Mechanisms of Action of Lidocaine and Quinidine on Action Potential Duration in Rabbit Cardiac Purkinje Fibers
An Effect on Steady State Sodium Currents?

THOMAS J. COLATSKY

SUMMARY  The effects of lidocaine and quinidine on rabbit cardiac Purkinje fibers were compared at 37 °C, using action potential and voltage clamp measurements. At therapeutic concentrations (5 μg/ml), lidocaine shortened the duration of the action potential while quinidine lengthened it. When membrane potential was held constant between —40 and —50 mV by the application of the two-microelectrode voltage clamp, holding current consistently became more outward with lidocaine (+2.3 ± 0.7 nA, mean ± SEM, n = 5), but it either did not change or became more inward with quinidine (—2.1 ± 1.4 nA, n = 5). Both drugs reduced the outward tails generally associated with deactivation of the delayed rectifier current, i_o. The depression of delayed rectification by quinidine (—68 ± 6%, n = 5) was greater than that produced by lidocaine (—12 ± 4%, n = 5), and can explain the observed prolongation of the action potential. To evaluate the possibility that lidocaine blocks steady state sodium channels, experiments were performed with tetrodotoxin (TTX), a specific blocker of sodium channels in a variety of excitable membranes. TTX at concentrations of 0.1-5 μM shortened the action potential, reducing its duration to 50% of control at 1.6 μM. Voltage clamp experiments revealed a small TTX-sensitive component of steady state current flowing at membrane potentials positive to —80 mV. In the presence of 10 μM TTX, lidocaine failed to produce additional steady outward membrane current. These data suggest that (1) lidocaine and quinidine differ substantially in their modification of membrane channels, with quinidine having a strong effect on delayed rectification, (2) a TTX-sensitive “window” current exists in the rabbit Purkinje fiber and helps maintain the action potential plateau, and (3) the effect of lidocaine on the cardiac action potential results primarily from block of TTX-sensitive sodium channels, rather than from an enhancement of potassium conductance.


LIDOCAINE and quinidine are used widely in the clinical management of ventricular arrhythmias. However, the mechanisms by which these agents exert their therapeutic effects remain unclear (for a recent review, see Hauswirth and Singh, 1979). Available experimental evidence suggests that both lidocaine and quinidine depress the excitatory sodium current underlying the action potential upstroke and delay its reactivation during diastole (Ducouret, 1976; Hondeghem and Katzung, 1977; Lee et al., 1981). Additional effects on the cardiac membrane have also been indicated. At clinically relevant concentrations (2-5 μg/ml), lidocaine is known to shorten the cardiac action potential (Davis and Temte, 1969; Bigger and Mandel, 1970), whereas quinidine generally produces a lengthening or no change (Vaughan Williams, 1958; Hoffman, 1958; Mirro et al., 1981). The basis for the quinidine effect on action potential duration has not been defined, whereas an increase of potassium conductance has been proposed to mediate the action of lidocaine (Arnsdorf and Bigger, 1972; Weld and Bigger, 1976).

The question of whether effective antiarrhythmic action involves multiple drug target sites or interaction with one specific ionic channel is of importance. In the present study, experiments were performed to investigate the ionic basis for effects of lidocaine and quinidine on cardiac action potential duration. Membrane currents were measured directly by applying the two-microelectrode voltage clamp technique to short strands of rabbit Purkinje fibers, a multicellular mammalian cardiac preparation having relatively simple morphology (Johnson and Sommer, 1967; Sommer and Johnson, 1968). Previous studies (Colatsky and Tsien, 1979a, 1979b) have shown the rabbit Purkinje fiber to be largely free from the problems of nonuniformity in membrane potential and external ion concentration which can occur during voltage clamp polarizations in other cardiac tissue (Johnson and Lieberman, 1971).

The data indicate that lidocaine and quinidine...
exert different effects on the membrane currents underlying the cardiac action potential plateau. The lengthening of the action potential at therapeutic concentrations of quinidine appears to be the result of a dramatic suppression of the delayed rectifier current, whereas the decrease in action potential duration seen with lidocaine results primarily from inhibition of steady state TTX-sensitive sodium channels. No compelling evidence that lidocaine enhances potassium conductance was found in these experiments. A preliminary report of this work has already appeared (Colatsky, 1981).

