltzmann function to the 21°C curve yields slope factor and half-maximal potential values of -15.2 mV and -101.7 mV. Thus, as the temperature is increased, the model produces a rightward shift of 5.3 mV per 8°C. This shift is similar to that measured experimentally, which is 7 mV per 10°C (Murray, et al., 1990). Experimental data also predict that the slope factor increases slightly (0.5 mV per 10°C) over the temperature range 16°C to 26°C (Murray, et al., 1990). The additional increase in slope factor over that predicted by the experimental data probably results from the exponential form of the rate constants, which prevents them from becoming constant except at the extremes of the voltage range. The non-saturating rate constants prevent a true plateau of the curve at very negative potentials and thus, as temperature is increased, the curve cannot simply be shifted to the right. Nevertheless, the model is able to reproduce well the level of inactivation over a large voltage and temperature range.

Figure 2.7: Steady-state availability curves. *A.* At 13°C for experimental data (O) (provided by Hanck and Sheets similar to (Sheets, et al., 1996)) and the model (●). Curves are the best fits of a Boltzmann function where the slope factor and half-maximal potential values are 10.6 mV and 106.1 mV for the experimental data and 10.6 mV and 107 mV for the model respectively. *B.* Model's steady-state availability curves at 13°C (●) and at 21°C (●). The slope factor and half-maximal potential values for 21°C are 15.2 mV and 101.7 mV respectively.

In addition to ionic currents, gating charge, and steady-state availability, the model can reproduce recovery from inactivation. Shown in Fig. 2.8A is the rate at which the model recovers from inactivation at 13°C in comparison to experimental data (Sakakibara, et al., 1993). The model recovers from inactivation at a similar rate as the experimental data for a holding potential of -100 mV and, as the holding potential is decreased, the model recovers faster. For -100 mV, both the experimental data and the model show a delay of 10 ms before recovery occurs. Sakakibara and coworkers fit the experimental data using the sum of two exponentials. However, their data can be fit just as well with a single exponential and this form yields better fits to the model results. For -100 mV the time constants are 164.9 ms and 177.7 ms, for -120 mV they are 42.9 ms and 46.7 ms, and for -140 mV they are 6.9 ms and 21.9 ms for the experimental and model data respectively. The model's time constants are generally larger than those of the experimental data and, as the holding potential is decreased, the difference between the time constants increases. One possible explanation for the model's recovery rate being too slow is a lack of voltage dependence of the rate constants governing transitions between the closed and closed-inactivated states. Adding voltage dependence here could allow the model to more correctly approximate the time constant of recovery as holding potentials are lowered, but such a change would increase model complexity substantially by adding additional loops for which microscopic reversibility must be satisfied. Figure 2.8B compares the rates of recovery from inactivation at 13°C and

Figure 2.8: Rates of recovery from inactivation. *A.* At 13°C for experimental data (Sakakibara, et al., 1993) and the model. Data is plotted for holding potentials of -100 mV (experimental data $\langle \gg \rangle$, model $\langle \gg \rangle$), -120 mV (experimental data (O), model $\langle \gg \rangle$), and -140 mV (experimental data (?), model $\langle \gg \rangle$). Model curves are fit using a single exponential with time constants of 177.7 ms, 46.7 ms, and 21.9 ms for -100 mV, -120 mV, and -140 mV respectively. *B.* Model's recovery from inactivation curves for a holding potential of -120 mV at 13°C ($\langle \gg \rangle$) and 21°C ($\langle \gg \rangle$). The time constant at 21°C is 13.3 ms

21°C for a holding potential of -120 mV. As temperature is increased, the recovery rate is increased significantly. Fitting a single exponential to the recovery data at 21°C yields a time constant of 13.3 ms. This rate is similar to that found by refitting published data at 21°C (Wang, et al., 1996a) with a single exponential, which yields a time constant of 15 ms. The model presented here thus differs from existing Markov models of the cardiac sodium channel (Benndorf, 1988; Berman, et al., 1989; Scanley, et al., 1990) in that it recovers from inactivation with the correct voltage dependence.

The model also reproduces the rate at which an open channel deactivates. Plotted in Fig. 2.9 are the time constants from a single exponential fit of the current decay at 13°C upon stepping from 40 mV to the test potential. The model has two deactivation pathways ($O_1 \rightarrow C_4$ and $O_2 \rightarrow C_4$) and therefore will have a biexponential tail current. For potentials of -100 mV and below, the model's time constants are similar to those measured experimentally (Hanck and Sheets, 1995) and there is little difference between a monoexponential and a biexponential fit to the data. At these potentials, the $O_1 \rightarrow C_4$ pathway, which is faster, appears to dominate. At more depolarized potentials, the model predicts larger time constants than measured experimentally and the current decay is much better fit using two exponentials. Thus, as the test potential is increased, channels more readily exit the open states using both deactivation pathways. At 21°C, the current decay produced by the model is best fit using a single exponential at all potentials. At this temperature, the $O_1 \rightarrow C_4$ pathway

Figure 2.9: Time constants from a single exponential fit of the tail current relaxations at 13°C for experimental data (Hanck and Sheets, 1995) (O) and the model (●) and at 21°C for the model (●).

dominates because of the low probability of occupying the second open state. As expected, as the temperature is increased, the rate of deactivation increases.

The final data set used to determine the model parameters is single channel open durations. Figure 2.10 shows the model's densities of single channel open durations for both 13°C and 21°C. Twelve hundred channels are simulated as described in METHODS for a 40 ms sweep and their open durations measured. At 13°C, for a clamp voltage of -50 mV, a histogram of open durations shows a wide variation of open times including a significant fraction greater than 2 ms. In contrast, for a clamp voltage of -15 mV at 13°C, most of the channels have open times of less than 2 ms. These densities are clearly biexponential because the model has two open states and the dwell times in each open state are significantly different. At 21°C, for both clamp potentials, almost all of the channels have open times of less than 2 ms, but the densities are still biexponential. Experimental data for the densities and distributions of open times are usually fit with a single exponential. In order to compare the model and the experimental data, the distributions are calculated from Eq. A2.14 and are fit with a single exponential. Fitting the densities calculated from stochastic channel simulations yields the same results. Figure 2.11 shows the open time distribution time constants versus voltage at 13°C and 21°C and the model's prediction at 17°C. Experimental data by Scanley (Scanley, et al., 1990) are plotted for 13°C and by Benndorf (Benndorf, 1988) for 21°C. For the entire voltage and temperature range depicted, the model data agree well with the experimental data. As

Figure 2.10: Densities of single channel open durations. Twelve hundred channels are simulated for 40 ms as described in the text. Bin size is 0.5 ms. *A.* At 13°C for +50 mV. *B.* At 13°C for +15 mV. *C.* At 21°C for +50 mV. *D.* At 21°C for +15 mV.

Figure 2.11: Model-predicted time constants for the open time distributions calculated from Eq. A2.14 at 13°C (—), 17°C (----), and 21°C (·····). Experimental data by Scanley (Scanley, et al., 1990) (⊗) at 13°C and by Benndorf (Benndorf, 1988) (⊕) at 21°C is also plotted.

temperature is increased, the model's open times become shorter and the peak open time is shifted rightward, both of which are supported by the experimental data. It should be emphasized that time constants obtained by fitting a single exponential to the open time distributions are not equal to the mean open times of the model. (The mean open times can be calculated using the probability density function in Eq. A2.13.) The mean open time at each potential is larger, particularly at very depolarized potentials, due to rare long occupancies of the second open state (see Fig. 2.10). Despite its larger mean open times, the model reproduces the distributions of the open durations well for a large voltage and temperature range.

The majority of the data presented to this point were used to determine the model's parameters. While it is important that the parameters adequately reproduce all the data used to determine them, it is also important that the parameters can be used to predict data not used in the fitting process. The ability of the model to fit data not used in determining the parameters is an independent test of how well the model approximates reality. In developing the model, ionic currents obtained with different voltage clamp protocols were used. The model was thus constructed so that it could reproduce the ensemble behavior of many sodium channels. In testing the model, therefore, measures of single channel behavior were chosen to see if the model could represent one channel as well as the average of many channels.

Figure 2.12 shows the first latency densities for clamp potentials of -50 mV and -15 mV at 13°C and 21°C . Single channel simulations are done as described

Figure 2.12: Densities of single channel first latencies. Twelve hundred channels are simulated for 40 ms as described in the text. Bin size is 0.5 ms. *A.* At 13°C for ?50 mV. *B.* At 13°C for ?15 mV. *C.* At 21°C for ?50 mV. *D.* At 21°C for ?15 mV.

previously. At 13°C, for a clamp voltage of -50 mV, there is a wide variation of first latencies with many longer than 10 ms. In contrast, for a clamp voltage of -15 mV at 13°C, almost all of the channels have first latencies of less than 5 ms. The probability that a channel first opens after time t was computed from these histograms and plotted versus time. The plots have a nonzero plateau that describes the probability of not opening during the channel simulation as well as a single time constant with which the probability relaxes to this plateau value. The plateau and time constant values are 0.34 and 6.41 ms for -50 mV and 0.22 and 1.65 ms for -15 mV, respectively. At 21°C, for a clamp voltage of -50 mV, a much larger fraction of channels have first latencies less than 5 ms, although there is still a wide variation in latencies. For -15 mV, almost all of the latencies are less than 2 ms. The plateau and time constant values at 21°C are 0.76 and 3.69 ms for -50 mV and 0.52 and 0.63 ms for -15 mV, respectively. Experimental data at 21°C for a clamp potential of -50 mV yield a time constant of 1.15 ms (Berman, et al., 1989). The corresponding plateau value is not available. The model probably has a larger time constant because of the wide variation in the latencies. The long latencies are due to channels inactivating from a closed state for a considerable time and then returning to a closed state from which the channel can then open. The long latencies might be eliminated by adding voltage dependence to the transitions between the closed and closed-inactivated states. However, as discussed previously, such a change would greatly increase the complexity of the model. Although experimental data with which to compare the

model's data are limited, first latencies are related to the time to peak current and thus, like these values, should decrease with temperature at all voltages, as the model predicts.

Finally, the model is used to predict the fraction of channels that do not open and the number of channels that reopen during a voltage clamp of 40 ms as additional tests of the model's ability to predict single channel behavior. Figure 2.13A shows the probability of not opening as a function of voltage at 13°C and 21°C. The probability of not opening is high at very negative potentials, while, at depolarized potentials, it is much lower. Even at these depolarized potentials, though, there is still a significant fraction of channels that do not open at both temperatures. The model's predictions for 13°C are generally lower than the experimental data reported by Scanley (Scanley, et al., 1990), although the overall trend of the curve is similar. The tendency for the model to predict slightly lower probabilities than those measured experimentally may be due to brief and missed openings in the experimental data. Experimentally, openings shorter than 178 μ s cannot be detected (Scanley, et al., 1990). If these openings are excluded from the model simulation, the resulting probabilities of a null sweep are increased by 5-20% depending on the clamp potential. The probability a channel does not open increases as the temperature is increased as expected from data in the literature (Correa, et al., 1992). This result suggests that as the temperature is increased, a larger fraction of channels are inactivated before they reach the open state.

Figure 2.13: Single channel probabilities for experimental data (O) at 13°C (Scanley, et al., 1990), model data at 13°C (●), and model data at 21°C (●). *A.* The probability of not opening versus clamp voltage. *B.* The number of channel openings, normalized by the number of channels that open, versus clamp voltage.

Figure 2.13B shows the number of channel openings, normalized by the number of channels that open, versus voltage. At 13°C, there are a significant number of reopenings for -60 mV and -50 mV with the number of reopenings decreasing as the potential is increased. The model predicts that 32% of opening events are reopenings at -60 mV. This percentage is similar to that of the experimental data; by Scanley's calculations, at -57 mV, 39% of opening events are reopenings (Scanley, et al., 1990). While the exact number of reopenings differs from Scanley's measurements (Scanley, et al., 1990), the overall trend of the curve is nevertheless similar. As the temperature is increased from 13°C to 21°C, the number of reopenings generally decreases and the peak of the reopening curve is shifted to the right. At 21°C a channel spends less time in the open state and thus, has less opportunity to close and reopen. These results are consistent with the reduction in open time and shift of the open time curve rightward as temperature is increased. Therefore, not only does the model fit well the ensemble channel behavior used to constrain the parameters, but it also predicts single channel behaviors not used in the fitting process.