Methods

Male New Zealand white rabbits, 3–5 pounds, were killed by cervical dislocation and the hearts quickly removed. Strands of Purkinje fibers, 0.6–1.3 mm in length, were excised from either ventricle following the procedure described previously (Colatsky and Tsien, 1979a; Colatsky, 1980). The fibers were stored in normal Tyrode's solution at room temperature until used. On initial impalement, the fibers were found to be depolarized (range −32 to −41 mV) and had input resistances of 1–11 MΩ. Some preparations recovered resting potentials near −85 mV during the course of the experiment.

Experiments were performed at 36–37°C, with Tyrode's solution having the following composition (mM): 140 NaCl, 4 KCl, 2.7 CaCl2, 0.5 MgCl2, 5.5 dextrose, oxygenated with 100% O2. Solutions were maintained at pH 7.3–7.4 by 20 mM Na-HEPES buffer. Fresh stock solutions of quinidine were made for each experiment by dissolving 0.2 g of crystalline Quinidine HCl (Sigma Chemical Co.) in 100 ml of deionized water. Lidocaine was used as a commercially prepared solution of 0.5% Xylocaine HCl (Astra Pharmaceuticals). Small amounts of each stock were added to Tyrode's solution to give final bath concentrations of 5 µg/ml for both drugs or, equivalently, 13.2 µM quinidine and 213 µM lidocaine.

Preparations were exposed to drug-containing solution for 10–20 minutes before records were taken. Reversal of the drug effect was attempted in every experiment. The effects of lidocaine were readily reversed within 10–15 minute after return to the control Tyrode's solution, whereas those of quinidine were usually only partially reversed even after 45 minutes. In most experiments, both drugs were applied to the same preparation in consecutive runs separated by a suitably long recovery period. Similar results were obtained in experiments involving exposure to only a single drug.

Membrane potential was recorded by means of a glass microelectrode filled with 3 M KCl. Current electrodes contained 1.7 M potassium citrate and 0.7 M KCl, acidified to pH 6 with citric acid. The voltage clamp circuitry was similar to that described by Colatsky and Tsien (1979a). Records were taken on a Brush 2200 recorder or digitized at sampling rates of 1–10 msec/point and stored on a PDP-11/03 laboratory computer (Digital Equipment Corp.). Traces could subsequently be displayed on a Tektronix 620 display monitor for analysis. Action potentials were elicited by field stimulation using thin platinum electrodes located on the floor of the tissue chamber. Both stimulus pulses and voltage clamp command pulses were delivered by a W.P.I. model 301 single channel stimulator (W. P. Instruments): in some voltage clamp experiments, slowly rising ramps were used to command membrane potential with a Tektronix FG 501 function generator. The tissue bath was held at virtual ground by a W.P.I. model 180 virtual ground amplifier.

Test depolarizations lasting 1 second were used to activate delayed rectification. This duration was chosen so that the effects of lidocaine and quinidine on the delayed rectifier could be evaluated over a time period relevant to the duration of the action potential. The curves relating the magnitude of the outward tails to the membrane potential during the test pulse (generally termed isochronal activation curves for the delayed rectifier current, i.e. Kass and Tsien, 1975), were fitted by computer using a nonlinear regression routine that varied the midpoint, slope, and asymptote to obtain the best fit of the data. In all experiments except one, the asymptotic value generated by the curve-fitting procedure was used to quantify the degree of pharmacological inhibition of the delayed rectifier. No attempt was made to distinguish between suppression of delayed rectification due to a slowing of the activation time course as opposed to changes in the fully activated current-voltage relation. Additional components of the outward tail currents resembling ir2 (Noble and Tsien, 1968) were not routinely identified.

Fiber diameter and length were measured under the dissecting microscope (80×), and the recorded values were used to estimate an apparent surface area, assuming right cylindrical geometry. In experiments involving measurements of the TTX-sensitive current, total membrane surface area was estimated by integrating capacity current transients for voltage steps which did not elicit time-dependent ionic currents. The capacity current records were corrected for leakage current by scaling the voltage step in proportion to the steady state value of current and then subtracting the remainder from the total current record. Membrane current density is referred to the effective membrane capacitance calculated in this way. Translation to current density per unit membrane surface area can be made by assuming the appropriate specific membrane capacitance. Whenever possible, results have been collected and presented as mean ± SEM.