DISCUSSION

Three Hodgkin-Huxley models have been formulated to describe the kinetics of cardiac sodium channels. They are the Beeler-Reuter model (Beeler and Reuter, 1977), the Ebihara-Johnson model (Ebihara and Johnson, 1980), used in the Luo-Rudy ventricular cell model (Luo and Rudy, 1991), and the DiFrancesco-Noble

model (DiFrancesco and Noble, 1985). All of these models were constructed by fitting empirically-derived mathematical functions to channel activation and inactivation data. They all reproduce ionic currents in response to voltage clamp protocols and reproduce recovery from inactivation. As evidenced by their widespread use, these models are considered to provide accurate representations of the ensemble-average behavior of cardiac sodium channels.

The Markov model reported in this chapter reproduces the same range of experimental data as that reproduced by these Hodgkin-Huxley models. In addition, the Markov model has two advantages over previous Hodgkin-Huxley models. First, the rate constants are not empirical functions, but rather, are based on the thermodynamic principles of Eyring rate theory (Hille, 1992). This formulation gives physical meaning to the rate constants, allows a direct comparison of certain rate constants to experimental data, and facilitates the explicit incorporation of temperature dependence into the model. Second, the Markov model, as shown in RESULTS, can not only reproduce ensemble-average behavior, but can also predict single channel behaviors. The Beeler-Reuter, Ebihara-Johnson, and DiFrancesco-Noble models, in their original formulations, were intended only to represent channel ensemble behavior, not that of individual channels. These Hodgkin-Huxley models can be expanded to have multiple resting and inactivated states and thus, can be made to represent a single channel. However, it is unclear whether such an expanded Hodgkin-Huxley model would predict these single channel behaviors as well as the Markov model. Studies done to compare the predictive ability of expanded Hodgkin-

Huxley models versus Markov models are contradictory. Chay compared a five-state Markov model with an eight-state Hodgkin-Huxley model, both of which had absorbing inactivated states, and found that the Hodgkin-Huxley model was not inferior to the Markov model in its ability to predict single channel behaviors (Chay, 1991). However, this result differs from that of Horn and Vandenberg, who compared a five-state Markov model with a five-state Hodgkin-Huxley model. They found that the Hodgkin-Huxley model was not able to predict single channel behaviors as well as the Markov model (Horn and Vandenberg, 1984). In any case, multi-state models clearly have an advantage over the original Hodgkin-Huxley models in their ability to predict single channel behaviors. The Markov model in this chapter therefore reproduces a wider range of experimental data than Hodgkin-Huxley models and is thus a more complete representation of the cardiac sodium channel.

The new Markov model improves on existing Markov models of the cardiac sodium channel (Benndorf, 1988; Berman, et al., 1989; Scanley, et al., 1990) as well. These models were constructed to reproduce a subset of single channel data. To this end, they were made to have four or five states. They were not meant to closely resemble channel structure. In contrast, the Markov model reported here more closely follows the current hypotheses of channel structure. The model is similar to that proposed by Kuo and Bean for sodium channels in CA1 hippocampal neurons (Kuo and Bean, 1994). It is designed so that each closed and closed-inactivated state represents a specific combination of the positions of the four voltage sensors and the

position of the inactivation particle (Hille, 1992). Having a model that follows the channel structure as closely as possible is important because it facilitates investigations of the structure-function relationship.

The Markov model reported here also improves on existing Markov models of the cardiac sodium channel because it can recover from inactivation. Previous Markov models of the cardiac sodium channel have an absorbing inactivated state and thus cannot recover from inactivation. Recovery from inactivation is necessary for any model to be used in studies of antiarrhythmic drug action. Antiarrhythmic drugs exhibit use-dependence, a property thought to determine their effectiveness (Hondegem and Katzung, 1977; Starmer, et al., 1984), that requires the application of a pulse train to measure. Models with an absorbing inactivated state cannot reproduce channel activity in response to a pulse train and thus will not reproduce use-dependence. Therefore, the Markov model reported here improves on previous Markov models of the cardiac sodium channel by more closely resembling the channel's structure and by reproducing recovery from inactivation.

There are two features of the Markov model in this chapter that differ from Kuo and Bean's model (Kuo and Bean, 1994) and are necessary to model the cardiac isoform of the sodium channel. First, although many neuronal models are able to reproduce ionic currents assuming voltage independent open-inactivated transitions, such is not the case with the model presented here. The open-inactivated transitions are associated with the movement of 0.66 charges. This value is close to the range of 0.75 to 1.9 charges reported for these transitions by others (Horn, et al., 1984;

Vandenberg and Horn, 1984; Sheets and Hanck, 1995). Sheets and Hanck measure a significant component of gating current due to this transition (Sheets and Hanck, 1995). The inclusion of voltage dependent open-inactivated transitions is also supported by Yue, Lawrence, and coworkers' finding that voltage dependence is necessary to produce the correct voltage dependence of channel reopenings and mean open times (Yue, et al., 1989; Lawrence, et al., 1991). Without voltage dependence of the $O \rightarrow I$ transition, the mean open times increase monotonically as a function of voltage, instead of reaching a peak and then decaying.

A second difference from the model of Kuo and Bean is that the present model has two open states with the same conductance. This addition provides a second pathway by which the channel can open ($C_4 \rightarrow O_2$) and yields a better fit to the current decay by providing a temporary store of channels in the open state. Upon entering state O_1 the channel can either inactivate immediately or transition to O_2 before inactivating through O_1 . To show the difference between a single open state model and a two open state model, a single open state model was developed at 13°C using the same procedure as described in METHODS for the two open state model. Figure 2.14A compares several ionic currents from this single open state model with those of the experimental data. While the data for -50 mV are very similar, the model's decay is much too fast for all the other potentials. In addition, the model does not decay to zero, but rather to some plateau level of current. These plateaus are the result of trying to fit the decay without greatly increasing the open times

Figure 2.14: Single open state model results at 13°C. *A.* Comparison of voltage-clamped sodium current tracings for clamp voltages of -50 mV, -30 mV, -10 mV, and 10 mV for experimental data (----) and the model (—). *B.* Model-predicted time constants of the open time distribution (—) compared to experimental data by Scanley (O) (Scanley, et al., 1990).

(Fig. 2.14B). A comparison of the single open state model results to those of the two open state model (Figs. 2.2A and 2.10) shows that the two open state model does a much better job of reproducing the experimental data. Previous models of the cardiac sodium channel have had only a single open state because single channel open time distributions are well fit by a single exponential decay (Patlak and Oritz, 1985; Berman, et al., 1989; Scanley, et al., 1990). However, single channel open time distributions can also be well fit by multiple exponentials, particularly in the presence of toxins (Kunze, et al., 1985; Nagy, 1987; Schreibmayer and Jeglitsch, 1992; Correa and Bezanilla, 1994). These toxins may be exposing open states that are always present but not readily distinguishable except in the presence of toxin. A second open state is also supported by the biexponential decay of the tail currents, which requires two pathways for deactivation and thus two open states (Elinder and Arhem, 1997). Additional transitions from O_2 directly to an inactivated state could have been added, but these transitions added unnecessary, ill-constrained parameters. The decay could also be fit using an additional inactivated state, but such a model requires the channel to reopen from the inactivated state throughout the voltage range tested in order to fit the data. From single channel studies, it is known that such reopening does not occur for very depolarized potentials (Berman, et al., 1989; Scanley, et al., 1990). Therefore, the addition of a second open state and voltage dependence to the open-inactivated transitions are modifications which are unique to modeling of the cardiac sodium channel isoform and are necessary to reproduce a full range of channel activity.

A final feature of the Markov model presented here that provides an advantage over previous models is its ability to reproduce experimental data over a range of temperatures. Hodgkin-Huxley models can be made to reproduce channel activity at multiple temperatures by modifying the conductance and the time constants of activation and inactivation by a Q_{10} factor and by shifting the steady-state activation and inactivation curves (Colatsky, 1980; Murray, et al., 1990; Milburn, et al., 1995). However, such models still have the disadvantages, which were discussed previously, of any Hodgkin-Huxley model as compared to the Markov model in this chapter. No Markov models exist for sodium channels that can reproduce ensemble-average and single channel behaviors over a range of temperatures. To do so, each term in the model's rate constants needs to have its own temperature dependence or its own Q_{10} factor (Kohlhardt, 1990). Formulating the rate constants as exponential functions of the change in enthalpy and entropy allows the Markov model presented here to have explicit temperature dependence. As shown in RESULTS, this formulation yields a model that reproduces ensemble-average and single channel behavior over a temperature range of 10°C to 25°C. A model with explicit temperature dependence is advantageous in characterizing a channel's behavior because it allows predictions to be made about how drugs or toxins differentially affect channel states as the temperature is changed. Therefore, the explicit temperature dependence of the Markov model presented here is an additional improvement on all previous models of the cardiac sodium channel.

Although the Markov model in this chapter improves on existing models, there are several potential sources of uncertainty in the model's parameters. First, all of the data used to determine the parameters were obtained from single cells rather than the averaged behavior of many cells. Therefore, any deviation of the data from the averaged behavior will be reflected in the model's parameters. Second, data from several different laboratories were used to determine the parameters and their temperature dependence. Care was taken to correct for differences in solutions and temperatures, but, even so, differences in experimental technique and cell type could produce error in the parameters.

In addition to the experimental sources of uncertainty in the model's parameters, there are also sources of uncertainty in the fitting procedure. First, the parameters are sensitive to the relative weights of the cost function terms used in the minimization algorithm. By changing these weights, another set of parameters with a similar cost function value can be obtained. Therefore, the set of parameters presented here is not the only possible solution to the minimization problem. This set of parameters is, however, an acceptable solution because the amount of charge movement associated with activation and inactivation is similar to the values measured experimentally (Hodgkin and Huxley, 1952a; Oxford, 1981; Horn, et al., 1984; Vandenberg and Horn, 1984; Hirschberg, et al., 1995; Sheets and Hanck, 1995). It is also an acceptable set of parameters because the enthalpy, entropy, and effective valence values for the $O_1 \rightarrow C_4$ and $O_1 \rightarrow I$ transitions are similar to those

measured experimentally (Benndorf and Koopmann, 1993). Second, only a limited amount of experimental data can be used in the fitting process. By not including certain data in determining the model parameters, inevitably there will be data that the model does not predict. Enough data were used to constrain the model as fully as possible and to reproduce as much channel activity as possible, but not so much as to prevent timely convergence of the minimization algorithm. Moreover, as models become increasingly complex and include additional states and rate constants, inevitably there will be some parameters that are not as well constrained. The potential of unconstrained parameters was minimized by using microscopic reversibility, by assuming cooperative movement of the voltage sensors, and by requiring transitions between closed-inactivated states to have rate constants similar to those of transitions between closed states. Enough data were used to constrain the model since, as shown in RESULTS, the model's rate constants are well determined and the model reproduces a broad range of experimental data.

There are many potential applications for the cardiac sodium channel Markov model in this chapter, but there are also some limitations in the model's ability to reproduce channel activity. First, as shown in RESULTS, the model can deviate significantly from the experimental data at the extremes of the potential range. Thus, caution should be exercised in using the model outside of the -150 mV to 20 mV range for which it was developed. Second, in order to have a model that reproduces the widest range of data possible, the best fit of a particular data set was sacrificed for

good fits to all of the data sets. Applications that require the best fit of one particular data set will thus require adjustment of the model's parameters. Finally, as the temperature increases above 25°C, multiple conductance levels of the cardiac sodium channel emerge (Benndorf, 1994). The kinetics underlying these multiple conductance levels are not known and therefore, the same channel model may not apply at temperatures above 25°C. Despite these limitations, the cardiac sodium channel model reported here has many potential applications and its development is an important step in the further characterization of this channel.

APPENDIX: Calculation of the open time distributions

Figure 2.15 shows the simplified model from which the open time distributions are calculated. The two open states and the transitions between them are the same as for the full model shown in Fig. 2.1. Transitions out of each open state are lumped into a single transition from each open state to an absorbing, non-open state. It is assumed that at time zero the channel is in either O_1 or O_2 . The probability of occupying each open state at time zero is equal to the transition rate from C_4 to the respective open state (α or β) divided by the sum of these rates ($\alpha + \beta$). The probability of occupying each open state, denoted by $P_{O1}(t)$ and $P_{O2}(t)$, is then given by the solution of the following equation:

$$\frac{P_{O1}(t) \quad P_{O2}(t)}{t} = \frac{\alpha}{\alpha + \beta} \frac{e^{-\lambda t}}{\lambda} + \frac{\beta}{\alpha + \beta} \frac{e^{-\lambda t}}{\lambda} \quad (A2.1)$$

Figure 2.15: Simplified model for the computation of the open time distributions where O_1 and O_2 are the open states from the model in Fig. 2.1 and CI is an absorbing, non-open state. The rate constants have the same definitions as in Fig. 2.1.

The solution of Eq. A2.1 has the form

$$\begin{bmatrix} P_{o1}(t) \\ P_{o2}(t) \end{bmatrix} = \begin{bmatrix} \frac{?}{?} \\ \frac{?}{?} \end{bmatrix} \exp(\mathbf{M}t) \quad (\text{A2.2})$$

where \mathbf{M} is the 2x2 matrix in Eq. A2.1. The matrix exponential can be computed using Laplace transforms and partial fraction expansions. The resulting equations for $P_{o1}(t)$ and $P_{o2}(t)$ are as follows:

$$P_{o1}(t) = \frac{?}{?} A \exp(?_1 t) + B \exp(?_2 t) + \frac{?}{?} C \exp(?_1 t) + D \exp(?_2 t) \quad (\text{A2.3})$$

$$P_{o2}(t) = \frac{?}{?} E \exp(?_1 t) + F \exp(?_2 t) + \frac{?}{?} G \exp(?_1 t) + H \exp(?_2 t) \quad (\text{A2.4})$$

where $?_1$ and $?_2$ are the eigenvalues of the matrix \mathbf{M} and the constants are as follows

$$A = \frac{?_1 - ?}{?_2 - ?_1} \quad (\text{A2.5})$$

$$B = \frac{? - ?_2}{?_2 - ?_1} \quad (\text{A2.6})$$

$$C = \frac{?}{?_2 - ?_1} \quad (\text{A2.7})$$

$$D = \frac{??}{?_2 - ?_1} \quad (\text{A2.8})$$

$$E = \frac{?}{?_2 - ?_1} \quad (\text{A2.9})$$

$$F = \frac{??}{?_2 - ?_1} \quad (\text{A2.10})$$

$$G = \frac{\lambda_1 \lambda_2 \lambda_{on}}{\lambda_2 \lambda_1} \quad (\text{A2.11})$$

$$H = \frac{\lambda_2 \lambda_1 \lambda_{on}}{\lambda_2 \lambda_1}. \quad (\text{A2.12})$$

The probability of leaving the open state and entering the absorbing, non-open state is then the solution to the equation:

$$\frac{dCI}{dt} = (\lambda_1 \lambda_{on})P_{O1}(t) - \lambda_2 P_{O2}(t) \quad (\text{A2.13})$$

where CI is the probability of occupying the absorbing state in Fig. 2.15. Integrating Eq. A2.13 gives the distribution of the times of entering the absorbing state, which is the complement of the distribution of the open times. The distribution of the open times is given by the equation:

$$P_{open}(t) = 1 - \frac{\lambda_1 (1 - \exp(-\lambda_1 t))(A \lambda_{on} + E)}{\lambda_1} - \frac{(1 - \exp(-\lambda_2 t))(B \lambda_{on} + F)}{\lambda_2} - \frac{\lambda_1 (1 - \exp(-\lambda_1 t))(C \lambda_{on} + G)}{\lambda_1} - \frac{(1 - \exp(-\lambda_2 t))(D \lambda_{on} + H)}{\lambda_2}. \quad (\text{A2.14})$$

CHAPTER 3:
MODULATED RECEPTOR AND ALLOSTERIC
EFFECTOR MODELS OF DRUG ACTION

INTRODUCTION

Several hypotheses have been formulated to explain the block of cardiac sodium channels by class I antiarrhythmic drugs. They are the guarded receptor (GR) hypothesis (Starmer, et al., 1984; Starmer and Grant, 1985; Starmer and Courtney, 1986; Starmer, 1987), the modulated receptor (MR) hypothesis (Hille, 1977; Hondeghem and Katzung, 1977), and the allosteric effector (AE) hypothesis (Balsler, et al., 1996). 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 as the channel changes state, while access to the receptor is constant. Drug can bind to any of three channel states (resting, activated, or inactivated), 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 (Balsler, et al., 1996). Drug binding reduces the Gibbs free energy for the inactivated channel conformation, thereby stabilizing it and rendering the channel unavailable to conduct ions. Drug causes channels to inactivate more readily and to remain

inactivated for an extended period of time (Balsler, et al., 1996). Therefore, the AE hypothesis also 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 reduced the slope of the charge-voltage relationship, from which they concluded gating of drug bound channels was 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 Hanck's experimental data.

Quantitative models have been formulated from the MR (Hille, 1977; Hondeghem and Katzung, 1977), the GR (Starmer, et al., 1984; Starmer and Grant, 1985; Starmer and Courtney, 1986; Starmer, 1987) and the AE (Balsler, et al., 1996) hypotheses, but no extensive, quantitative analysis of these models' ability to simultaneously reproduce a wide range of drug effects has been performed. In this chapter, models of lidocaine's action on the cardiac sodium channel are developed based on both the MR and AE hypotheses. 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 simultaneously 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 building improved antiarrhythmic drug models.

METHODS

Modulated Receptor Model

The MR hypothesis is tested using the Hondeghem-Katzung model (Hondeghem and Katzung, 1977). As shown in Fig. 3.1, 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 states in which drug is bound (RD, AD, and ID). Hondeghem and Katzung assume 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 ions (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 channel are taken from the DiFrancesco-Noble ventricular cell model (DiFrancesco and Noble, 1985). These equations apply to sodium channels at 37°C, but the experimental data describing lidocaine's effects are at 17°C. Therefore, to revise these equations for channels at 17°C, experimental data shows that the steady-state activation and inactivation curves should be shifted towards more negative potentials and the time constants of activation and inactivation should be increased (Colatsky, 1980; Murray, et al., 1990). The shifts in the steady-state activation and inactivation curves, the time constants of activation and inactivation, and the maximal sodium conductance are determined by fitting model responses to

Figure 3.1: The MR model (Hondegheem 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.

experimentally measured ionic currents at 17°C (provided by Wasserstrom, similar to (Sakakibara, et al., 1993). The resulting modified equations are as follows:

$$g_m = \frac{0.2(V - 48.8)}{1 - \exp[-0.1(V - 48.8)]} \quad (3.1)$$

$$g_m = 8 \exp[-0.056(V - 73.8)] \quad (3.2)$$

$$g_h = 0.02 \exp[-0.125(V - 88.6)] \quad (3.3)$$

$$g_h = \frac{2}{1 + 320 \exp[-0.1(V - 88.6)]} \quad (3.4)$$

$$g_m = \frac{1.2}{g_m + g_m} \quad (3.5)$$

$$g_h = \frac{3.9}{g_h + g_h} \quad (3.6)$$

The maximal sodium conductance, G_{Na} , is 0.085 μ S. These equations produce ionic currents that resemble those measured experimentally at 17°C. Figure 3.2A 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 3.2B compares the current-voltage relationships of the model and experimental data. The peak current values as well as the time courses of activation and inactivation are well fit by the model throughout the voltage range tested. Both the model and experimental current-voltage curves are fit using 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

Figure 3.2: Sodium currents at 17°C produced with Eqs. 3.1-3.6. 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.

the model, respectively. Although the model has a slightly steeper slope, the conductance and half-maximal voltages are similar. Thus, the modified DiFrancesco-Noble sodium current model reproduces currents at 17°C well.

Allosteric Effector Model

The AE hypothesis is tested using the Balser model (Balser, et al., 1996). In this model, binding and unbinding of drug are assumed to be faster than changes in the channel's state. Therefore, drug bound states are not explicitly included. 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 Balser model has as its basis a Markov model of the skeletal muscle sodium channel. In order to compare this model with a MR model, the underlying Markov model should be that of a cardiac sodium channel. Our implementation of the AE model, therefore, has as its basis the thirteen-state model of the cardiac sodium channel presented in Chapter 2 (Fig. 3.3). Drug effect is incorporated into the model by modifying the transition rates between the top and bottom rows (Balser, et al., 1996):

$$D_n \rightarrow C_n(1 - A_{on}[lidocaine]) \quad (3.7)$$

$$D_f \rightarrow C_f(1 - A_{off}[lidocaine]) \quad (3.8)$$

$$DO_n \rightarrow O_n(1 - A_{on}[lidocaine]) \quad (3.9)$$

Figure 3.3: The AE model (Balser, et al., 1996) based on the thirteen-state model of the cardiac sodium channel presented in Chapter 2. 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. 3.7-3.10.

$$DO_f \cdot O_f (1 + A_{off} [\textit{lidocaine}]) \quad (3.10)$$

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

Determination of the 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

$$\frac{d\mathbf{H}(t)}{dt} = \mathbf{W}\mathbf{H}(t) + \mathbf{B} \quad (3.11)$$

where $\mathbf{H}(t)$ is a vector containing the Hodgkin-Huxley variables m , h , and h' , \mathbf{W} is the transition matrix, and \mathbf{B} is a vector of constants. The experimental protocols can be divided into constant voltage segments. Therefore, \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 Balsler model, the probability of occupying any particular channel state can be written as a set of differential equations of the form of Eq. 3.11 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) and 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 versus the 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).

For the MR model, 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 (ΔV) are parameters to be determined by fitting model responses to experimental data. For the AE model, the scaling factors A_{on} and A_{off} are determined in the same manner. Parameters are determined using the simulated annealing algorithm described in Chapter 2.

RESULTS

Parameters of the MR model that reproduced individual data sets were determined first. The results are shown in Fig. 3.4. For each of the four data sets, parameters could be found for which the model response (○) fit the experimental data (○) well. Although each data set could be fit separately, the resulting rate constants could not be used to predict any of the other data sets. For example, Fig. 3.5 shows that the rate constants that yielded good fits to the use-dependence data did not yield good fits to the onset, recovery, and dose-response data. These results suggest that each data set is dependent on different combinations of parameters. For example, the use-dependence data are critically dependent on the ratio of drug binding and unbinding rates and not so dependent on the absolute value of each rate. In contrast, the onset data are critically dependent on the absolute value of the binding rates. Therefore, in order to develop a model that reproduces a range of drug effects and is well-constrained, more than one data set must be fit.

Parameters of the MR model that reproduced all four data sets simultaneously were then determined. This set of parameters provided a good fit of the model response (○) to all four data sets (○) (Fig. 3.4). The resulting shift in the steady-state inactivation curve (V) was 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 were 529 μ M, 9.8 μ M, and 32.6 μ M, respectively. The resting and inactivated state affinities were similar to

Figure 3.4: Comparison of experimental data (O) (Jia, et al., 1993, Furukawa, et al., 1995) and the 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 3.5: Comparison of experimental data (O) (Jia, et al., 1993, Furukawa, et al., 1995) and the MR model responses with parameters determined by fitting the use-dependence data only (\otimes). 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.

those reported by Bean (440 μ M and 10 μ M, respectively) (Bean, et al., 1983). However, the activated state affinity was 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., 1995a). 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). Better estimates of the activated state affinity can be obtained by disabling inactivation (Bennett, et al., 1995a), 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, which is not the case in the MR model.

Three possible explanations exist for the discrepancies in the model's activated state affinity and the model's shift of the steady-state inactivation curve. Despite use of methods to prevent it, the minimization algorithm could have converged on a local minimum, which could have been avoided by using a different starting point. However, even when a different starting point was chosen, the algorithm could not find a set of parameters having the correct activated state affinity. A second possibility was that the activation and shift parameters were ill conditioned by this choice of data sets. Drug binding and unbinding rate constants cannot be measured directly for the activated state because of the brief time a channel occupies

this state. However, any choice of data sets would be subject to this limitation. There was a greater possibility that the shift of the steady-state inactivation curve was not well constrained because the data sets used do not directly measure this shift. To test this hypothesis, the shift in the steady-state inactivation curve was held fixed at 10 mV and the drug binding and unbinding rate constants were determined using all four data sets simultaneously. The resulting parameters provided as good a fit of all four data sets as when the shift of the steady-state inactivation curve was not held constant (data not shown). The resulting affinities of the resting, activated, and inactivated states were 547 μ M, 6.2 nM, and 27.2 μ M, respectively. Once again, while the resting and inactivated state affinities were similar to those measured experimentally (Bean, et al., 1983), the affinity of the activated state was much too large (Bean, et al., 1983; Kodama, et al., 1990; Bennett, et al., 1995a). Therefore, while it was possible to find a set of parameters that reproduced all four data sets and the shift of the steady-state inactivation curve, the activated state affinity still differed from the experimentally measured value. Since neither of the previous explanations seemed adequate to explain the discrepancy in the activated state affinity, the only other possible explanation was that the mechanism by which the model reproduced the drug effect is not correct.

Additionally, the 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 clockwise

must be equal to the product of rate constants when traversing the same loop counterclockwise (Hille, 1992). All chemical reactions, including drug binding and unbinding, must satisfy microscopic reversibility. To examine microscopic reversibility in the MR model, the underlying Hodgkin-Huxley model is expanded to an eight-state Markov model (Fig. 3.6A) (Chay, 1991) and eight corresponding drug bound states are added (Fig. 3.6B). To maintain the spirit of the MR model, all resting states (R_1 , R_2 , and R_3) have the same drug binding and unbinding rates (K_R and L_R respectively) and all inactivated states (I_1 , I_2 , I_3 , and I_4) have the same drug binding and unbinding rates (K_I and L_I respectively) (Fig. 3.6C). Also, drug bound channels have their voltage dependence of inactivation shifted to more negative potentials (denoted by $\phi_{h'}$ and ϕ_h in Fig. 3.6B). In this model, there are three loops that do not automatically satisfy microscopic reversibility (Fig. 3.6C): 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:

$$\frac{K_A}{L_A} \gamma \frac{K_R}{L_R} \quad (3.12)$$

$$\frac{K_A L_I}{K_I L_A} \gamma \frac{\phi_{h'} \phi_h}{\phi_h \phi_{h'}} \quad (3.13)$$

$$\frac{K_R L_I}{K_I L_R} \gamma \frac{\phi_{h'} \phi_h}{\phi_h \phi_{h'}}. \quad (3.14)$$

Figure 3.6: Expanded 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. 3.1-3.4. 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.

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. 3.13 and 3.14. 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 exists for inactivated state block (Furukawa, et al., 1995). However, Eqs. 3.13 and 3.14 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. 3.12-3.14 to satisfy microscopic reversibility.

The ability of the expanded 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 . The remaining rates of drug binding and unbinding 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 could be found for which the model response (?) fit the experimental data (O) (Fig. 3.7). Parameters

Figure 3.7: Comparison of experimental data (O) (Jia, et al., 1993, Furukawa, et al., 1995) and the expanded MR model responses with microscopic reversibility satisfied by constraining K_R and K_A . Parameters were determined by fitting each data set individually (?) and by fitting all four data sets simultaneously (\otimes). 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. 3.4. 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.

of the MR model that reproduced all four data sets simultaneously were then determined. This set of parameters provided a reasonable fit of the model response (\ominus) to all four data sets (O) (Fig. 3.7). The resulting shift of the steady-state inactivation curve was 38.5 mV. The resulting affinities of the resting, activated, and inactivated states were 539 μ M (at -140 mV), 15 μ M (at -20 mV), and 93 nM, respectively. Even though the model satisfied microscopic reversibility and reproduced all four data sets simultaneously, the affinities of the activated and inactivated states and the shift of the steady-state inactivation curve all differed significantly from their experimentally measured values (Bean, et al., 1983; Kodama, et al., 1990; Bennett, et al., 1995a; 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 other 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 could be found that provided a good fit of all four data sets simultaneously (data not shown). These sets of rate constants yielded approximately the same affinities of the resting, activated, and inactivated states as when K_R and K_A were constrained by microscopic reversibility. Also, for each pair, setting the shift of the steady-state inactivation curve to 10 mV resulted in a model that did not fit all four data sets (data

not shown). Therefore, since microscopic reversibility must be satisfied for all chemical reactions, the inability of the 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 MR model, first, parameters of the AE model that reproduced each data set individually were determined. The results are striking and are summarized in Fig. 3.8. Only parameters that reproduced the dose-response curve could be found. These parameters were $A_{\text{on}}=8542 \text{ M}^{-1}$ and $A_{\text{off}}=1.0256 \text{ M}^{-1}$. Using the parameters that reproduced the dose-response curve and a lidocaine concentration of $200 \text{ }\mu\text{M}$, the steady-state inactivation curve was computed and compared to that in the absence of drug. Figure 3.9A shows that the AE model produced 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 were -104.2 mV and -12.5 mV for no drug and -116.5 mV and -14 mV for the AE model. Therefore, the model predicted that lidocaine shifts the steady-state inactivation curve by 10.7 mV , which agrees 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 using Eq. 2.12. Figure 3.9B shows the charge-voltage curves in the presence and absence of lidocaine. The AE model reduced the maximum charge slightly ($Q_{\text{max}}=0.958$) and significantly reduced the slope of the

Figure 3.8: 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. 3.4. The IC_{50} values were 120.3 μ M for the experimental data, 122.4 μ M for the AE model, and 120.6 μ M for the AE model plus an open drug bound state.

Figure 3.9: A. Steady-state availability curves for no drug (- - -) and for drug action modeled with the AE model (—). Parameters of the AE model were $A_{on} = 8542 \text{ M}^{-1}$ and $A_{off} = 1.0256 \text{ M}^{-1}$. 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.

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 were -75 mV and 19 mV, respectively. For the AE model, the corresponding values were -75.3 mV and 25.4 mV, respectively. Hanck and coworkers found that QX-222, a permanently positively charged form of lidocaine, reduced the maximum charge and shifted 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 the shift of the steady-state inactivation curve, was unable to reproduce the changes in the charge-voltage curve due to lidocaine.

The AE model failed to reproduce the use-dependence, onset of block, and recovery from block data. The best fit of the model to the experimental data produced the same amount of current for each pulse or at each time for both the use-dependence and onset protocols (Fig. 3.8). For the recovery protocol, the AE model produced a small increase in current during the first 100 ms, but then maintained this current for all longer recovery intervals. The AE model was therefore able to reproduce steady-state drug effects (ie., the dose-response curve and the shift of the steady-state inactivation curve), but was unable to reproduce drug effects that evolved over time.

It was hypothesized that the AE model could not reproduce the time-dependent drug effects because the model does not have explicit drug bound states. This lack of drug bound states results from the assumption, implicit in the AE model,

that drug binding and unbinding are significantly faster than channel state transitions. Therefore, drug effect is modeled by simply scaling transition rate constants by the drug concentration. As illustrated in Fig. 3.1, 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, some channels enter the drug bound states, in which the channels are non-conducting. Exit from these states (ie. drug unbinding) occurs more slowly than recovery from inactivation. With each depolarization, some channels bind drug. Since unbinding is slow, these channels tend to remain drug bound and thus are unavailable to conduct ions 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.

Therefore, 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 ions with the next pulse. Therefore, for each depolarization, the same number of channels are available to produce the sodium current. Channels are not accumulating in the inactivated states in the AE model and therefore, 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 parameters were determined by fitting each data set individually. The results of adding a drug bound state from the open state O_1 are shown in Fig. 3.8 (↵). By adding this single drug bound state, the fits to the use-dependence and recovery from block data were significantly improved. The dose-response curve was fit just as well as with the original AE model. The fit to the onset data was improved slightly, although the fit was not optimal. The fit was probably not optimal as a result of choosing to connect the drug bound state only to the open state. Fitting the onset data requires that drug binding increase as the clamp duration is increased. Drug binding cannot increase as the clamp duration is increased using this configuration,

because channels rapidly enter the inactivated state, from which they cannot bind drug.

Similar improvements in the fits to all four data sets were obtained using a closed drug bound state (C_4D) or an inactivated drug bound state (ID). One could argue that the fits were improved only because there were more free parameters. However, in the AE model, when the coupling between the open and inactivated states was assumed 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}), then the fits to the use-dependence, onset of block, and recovery from block data were not improved (data not shown). Therefore, having more free parameters was not an adequate explanation for the improved fit of the data sets. Thus, the AE model failed to reproduce the time-dependent drug effects because it lacks drug bound states and at least one drug bound state is needed to reproduce this data.

Although adding a single drug bound state to the AE model improved the fit to each data set individually, it did not produce an accurate drug model. Parameters for the AE model plus an open drug bound state were determined by fitting all four data sets simultaneously. As shown in Fig. 3.10, a set of parameters could not be found which yielded a good fit of the model response (\otimes) to all four data sets (O). For each data set, the parameters determined by fitting all four data sets simultaneously yielded a much worse fit to the experimental data than did the parameters determined by fitting each data set individually (?). Therefore, neither the

Figure 3.10: 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. 3.4. The IC_{50} values were 120.3 μ M for the experimental data, 120.6 μ M for parameters determined by fitting the dose-response data only, and 169.7 μ M for parameters determined by fitting all four data sets.

AE model nor the AE model plus a single drug bound state can fully reproduce the mechanism of drug action.

DISCUSSION

The 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 this model reproduces lidocaine's effect is not correct. Furthermore, as noted in the paper describing the MR model (Hondeghe and Katzung, 1977), this model does not satisfy microscopic reversibility. Hondeghe and Katzung claim 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. Therefore, the MR model does not correctly reproduce the mechanism of drug action in that it violates the law of conservation of energy and allows too much block in the activated state.

The 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 because 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 L_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, 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 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 flaw in the model. We suggest that the flaw is not in the MR hypothesis itself, but rather in the inability of the MR model to reproduce the mechanism of drug action.

Measurements of gating currents support the conclusion that the 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 interference in 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):

$$Q(V) = Nq_m m_\infty(V) \quad (3.15)$$

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 MR model assumes that drug binding does not affect activation and thus does not change $m_\infty(V)$ (Hondegheem and Katzung, 1977). Therefore, the MR 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 this model does not fully reproduce the mechanism of lidocaine's action.

While the MR model may not fully reproduce the mechanism of lidocaine's action, it reproduces more of lidocaine's effect than does the AE model. The AE model reproduces the dose-response curve and the shift of the steady-state

inactivation curve. It cannot reproduce use-dependence, onset of block, or recovery from block because it does not have explicit drug bound states. Drug bound states are needed so that when drug is bound, channels are unable to conduct for a longer time than when drug is not bound. When a single drug bound state is added to the AE model, the model reproduces time-dependent drug effects. Thus, to reproduce the mechanism of lidocaine's action, at least one drug bound state is needed.

The conclusion that drug bound states are needed to model lidocaine's action contradicts the conclusion drawn by the authors of the paper describing the AE model (Balsler, et al., 1996). The authors claim that AE models can reproduce the dose-response curve, while MR models cannot. They demonstrate this by examining the ability of two different MR models to reproduce the dose-response curve. They define response as the peak current produced on stepping from -120 mV to -20 mV for 20 ms. Their definition of response produces a tonic block dose-response curve, rather than a use-dependent block dose-response curve, as used in the present study. Tonic block is produced by drug binding to the resting and inactivated states. First, they examine a model in which four closed drug bound states and one open drug bound state are added to the sodium channel model and the affinity for lidocaine is assumed to increase as the channel progresses towards opening. This model produces a dose-dependent reduction in current, but the percent reduction in current is not correct for all drug concentrations. Although the authors do not give the rate constants for drug binding and unbinding to each of these states, examination of the

results suggests that the underlying behavior of the model is correct. With some adjustment of the rate constants, this model could perform as well as the AE model. 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 examine a model in which four closed-inactivated drug bound states and one inactivated drug bound state are added to the sodium channel model. The model is tested for an infinite lidocaine concentration and drug bound states are assumed to be absorbing. This model produces no reduction in peak current for large lidocaine concentrations. The authors therefore conclude that MR models with inactivated state block cannot reproduce the tonic block dose-response curve. However, the model may fail because of deficiencies in the underlying sodium channel model. Although not stated, it is assumed that at -120 mV all of the channels are 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. Therefore, the conclusion that MR models are incapable of reproducing the tonic block dose-response curve, and are thus inferior to AE models, is not well supported.

An additional criticism of the AE model is that drug binding and unbinding are assumed to be faster than the channel kinetics. This assumption produces a model in which there are no drug bound states and in which drug concentration changes immediately alter certain transition rates, as described by Eqs. 3.7-3.10. This assumption, however, contradicts experimental data. Bennett and coworkers measured the rate at which lidocaine block develops (Bennett, et al., 1995a). 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. Therefore, the assumption that drug binding is faster than the channel kinetics is false. If drug binding is not faster than inactivation, then one cannot neglect the kinetics of drug binding in developing a model of drug action.

While this chapter has focused on the shortcomings of the MR and AE models, it is important to acknowledge that each model provides information useful in building better models of antiarrhythmic drug action. The basic tenets of the MR model 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.

CHAPTER 4:
MODE-SWITCHING MODEL OF
LIDOCAINE'S ACTION

INTRODUCTION

The modulated receptor (MR) hypothesis states that the affinity of a receptor for drug changes as the channel changes state (Hille, 1977; Hondeghem and Katzung, 1977). In order to preserve energy balances while permitting state-dependent binding, this hypothesis requires gating of drug bound channels to be modified. Recent experiments show that for lidocaine, gating of drug bound channels is indeed altered. QX-222, a quaternary derivative of lidocaine, dramatically reduces the slope and maximum charge of the charge-voltage relationship (Hanck, et al., 1994). Lidocaine prevents bursting, and thus decreases the probability of occupying the slow-inactivating mode, and, as evidenced by an increase in null sweeps, increases the probability of occupying a “non-conducting” mode (Nilius, et al., 1987). Finally, lidocaine not only slows the rate of recovery from inactivation, it also prolongs the initial delay associated with deactivation before recovery occurs (Nuss, et al., 1995).

A quantitative model has been formulated from the MR hypothesis (Hondeghem and Katzung, 1977). As discussed in the previous chapter, this model reproduces a wide range of drug actions including use-dependence, rate of block onset, rate of recovery from block, and the dose-response curve. However, the model parameters that reproduce these data do not yield reasonable drug affinities for each state, do not preserve microscopic reversibility, and do not reproduce the changes in the charge-voltage relationship. We suggest that the model fails because it does not describe the mechanism of drug action.

Here the mechanism of drug action is proposed to be that of mode-switching. Experimentally, a single channel is said to exhibit different gating modes when the following criteria are satisfied (Nilius, 1988):

- (1) distinct patterns of channel openings and closings (referred to as gating schemes) coexist within the same recording;
- (2) transitions between these different gating schemes are slow;
- (3) chemical or physical agents exist which favor a gating scheme different from the normal scheme.

Although mode-switching has not been proposed previously to explain the effects of sodium channel blockers, it has been used to explain a variety of agent-induced changes in gating kinetics including: a) calcium-mediated inactivation of the calcium current (Imredy and Yue, 1994); b) the action of dihydropyridine calcium agonists and antagonists on L-type calcium channels (Hess, et al., 1984); and c) the effect of luteinizing hormone-releasing hormone on N-type calcium channels (Boland and Bean, 1993). As explained above, several experimental studies show that lidocaine induces changes in gating kinetics. Additionally, a study by Bennett and coworkers shows that for 25 μ M lidocaine, the time constant of drug block is 589 ms – roughly two orders of magnitude larger than that of any process associated with normal gating (Bennett, et al., 1995a). These observations support a mode-switching mechanism of action for lidocaine.

In this chapter, a mode-switching model of lidocaine's action is formulated. Lidocaine induces sodium channels to switch from the fast-inactivating mode (the normal mode) to another mode (the drug mode), distinct from the slow-inactivating mode, in which the channel gates, but fails to open. The drug mode is an additional set of states with altered kinetics that can be entered only in the presence of drug. Using the normal mode, the drug mode, and the transitions between them, a wide range of drug effects are reproduced and new understanding into the mechanism of lidocaine's action is gained.

METHODS

Model

Figure 4.1 shows the normal mode of channel gating (A), the drug mode of channel gating (B), and transitions between the two modes (C). The normal mode is the same as the model of the cardiac sodium channel discussed in Chapter 2. Transitions between modes (ie. drug binding and unbinding) are permitted only between the open states, the closed-inactivated states, and the inactivated state (Fig. 4.1C). The affinities of the closed states for drug are low. Thus, transitions between modes rarely occur from these states and closed states in the drug mode are rarely occupied. Therefore, transitions between modes from the closed states and closed-drug bound states are excluded from the model.

To minimize the number of free parameters, the rates of drug binding and unbinding to the closed-inactivated states are assumed to increase by a scaling factor

Figure 4.1: State diagram for the mode-switching model of lidocaine's action. *A.* The normal mode of channel gating occupied by non-drug bound channels. C_0 to C_4 are the closed states, O_1 and O_2 are the open states, C_0I to C_4I are the closed-inactivated states, and I is the inactivated state. Rate constants are as in Chapter 2. *B.* The drug mode of channel gating occupied by drug bound channels. D_1 and D_2 are the drug bound "open" states, C_0ID to C_4ID are the drug bound closed-inactivated states, and ID is the drug bound inactivated state. Rate constants are modified from those in the normal mode to preserve microscopic reversibility. *C.* Transitions between the normal and drug modes of gating. A channel can enter and exit the drug mode from the open states, the closed-inactivated states, and the inactivated state only. Each of these states has unique rates of drug binding and unbinding in accordance with the modulated receptor hypothesis (Hille, 1977; Hondeghem and Katzung, 1977). K and L are the rates of drug binding and unbinding, respectively, and g and h are scaling factors.

(g) with transition towards the inactivated state. Similarly, the rates of drug unbinding from the closed-inactivated states are decreased by a scaling factor (h) with transition towards the inactivated state. As a result of scaling the binding and unbinding rates, the drug affinities of the closed-inactivated states increase with transition towards the inactivated state.

To satisfy microscopic reversibility around the closed-inactivated-closed-inactivated-drug bound loop, the rate constants within the drug mode must be modified. Microscopic reversibility can be satisfied using two different modifications. Figure 4.1B shows the modifications that yield the better prediction of the charge-voltage curve. Using these modifications, forward rate constants are multiplied by the scaling factor h , while reverse rate constants are divided by the scaling factor g . Microscopic reversibility must also be satisfied around the open-inactivated-drug bound loop. The rate of inactivating from the “open”-drug bound state (D_I) is assumed to be a constant f times the rate of inactivation in the normal mode (O_n). The rate of reopening from the inactivated drug bound state is then

$$O_f^* = \frac{K_A L_I f O_f}{K_I L_A} \quad (4.1)$$

where K_A and L_A are the drug binding and unbinding rates, respectively, for the open states, K_I and L_I are the drug binding and unbinding rates, respectively, for the inactivated state, O_f is the rate of reopening from the inactivated state in the normal mode, and f is as defined above.

Determination of the Model Parameters

Parameters of the model are determined by fitting a variety of experimental data sets describing lidocaine's action. The probability of occupying any particular channel state is described mathematically by a set of ordinary differential equations. The equations and their solutions are as given in Eqs. 1.8 and 1.9. Five different data sets at a lidocaine concentration of 200 μ M (Furukawa, et al., 1995) and the dose-response curve (Jia, et al., 1993) are used to determine the model parameters. 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 and third data sets describe the rate of block onset due to a -80 mV and 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 fourth 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 fifth data set describes the steady-state availability curve in the presence of lidocaine. A 500 ms conditioning prepulse to a potential between -150 mV and -20 mV is applied. The current is then measured at 0 mV. Finally, the dose-response curve is defined as the fraction of unblocked sodium current elicited by the twentieth pulse of a 2 Hz train versus the

drug concentration. Pulses are from -140 mV to -20 mV for 10 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_A , K_I) and unbinding rates (L_R , L_A , L_I) and the scaling factors f , g , and h are parameters to be determined by fitting model responses to experimental data. The rate of drug binding to the closed-inactivated states (K_R) is determined by satisfying microscopic reversibility around the closed-inactivated-inactivated-drug bound loop according to the following equation:

$$K_R = \frac{K_I L_R}{L_I g^5 h^5}. \quad (4.2)$$

Parameters are determined using the simulated annealing algorithm described in Chapter 2.

RESULTS

Several sets of parameters were found that yielded good fits of model responses to experimental data. The parameters that gave affinities closest to the experimental affinities were chosen and are listed in Table 4.1. The resulting affinity of the inactivated state for lidocaine is $11 \mu\text{M}$. The inactivated state affinity is within the range of experimental values (10 to $27 \mu\text{M}$) (Bean, et al., 1983; Furukawa, et al., 1995; Nuss, et al., 1995). Affinities of the closed-

inactivated states for lidocaine range from 628 μ M for C₀I to 24 μ M for C₄I.

The closed-inactivated state (C₀I) has

Table 4.1

Parameter	Value
K_R	$1.6502 \text{ M}^{-1} \text{ ms}^{-1}$
L_R	$0.10367 \times 10^{-2} \text{ ms}^{-1}$
K_A	$4185.4 \text{ M}^{-1} \text{ ms}^{-1}$
L_A	0.09512 ms^{-1}
K_I	$3.8661 \text{ M}^{-1} \text{ ms}^{-1}$
L_I	$0.4154 \times 10^{-4} \text{ ms}^{-1}$
f	0.2899
g	1.9981
h	1.1292

Table 4.1: Parameters of the mode-switching model of lidocaine's action.

an affinity similar to that of the resting state in the literature (402 to 669 μM) (Bean, et al., 1983; Furukawa, et al., 1995; Nuss, et al., 1995). The model's open state affinity is 23 μM . This affinity is difficult to compare with experimental values. Bennett disabled inactivation by mutating three amino acids in the III-IV interdomain to glutamines and calculated the open state affinity to be 600 μM (Bennett, et al., 1995a). However, it is unclear whether the open state affinity is unchanged if inactivation is disabled. Another study estimates the open state affinity to be 47 μM , which is similar to the model's value (Furukawa, et al., 1995). Although the exact affinity of the open state is difficult to measure, experimental studies agree that it is less than that of the inactivated state (Bean, et al., 1983; Kodama, et al., 1990; Bennett, et al., 1995a;). Therefore, the affinities of the open, inactivated, and closed-inactivated states are similar to their respective experimental values.

The model is able to reproduce a wide range of experimental data. Figure 4.2 shows the model's fit to three of the data sets used to determine the model parameters. The model reproduces use-dependence (Fig. 4.2A), the rate of recovery from block at -140 mV (Fig. 4.2B), and the dose-response curve (Fig. 4.2C) well. The use-dependence and recovery curves are fit using a single exponential. For the use-dependence data, the time constants are 2.5 and 3 pulses for the experimental data and the model, respectively. For the recovery data, the time constants are 793 ms and 794 ms, respectively.

Figure 4.2: Comparison of the experimental data (O) and the model responses (\ominus) for three data sets used to determine the model's parameters. *A.* Use-dependence. Each curve is fit using a single exponential. The time constants are 2.5 and 3 pulses for the experimental data and the model, respectively. *B.* Recovery from block. Each curve is fit using a single exponential. The time constants are 793 ms and 794 ms for the experimental data and the model, respectively. *C.* Dose-response. The dose-response curves are fit using Eq. 4.3. The IC_{50} values are 166 μ M and 193 μ M for the experimental data and the model, respectively.

The dose-response curves are fit using a sigmoidal curve of the form:

$$\frac{I}{I_{\text{nodrug}}} = \frac{1}{1 + \frac{[\text{lidocaine}]}{IC_{50}}} \quad (4.3)$$

where the IC_{50} is the drug concentration at which the current is reduced by 50%. The IC_{50} values are 166 μM and 193 μM for the experimental data and the model, respectively.

Figure 4.3 shows the model's fit to the data sets describing the rate of block onset, which were also used to determine the model parameters. The rate of block development at both potentials is reproduced by the model. For -20 mV (Fig. 4.3A), the data is best fit using the sum of two exponentials. The time constants are 5.7 ms and 181 ms for the experimental data and 6.2 ms and 964 ms for the model, respectively. The fast time constants are similar, but the model's slow time constant is much too large. This time constant can be decreased by making the affinity of the open state greater than that of the inactivated state. However, such a change results in affinities that contradict the experimental data (Bean, et al., 1983; Bennett, et al., 1995a; Kodama, et al., 1990). For -80 mV (Fig. 4.3B), the data is fit using a single exponential. The time constants are 360 ms and 357 ms for the experimental data and the model, respectively.

The final data set used to determine the model parameters is steady-state availability. Figure 4.4A shows the steady-state availability curves in the absence of drug and in the presence of 200 μM lidocaine. Both curves are fit using a Boltzmann

Figure 4.3: Comparison of the experimental data (O) and the model responses (\ominus) for the rate of block development due to a conditioning pulse of varying duration. *A.* -20 mV conditioning pulse. The curves are fit using two exponentials. The time constants of the experimental data are 5.7 ms and 181 ms. The time constants of the model are 6.2 ms and 964 ms. *B.* -80 mV conditioning pulse. The curves are fit using a single exponential. The time constants of the experimental data and the model are 360 ms and 357 ms, respectively.

Figure 4.4: Simulated steady-state availability curves in the absence of drug (O) and in the presence of 200 μM (A) (\otimes) and 25 μM (B) (\otimes) lidocaine. The curves are fit using a Boltzmann function. In the absence of drug, the slope factor and half-maximal potential values are 12.5 mV and 104 mV . For 200 μM lidocaine, the slope factor and half-maximal potential values are 13 mV and 120 mV . For 25 μM lidocaine, the slope factor and half-maximal potential values are 13.1 mV and 111.5 mV .

function. The slope factor and half-maximal potential values are 12.5 mV and 104.2 mV for the experimental data and 13 mV and 120 mV for the model, respectively. The 200 μ M lidocaine concentration produces a 15.8 mV leftward shift of the steady-state inactivation curve without any significant change in the slope. This shift is similar to that measured experimentally (11 mV leftward) (Furukawa, et al., 1995). Figure 4.4B shows the steady-state availability curve in the presence of 25 μ M lidocaine. A Boltzmann fit to this curve yields slope factor and half-maximal potential values of 13.1 mV and 111.5 mV. The 25 μ M lidocaine concentration produces a much smaller shift of 7.2 mV. Thus, the model predicts that the shift in the steady-state availability curve is a function of drug concentration. This prediction is supported by experimental data which shows that the shift of the steady-state availability curve increases as the lidocaine concentration is increased (Bean, et al., 1983).

In addition to being able to reproduce all of the data used to determine the model parameters, the model can predict numerous data sets not used in the fitting procedure. Figure 4.5 shows the model's ability to predict the rate of block development for use-dependent protocols of different frequencies and pulse durations of 3 ms (Fig. 4.5A) and 10 ms (Fig. 4.5B). As the interstimulus interval (ISI) is decreased, it takes more pulses to reach steady-state and more block is produced. For any ISI, a longer pulse duration results in more block. These results can be compared to those of Furukawa and coworkers (Fig. 6b in Furukawa, et al., 1995). The number

Figure 4.5: The rate of development of use-dependent block for different interstimulus intervals (ISI) and pulse durations of 3 ms (*A*) and 10 ms (*B*) simulated with the mode-switching model. Pulses are from -140 mV to -20 mV. As the ISI is decreased, it takes more pulses to reach steady-state and more block is produced. For any ISI, a longer pulse duration results in more block.

of pulses needed to reach steady-state and the steady-state block for different ISIs are very similar in the model and the experimental data. However, there are some minor differences. At low frequencies, the model requires a few more pulses to reach steady-state than is shown by the experimental data. The model also predicts too little block at high frequencies for 10 ms pulse durations. Nevertheless, the ability of the model to predict use-dependence data for a range of frequencies and pulse durations provides evidence of the model's accuracy and supports a mode-switching mechanism of action for lidocaine.

Another data set predicted by the model is the reduction in sodium current due to lidocaine. Figure 4.6A compares current tracings for a clamp voltage of -30 mV in the absence of lidocaine and in the presence of 25 μ M and 200 μ M lidocaine. Lidocaine reduces the peak current without changing the kinetics of activation or inactivation. At this clamp potential, 25 μ M lidocaine reduces the peak current by 5.9%; 200 μ M lidocaine reduces the peak current by 34.8%. Furukawa and coworkers found that 200 μ M lidocaine reduced the peak current by $24.5 \pm 2.9\%$ (Furukawa, et al., 1995). Thus, the model predicts slightly more reduction in peak current at -30 mV than the experimental data shows. Figure 4.6B shows the current-voltage curves in the absence of lidocaine and in the presence of 25 μ M and 200 μ M lidocaine. Lidocaine reduces the magnitude of the current at each potential without altering the shape of the curve. Each curve is fit using a modified Boltzmann function. In the absence of lidocaine, the conductance (G_{Na}), slope factor (s), and

Figure 4.6: *A.* Comparison of simulated sodium current tracings at -30 mV in the absence of lidocaine (---) and in the presence of 25 μ M (- - -) and 200 μ M (??) lidocaine. *B.* Normalized current-voltage curves in the absence of lidocaine (O) and in the presence of 25 μ M (??) and 200 μ M (??) lidocaine simulated with the mode-switching model. Curves are the best fits to a modified Boltzmann function. The conductance, slope factor, and half-maximal potential are 0.0154 mV^{-1} , 7.0 mV, and -47.1 mV, respectively, in the absence of drug. For 25 μ M lidocaine, the conductance, slope factor, and half-maximal potential are 0.0147 mV^{-1} , 7.1 mV, and -46.6 mV and for 200 μ M lidocaine, the respective values are 0.0109 mV^{-1} , 7.6 mV, and -44.2 mV.

half-maximal potential ($V_{0.5}$) values are 0.0154 mV^{-1} , 7.0 mV , and -47.1 mV , respectively. For $25 \text{ } \mu\text{M}$ lidocaine, there is little change in these values; G_{Na} is 0.0147 mV^{-1} , s is 7.1 mV , and $V_{0.5}$ is -46.6 mV . For $200 \text{ } \mu\text{M}$ lidocaine, G_{Na} is 0.0109 mV^{-1} , s is 7.6 mV , and $V_{0.5}$ is -44.2 mV . Thus, for $200 \text{ } \mu\text{M}$ lidocaine, the model predicts a 29.2% reduction in conductance, a 0.6 mV increase in slope factor, and a 2.9 mV rightward shift in the half-maximal potential. The experimental data shows a $22.6 \pm 1.7\%$ decrease in conductance for $200 \text{ } \mu\text{M}$ lidocaine (Furukawa, et al., 1995). Thus, the model predicts a slightly larger decrease in conductance. The change in slope factor and half-maximal potential are small and are close to the range of variation observed experimentally. Therefore, the model agrees with the experimental data in predicting no significant change in the slope factor or half-maximal potential (Furukawa, et al., 1995). Thus, the mode-switching model can predict the reduction in current due to lidocaine for a large voltage range.

Additionally, the model predicts the experimentally observed changes in the charge-voltage curve due to lidocaine. Block is developed by applying a 130 pulse train from -150 mV to 0 mV at 10 Hz . After a 90 ms interval at -150 mV , a test pulse is applied and the gating charge is computed. Figure 4.7A compares the charge-voltage curves in the absence of drug and in the presence of $200 \text{ } \mu\text{M}$ lidocaine. A Boltzmann function is fit to each curve. In the absence of drug, the maximum charge is normalized to one and the slope factor and half-maximal potential values are 19 mV and -75 mV , respectively. In the presence of drug, the

Figure 4.7: Simulated charge-voltage curves in the absence of lidocaine (O) and in the presence of 200 μM (A) (●) and 25 μM (B) (●) lidocaine. The curves are fit using a Boltzmann function. In the absence of drug, the maximum charge, slope factor, and half-maximal potential values are 1, 19 mV, and -75 mV. For 200 μM lidocaine, the maximum charge, slope factor, and half-maximal potential values are 0.587, 22.3 mV and -92.1 mV. For 25 μM lidocaine, the maximum charge, slope factor, and half-maximal potential values are 0.84, 21.8 mV and -78.5 mV.

maximum charge moved is a fraction of the charge moved in the absence of drug and is a parameter to be determined when fitting the Boltzmann function. For 200 μ M lidocaine, the maximum charge is 0.587 and the slope factor and half-maximal potential values are 22.3 mV and -92.1 mV, respectively. Therefore, lidocaine reduces the amount of charge moved by 41.3%, reduces the slope of the charge-voltage curve, and shifts the half-maximal potential 17.1 mV leftward. Experimental data suggest that the lidocaine concentration at which the maximum charge is reduced by 50% is 200 μ M (Hanck, et al., 1992). In addition, although no data exist on the change in the slope factor and half-maximal potential of the charge-voltage curve for 200 μ M lidocaine, studies using 1 mM QX-222, a quaternary derivative of lidocaine, show almost a twofold reduction in slope factor and a leftward shift of the half-maximal potential of 27 mV (Hanck, et al., 1994). Therefore, the model's predictions of lidocaine's effect on the charge-voltage curve are similar to experimental data in the literature. Furthermore, experimental data suggest that the change in the charge-voltage curve is concentration-dependent (Hanck, et al., 1994; Josephson and Cui, 1994). Figure 4.7B shows the charge-voltage curve for 25 μ M lidocaine. The maximum charge, slope factor, and half-maximal potential values from a Boltzmann fit are 0.840, 21.8 mV, and -78.5 mV, respectively. Thus, at this lidocaine concentration, there is only a 26% reduction in charge and a leftward shift of the half-maximal potential of 3.5 mV. The model's prediction of a 26% reduction in charge for 25 μ M lidocaine is similar to the experimental data, which show a 24% reduction

in charge for 20 μ M lidocaine (Josephson and Cui, 1994). There are no data at this lidocaine concentration on the shift of the half-maximal potential. However, studies done with various concentrations of QX-222 show that the maximum charge is reduced and the slope factor and shift of half-maximal potential are increased as the drug concentration is increased (Hanck, et al., 1994). Therefore, the mode-switching model accurately predicts the changes in the charge-voltage curve due to various lidocaine concentrations.

Finally, the mode-switching model can predict how single channel behavior is altered in the presence of lidocaine. Twelve hundred channels are simulated for a 40 ms sweep using a Monte Carlo method described by Clay and DeFelice (Clay and DeFelice, 1983). Open durations and the probability of a null sweep are examined for a voltage range of -70 mV to -10 mV. Figures 4.8A and 4.8B show the open duration densities for a clamp voltage of -50 mV in the absence of drug and in the presence of 200 μ M lidocaine. In the absence of drug, most openings are less than 2 ms, although several openings are much longer. In the presence of 200 μ M lidocaine, the openings are shorter and there are almost no long openings. The reduction in long openings due to lidocaine is similar to that seen in single channel experiments (Nilius, et al., 1987). Fitting the open duration densities with a single exponential yields time constants of 0.62 ms and 0.42 ms for the no drug and 200 μ M lidocaine data, respectively. For 200 μ M lidocaine, the open durations are reduced throughout the voltage range tested. The greatest reduction occurs for voltages between -40 mV and

Figure 4.8: Simulated single channel open duration densities at -50 mV in the absence of drug (A) and in the presence of $200 \mu\text{M}$ lidocaine (B). Twelve hundred channels are simulated for 40 ms as described in the text. Bin size is 0.5 ms. The histograms are fit using a single exponential. The time constants are 0.615 ms and 0.421 ms for no drug and $200 \mu\text{M}$ lidocaine, respectively.

-60 mV, inclusive, where time constants fit to the open duration densities are reduced by approximately 30%. At these potentials, inactivation is relatively slow and channels often close and reopen before they inactivate. The presence of lidocaine provides an additional relatively fast path by which the channel can leave the open state and thus reduces the open duration. At more depolarized potentials, however, channels tend to inactivate quickly. Fewer channels bind lidocaine before inactivating and thus there is less reduction in open durations. For 25 μ M lidocaine, there is very little change in open durations throughout the voltage range tested. Experimental data show that lidocaine does produce a small reduction in open durations, although these studies have been done at low drug concentrations and the concentration-dependence of this reduction is not known (Nilius, et al., 1987; Bennett, et al., 1995a;). Close inspection of Fig. 4.8 also shows that the number of openings is reduced in the presence of 200 μ M lidocaine. The probability of a null sweep is increased an average of 10% across the voltage range tested. These results are similar to those of the experimental data, which show that lidocaine increases the number of null sweeps (Nilius, et al., 1987). Therefore, the mode-switching model not only reproduces well all of the data used to determine its parameters, but also predicts a large range of whole-cell and single channel data not used in the fitting procedure.

DISCUSSION

A mode-switching model of lidocaine's action on cardiac sodium channels is presented. The model is based on the observation that in the presence of drug, there is a population of drug bound channels and a population of non-drug bound channels. The drug concentration and the rate of drug binding and unbinding determine the relative size of each population and the observed effect of the drug. Rates of drug binding and unbinding are unique for each state of the channel in accordance with the modulated receptor hypothesis (Hille, 1977; Hondeghem and Katzung, 1977). The drug bound and non-drug bound channels are each represented by a mode of channel gating. To be classified as a mode-switching model, each mode must exhibit distinctly different gating and the transitions between modes must be slower than transitions within each mode (Nilius, 1988). The model presented satisfies these criteria. The dramatic reduction in maximum gating charge and the shift of gating to more negative potentials both provide evidence of a mode entered in the presence of lidocaine with gating kinetics different from those of the normal mode. The fastest transitions between modes are those between the open and drug bound "open" states. These transitions have a rate constant of less than 1 ms^{-1} for drug concentrations less than or equal to $200 \text{ } \mu\text{M}$. From Chapter 2, rate constants governing activation and inactivation are generally no smaller than 1 ms^{-1} . Thus, the model satisfies the criteria of mode-switching because the transitions between modes are slow and the drug mode exhibits distinctly different gating kinetics.

Several models of lidocaine's action on sodium channels have been formulated. They are the Hondeghem-Katzung modulated receptor model (Hondeghem and Katzung, 1977), the Starmer guarded receptor model (Starmer, et al., 1984), and the Balser allosteric effector model (Balser, et al., 1996). As shown in Chapter 3, only the Hondeghem-Katzung model has been shown to reproduce a number of drug effects including use-dependence, the rate of block onset, the rate of recovery from block, and the dose-response curve. However, the model parameters that reproduce these data do not yield reasonable drug affinities for each state and do not preserve microscopic reversibility.

The mode-switching model presented here improves on the Hondeghem-Katzung modulated receptor model. The mode-switching model not only reproduces all of the data reproduced by the Hondeghem-Katzung model, but also predicts data, such as the change in the charge-voltage curve, that the Hondeghem-Katzung model cannot predict. The mode-switching model has only one more parameter than the Hondeghem-Katzung model and thus its ability to predict more data is not due to a dramatic increase in the number of parameters. In addition, the mode-switching model has reasonable drug affinities for each state and preserves microscopic reversibility. Finally, and most importantly, the mode-switching model does not simply provide a mathematical means of reproducing drug effects, it describes a mechanism of drug action.

The power of reproducing the mechanism of drug action is clearly seen in the mode-switching model's ability to reproduce the shift in the steady-state inactivation

curve and the change in the charge-voltage curve due to lidocaine. Previous models of drug action have reproduced the shift in the steady-state inactivation curve by directly altering the rate of inactivation (Hondegem and Katzung, 1977; Balsler, et al., 1996). For the mode-switching model, though, the shift in the steady-state inactivation curve is produced as a result of the increase in the affinities of the closed-inactivated states for lidocaine with transition towards the inactivated state. At very negative potentials, few channels occupy the closed-inactivated states, there is little drug binding, and therefore, there is no difference in the steady-state availability curves. As the holding potential is increased, more channels enter the closed-inactivated states and drug binding occurs. Channels return to the normal mode slowly, so fewer channels are available than in the absence of drug. With further increases in membrane potential, channels begin to occupy the closed-inactivated states having greater drug affinity and there is an even greater decrease in channel availability.

The reduction in gating charge is also an inherent feature of the mode-switching model. It is produced simply by excluding certain transitions from the drug bound mode and by altering the rates of transition within the drug mode so as to preserve microscopic reversibility. No changes in the valences are needed to reproduce the changes in the charge-voltage curve. This finding contradicts Hanck's suggestion that gating charge is reduced as a consequence of a reduction in the valence of transitions between drug bound states (Hanck, et al., 1994). Instead, the model suggests that lidocaine interacts with the voltage sensors to slow the channel's

entry into certain states and to prevent its entry into other states, thereby altering the charge-voltage curve. Molecular studies to determine how lidocaine interacts with various amino acid residues of the channel are currently being performed. No study has yet determined whether there is an interaction between lidocaine and the voltage sensors. However, it has been shown that lidocaine binds in the pore of the channel (Ragsdale, et al., 1994; Ragsdale, et al., 1996) and, from studies on the *Shaker* potassium channel, that residues in the pore interact with residues in the S4 voltage-sensing segment (Cha and Bezanilla, 1998; Loots and Isacoff, 1998).

Although the mode-switching model improves on existing models of drug action, there are some sources of uncertainty in the model's parameters. As noted in RESULTS, several sets of parameters could be found which yielded good fits of the model responses to experimental data. While the affinities of the closed-inactivated and inactivated states were similar in all sets of parameters, the affinity of the open state varied significantly. Parameters were chosen so that the open state affinity was significantly less than that of the inactivated state in accordance with experimental data (Bean, et al., 1983; Kodama, et al., 1990; Bennett, et al., 1995a). However, it is not known by how much the open state affinity differs from that of the inactivated state because there are no good estimates of the open state drug affinity. Estimates range from 47 μ M to 600 μ M depending on the method of calculation (Bennett, et al., 1995a; Furukawa, et al., 1995). Better estimates of the open state drug affinity would

help to limit the number of possible solutions and would reduce the uncertainty in the model's parameters.

The mode-switching model reproduces a wide range of drug effects by postulating a mechanism of drug action. Such a model has many potential applications. It can be used to study lidocaine's effect on various arrhythmias and diseases such as the long-QT syndrome. In addition, since the mechanism of action of all class I antiarrhythmic drugs is thought to be similar (Hille, 1977; Hondeghem and Katzung, 1977), mode-switching models can be built for other antiarrhythmic drugs and a quantitative comparison of these drugs for the treatment of various arrhythmias can be performed. Using quantitative models to study drugs will not only provide more insight into the mechanisms of drug action, but will also lead to the development of more effective antiarrhythmic drugs.

CHAPTER 5:
CONCLUSION

Summary and Significance

The preceding chapters have discussed the formulation of a model of the cardiac sodium channel and the use of this model to study lidocaine's action. The cardiac sodium channel model is a thirteen-state Markov model with voltage and temperature dependent rate constants. It reproduces a wide range of both whole-cell and single channel experimental data for a temperature range of 10°C to 25°C. The model reproduces more experimental data than any of the previous models of this channel. In addition, unlike previous models that have empirical rate constants, the Markov model presented here has rate constants with physical meaning that are based on the thermodynamic principles of Eyring rate theory.

The formulation of such a model not only is significant because it improves on models of the cardiac sodium channel, but also because the methods used to develop it can serve as a template for building models of other channels. All channels are thought to have a similar structure and thus a similar function. They all have a pore that is formed from individual subunits or from repeated motifs within a single subunit. They all undergo changes in their ability to conduct ions. Some channels regulate access to the pore in response to ligand binding, while others regulate access to the pore in response to changes in membrane potential. Further similarities are seen within the subclass of voltage-gated channels. All voltage-gated channels have four alpha-helical segments containing many positively charged amino acids that are thought to act like voltage sensors. A change in membrane potential causes these

positively charged amino acids to move a fractional distance through the membrane producing channel activation. In addition to activation, most voltage-gated channels also undergo inactivation. Inactivation is the result of some part of the channel entering the pore and blocking it from the outside and/or the inside.

The similarities between sodium channels and other voltage-gated channels, such as potassium and calcium channels, suggest that insights gained into modeling a specific channel are generally applicable to modeling other voltage-gated channels. The state diagram for the cardiac sodium channel model is based on generalizations of channel structure that apply to all voltage-gated channels. Other channels may require different numbers of closed, open, and inactivated states, but models of voltage-gated channels will all have such states. The form of the rate constants will also be similar among all models of voltage-gated channels. The rate constants of the cardiac sodium channel model are exponential functions of enthalpy, entropy, and voltage. They are formulated based on the idea that significant energy barriers exist between favorable conformations of the channel. The rate of transition between states is the height of the energy barrier plus the additional energy required to move the voltage sensors in the membrane's electric field. All channels have energy barriers between favorable conformations and all voltage-gated channels have voltage sensors. Therefore, this form of the rate constants can be used to develop a model of any voltage-gated channel. Finally, a procedure and experimental data sets similar to those used to develop the cardiac sodium channel model can be used to develop models of other voltage-gated channels. Experimental data were chosen to be included in the

fitting procedure based on their potential to constrain certain rate constants. The ability of the cardiac sodium channel model to predict data that was not fit demonstrates that the amount of experimental data used to constrain the rate constants was sufficient. Similar experimental data should be sufficient for developing models of other voltage-gated channels, because these channels have similar state diagrams and rate constants and the transitions between states can be elicited using similar protocols. Therefore, the development of the cardiac sodium channel Markov model provides a basis for developing detailed kinetic models of all voltage-gated channels.

The development of more models of channel gating will aid our understanding of the structure-function relationship. The structure-function relationship is being studied experimentally in a variety of ways. Some researchers mutate or delete certain amino acids in cloned channels and then study the behavior of the mutant channel. This technique is useful when the mutation results in a change in the probability of channel opening. To study changes in channel conformation that do not result in a change in the probability of channel opening, other researchers have used voltage clamp fluorimetry. Modeling provides insight into not only the type and number of channel conformations that exist, but also into how each of these channel conformations interact to produce the complex gating behavior observed. It can help to guide experiments by suggesting certain changes in channel conformation for which to look. It can also help to analyze and interpret the results of such experiments. The knowledge gained as the result of studying channel structure-function relationships can then be used to develop even more detailed models.

Besides using channel models to help elucidate the structure-function relationship, models can be used to study drug action. Chapter 3 has described the analysis of two models of drug action, a modulated receptor model and an allosteric effector model. The modulated receptor model can reproduce use-dependence, onset of block, recovery from block, and the dose-response curve, but does not yield reasonable drug affinities for each state and does not preserve microscopic reversibility. The allosteric effector model can reproduce the dose-response curve and the drug-induced shift of the steady-state inactivation curve, but none of the time-dependent drug effects.

The analysis presented here is the first quantitative comparison of these models' ability to simultaneously reproduce a number of lidocaine's actions. This analysis can be extended to all class I antiarrhythmic drugs (ie. sodium channel blockers). Sodium channel blockers all appear to: a) shift the voltage dependence of inactivation to more negative potentials; b) exhibit use-dependence; and c) exhibit voltage dependent recovery from block (Hondeghe and Katzung, 1977). In fact, the similarity of these drugs' actions prompted Hille, Hondeghe, and Katzung to formulate the modulated receptor hypothesis. In addition, site-directed mutagenesis experiments have determined that all class I antiarrhythmic drugs probably have the same binding site (Ragsdale, et al., 1996; Ragsdale, et al., 1994). Therefore, the mechanism of drug action appears to be similar for all class I antiarrhythmic drugs. A model that cannot reproduce the mechanism of drug action for lidocaine will not be

capable of reproducing the mechanism of drug action for quinidine, flecainide, or other sodium channel blockers.

The inability of the modulated receptor and allosteric effector models to adequately reproduce the mechanism of drug action demonstrates the need for a better model. Chapter 4 has discussed the development of a mode-switching model of lidocaine's action. Lidocaine causes channels to switch from their normal gating mode to another mode, in which channels gate, but fail to open. The model implements the modulated receptor hypothesis in that the rates of drug binding and unbinding (ie. transitions between modes) are unique for each state of the channel. The mode-switching model reproduces use-dependence, onset of block, recovery from block, the dose-response curve, the steady-state inactivation curve, and the charge-voltage curve. It improves on the Hondeghem-Katzung modulated receptor model, because it reproduces more experimental data, has reasonable drug affinities for each state, and preserves microscopic reversibility. Most importantly, unlike the Hondeghem-Katzung model, which simply provides a mathematical means of reproducing drug effects, the mode-switching model describes a mechanism of drug action.

This work is the first to suggest a mode-switching mechanism of action for an antiarrhythmic drug. Mode-switching has previously been used to explain calcium-mediated inactivation of the calcium current (Imredy and Yue, 1994), the action of dihydropyridine calcium agonists and antagonists (Hess, et al., 1984), and the effect of luteinizing hormone-releasing hormone (Boland and Bean, 1993). As discussed

previously, the mechanism of action of all class I antiarrhythmic drugs is thought to be similar. Therefore, mode-switching probably also describes the mechanism of action of quinidine, flecainide, and other sodium channel blockers. However, a mode-switching mechanism of action is probably not limited to antiarrhythmic drugs. Local anesthetics, like procaine and etidocaine, and anticonvulsants, like phenytoin and carbamazepine, are thought to have a mechanism of action similar to that of antiarrhythmic drugs. They all shift the voltage dependence of the steady-state inactivation curve towards more negative potentials, exhibit voltage and frequency-dependent block, and preferentially bind to open and/or inactivated channels (Butterworth and Strichartz, 1990; Catterall, 1987; Rogawski and Porter, 1990). They are all thought to have a common receptor site within the pore of the sodium channel (Ragsdale, 1996). Therefore, mode-switching is not only the mechanism of lidocaine's action, but is also probably the mechanism of action of many drugs on voltage-gated sodium channels.

Models of drug action can help to guide experiments designed to further characterize the receptor site and the interactions of the drug, receptor, and other parts of the channel. For example, the mode-switching model suggests that lidocaine interacts with the voltage sensors to slow the channel's entry into certain states and to prevent its entry into other states. There have not yet been any reports of such an interaction. However, the tools exist by which such an interaction could be studied. The model provides an incentive for such studies to be done.

A better understanding of drug-receptor-channel interactions will lead to better structure-activity relations. It is known that the rate of block development for amide-linked drugs is related to their lipid solubility and that ether-linked drugs having equivalent solubility develop block faster (Courtney, 1987). It is also known that the unblocking rate is fastest for small drugs with high lipid solubility (Courtney, 1987). However, few studies have been done to correlate specific chemical groups with drug binding and activity. After the components of the receptor site have been determined, studies to determine the relationship between chemical groups, binding, and drug activity will be much easier. Such studies will provide the data necessary to do rational drug design. Drugs can then be synthesized that contain the chemical groups necessary for a certain potency and effect. Left to determine will be what effect is needed to treat specific diseases and arrhythmias.

Future Directions

There are many potential applications of the cardiac sodium channel and lidocaine models, some of which have been mentioned already. One of the most promising applications is the use of these models to study diseases and their treatments. Two diseases are known to result from mutations of the cardiac sodium channel, idiopathic ventricular fibrillation (Chen, et al., 1998) and the long-QT syndrome (LQTS).

LQTS is defined on an electrocardiogram as an abnormally long interval from the start of the QRS complex to the end of the T wave. A rate-corrected QT interval

of $460 \text{ ms}^{1/2}$ is highly suggestive of LQTS (Ackerman, 1998). Patients with LQTS often complain of fainting spells or seizures, particularly when under physical or emotional stress. If undiagnosed or untreated, these patients have a 50% chance of sudden death due to ventricular arrhythmia within ten years (Ackerman, 1998). Current treatments for LQTS include β -adrenergic blockers, implantation of a pacemaker or defibrillator, or left cervicothoracic sympathetic ganglionectomy. However, the effectiveness of these treatments varies widely among patients and even with treatment, an 8-10% chance of sudden death within five years remains (Ackerman, 1998).

Five mutations of the cardiac sodium channel that produce LQTS have been identified and characterized: β KPQ, R1644H, N1325S, D1840G, and R1623Q (Bennett, et al., 1995b; Wang, et al., 1995a; Wang, et al., 1995b; Dumaine, et al., 1996; Wang, et al., 1996b; An, et al., 1998; Kambouris, et al., 1998a; Kambouris, et al., 1998b). They all cause LQTS by producing a sustained, non-inactivating sodium current. The mechanism by which the current is sustained, however, differs depending upon the precise mutation (Dumaine, et al., 1996). The most severe mutation is thought to be β KPQ. This mutation increases the number of channel reopenings and long openings (bursts) (Dumaine, et al., 1996). β KPQ mutants exhibit faster onset of inactivation and recovery from inactivation than wild-type channels (An, et al., 1996; Wang, et al., 1996b). In addition, their steady-state activation and inactivation curves are shifted towards more positive potentials (Wang, et al., 1996b).

The Δ KPQ mutation occurs in the III-IV interdomain, a region of the channel thought to block the pore during inactivation. The Δ KPQ mutation is thus hypothesized to destabilize the interaction between the inactivation particle and its docking site allowing inactivation to be readily reversible (Bennett, et al., 1995b; Wang, et al., 1996b).

The cardiac sodium channel model can be used to study the LQTS. An approximation of the Δ KPQ mutation is introduced into the model by making the I \rightarrow O transition 150 times larger and the CI \rightarrow C transitions 1.25 times larger. The changes in the CI \rightarrow C transitions are necessary to produce a shift in the steady-state inactivation curve. As shown in Fig. 5.1, these changes produce a persistent current that is 2.46% of the peak current for a clamp potential of -20 mV. This amount of persistent current is similar to the experimental value of $2.57 \pm 0.27\%$ for the same mutation and clamp potential (Wang, et al., 1996b). The amount of persistent current is a function of clamp potential; it decreases from 6.91% at -40 mV to 0.95% at 40 mV. The experimental data show a similar decrease from 5% at -40 mV to 2% at 40 mV (Wang, et al., 1996b). Figure 5.2 shows that this approximation of the Δ KPQ mutation produces a rightward shift of the steady-state inactivation curve. A Boltzmann fit to the wild-type (WT) and Δ KPQ curves yields slope factor and half-maximal potential values of -15.2 mV and -101.7 mV for the WT channel and -15.9 mV and -98 mV for the mutant channel, respectively. Thus, the curve is shifted by 3.7 mV to the right, which is similar to the 3.6 mV rightward shift observed

Figure 5.1: Simulated sodium current tracings for the wild-type (WT) ($\alpha_1\beta_1$) and $\alpha_1\beta_1$ KPQ ($\alpha_1\beta_1$) channels for a 200 ms voltage-clamp at -20 mV. The persistent current for the $\alpha_1\beta_1$ KPQ mutation is 2.46% of the peak current.

Figure 5.2: Steady-state availability curves for the wild-type (O) and Δ KPQ (\ominus) channels. Curves are the best fits of a Boltzmann function where the slope factor and half-maximal potential values are -15.2 mV and -101.7 mV for the WT channel and -15.9 mV and -98 mV for the mutant channel, respectively.

experimentally (Wang, et al., 1996b). Finally, Fig. 5.3 shows that this approximation of the Δ KPQ mutation slightly increases the rate of recovery from inactivation. The WT and Δ KPQ curves are fit using a single exponential; the time constants are 9.28 ms and 8.39 ms, respectively. The experimental data show a larger increase in the recovery rate; the time constant of the mutant channel is two to three times smaller than that of the WT channel (Wang, et al., 1996b).

Since LQTS mutations of the sodium channel result in too much current, class I antiarrhythmic drugs have been used to block the channels and thus reduce the current. To be an effective treatment, however, these agents must preferentially block the sustained component of the current. Studies done with lidocaine and mexiletine show that block is indeed greater for the sustained current produced by the Δ KPQ mutants than for the peak currents (An, et al., 1996; Wang, et al., 1997).

The mode-switching model of lidocaine's action coupled with the approximation of the Δ KPQ mutation can reproduce these experimental results. Figure 5.4 shows the percent reduction in both peak current and persistent current for a range of lidocaine concentrations. The model predicts that at all lidocaine concentrations the persistent current will be reduced more than the peak current. Thus, the model agrees with the experimental data (An, et al., 1996; Wang, et al., 1997). The model also predicts that larger lidocaine concentrations will produce a much greater difference in the percent reduction of persistent versus peak current. An and coworkers suggest that mutant channels are blocked by lidocaine with higher

Figure 5.3: Rates of recovery from inactivation for the wild-type (O) and Δ KPQ (\ominus) channels. The membrane potential is stepped from -120 mV to 20 mV for 50 ms and then held at -120 mV for various intervals. The current is measured with a step to -20 mV. Curves are fit with a single exponential. The time constants are 9.28 ms and 8.39 ms for the WT and Δ KPQ channels, respectively.

Figure 5.4: Percent reduction in peak and persistent current of the Δ KPQ channels due to various lidocaine concentrations. The reduction in persistent current is much greater than the reduction in peak current for all concentrations.

affinity than WT channels (An, et al., 1996). However, the model reproduces the reduction in persistent current without a change in the affinity of any state for lidocaine. The rate of lidocaine binding from the inactivated state is faster than the rate of reopening. Therefore, channels bind lidocaine, which makes them incapable of reopening, and the persistent current is reduced. The persistent current is reduced much more than the peak current because the affinity of the inactivated state for lidocaine is much greater than that of the open state.

This approximation of the Δ KPQ mutation is a good start to reproducing the mutation, but this simple approximation does not reproduce all of the known changes in channel function due to the mutation. For example, changing the rates of the $I \rightarrow O$ and $CI \rightarrow C$ transitions does not produce enough of an increase in the rate of recovery from inactivation. These changes also do not reproduce the experimentally observed shift in the steady-state activation curve (Wang, et al., 1996b). The Δ KPQ mutation probably has multiple effects on activation and inactivation (Chandra, 1998), which, with a little work, can probably be reproduced using the cardiac sodium channel model presented here. Once a more complete model of the Δ KPQ mutation is formulated, further studies can be done on lidocaine's effect on the mutant channel.

In conclusion, models of channel gating are underutilized tools that have the potential to greatly increase our understanding of channels' structure and function. The continually rapid expansion of computer power makes the development of such biophysically-detailed models more feasible than ever before. This work has

provided a template for building such models and has shown just a few applications of one particular model. Thus, the tools and the motivation are in place. All that is needed are a few engineers, biophysicists, and mathematicians to do the work. Now is the time.

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