Results

Action Potential Studies

Previous reports indicate that lidocaine and quinidine exert different effects on the cardiac action
potential. At therapeutic concentrations (2-5 μg/ml), lidocaine shortens action potential duration (Bigger and Mandel, 1970), whereas quinidine usually produces a lengthening at fixed cycle length (e.g., Mirro et al, 1981). Qualitatively similar effects occur in rabbit cardiac Purkinje fibers, as illustrated in Figure 1. The records shown were obtained during consecutive runs in the same preparation stimulated repetitively at a fixed cycle length of 1 second. The shortened rabbit Purkinje fiber characteristically generates long-lasting action potentials with a prominent early phase of repolarization and no pacemaker activity (Colatsky and Tsien, 1979a). Lidocaine at 5 μg/ml shortens the action potential to 70% of its duration in normal Tyrode’s solution; this effect is accompanied by a reduction in amplitude of the plateau phase (Fig. 1A). In contrast, 5 μg/ml quinidine lengthens the action potential to 138% of its control duration (Fig. 1B). In this preparation, plateau amplitude remained largely unaffected by quinidine but, in other experiments, some depression was seen. No significant change in resting potential occurred with either drug. The concentrations of lidocaine and quinidine used are generally associated with little or no change in maximum upstroke velocity at slow rates of stimulation

(Johnson and McKinnon, 1956; Davis and Temte, 1969; Bigger and Mandel, 1970; Weld and Bigger, 1975; Chen and Gettes, 1976). This parameter, however, was not measured in the present experiments.

Effect of Quinidine on Plateau Membrane Currents

The ionic basis for the observed changes in action potential configuration was explored in voltage clamp experiments using short rabbit Purkinje fibers. Membrane currents were studied at the level of the action potential plateau using a reduced holding potential to inactivate the excitatory sodium current. Figure 2 illustrates the type of records generally obtained in normal Tyrode’s solution (panel A) and during exposure to 5 μg/ml quinidine (panel B). Depolarization from a holding potential of -44 mV activate several ionic currents that overlap in their time- and voltage-dependence. These include the slow inward current, the transient outward current, and the delayed rectifier current. On repolarization, slowly decaying outward tails are seen which previous reports indicate are associated with the deactivation of the delayed rectifier, i, (Noble and Tsien, 1968; Colatsky and Tsien, 1979a). Because of the difficulty in separating out the contribution of individual currents from the total current recorded during the test pulse, the analysis of drug action was confined to measurements of changes in holding current, in late (steady) current
at the end of a clamp step, and in the magnitude of the outward tail current.

The most prominent effect of quinidine was a strong suppression of the outward tails and a reduction in the steady level of current at the end of the clamp step (Fig. 2B). In this experiment, little change in holding current was seen. The effects of quinidine on holding current were variable; shifts both in the direction of more inward and in the direction of more outward current were seen. The dramatic inhibition of the outward tails, however, was a consistent finding: in five experiments, 5 μg/ml quinidine reduced the peak outward tail by 68 ± 6%. The depression of delayed rectification by quinidine is fully consistent with the observed prolongation of action potential duration.

The effects of quinidine on steady membrane currents at potentials negative to −40 mV were assessed in three additional experiments. Figure 3 presents the currents recorded using a 0.02-Hz, 87-mV ramp as the command signal for the voltage clamp. The current-voltage relation obtained in the absence of drug (labeled: −Q) has a characteristic N-shape, with a clear region of net inward current between −37 and −55 mV. The record obtained 13.5 minutes after addition of quinidine (labeled: +Q) shows a decrease in net outward current at more positive potentials, and little change at more negative potentials. In particular, the region of net inward current is not greatly changed. Similar results were obtained in two other experiments, and are consistent with the previously described changes in holding current measured near −40 mV.

**Effect of Lidocaine on Plateau Membrane Currents**

In contrast to the results with quinidine, application of 5 μg/ml lidocaine produced a small but consistent shift in holding current but only slightly depressed the outward tail currents. These effects are illustrated in Figure 4. The holding current becomes more outward by 5 nA in lidocaine, while the maximum tail current amplitude is reduced by 22.5% from 31 to 23 nA (panel C). The current at the end of the pulse appears largely unaltered. After returning to normal Tyrode’s solution, the holding current and outward tails returned to nearly normal values within 10 minutes (panel D). Table 1 summarizes the results of a total of five lidocaine experiments, together with data from similar experiments performed in the presence of quinidine. The experiment shown in Figure 4 yielded the largest decrease in tail current magnitude during exposure to lidocaine. In the four remaining lidocaine experiments, the average change in holding current near −40 mV (1.6 ± 0.3 nA) was 4 times greater than the average change in maximum outward tail amplitude (0.4 ± 0.1 nA).

Figure 5 presents the current-voltage relations for the late current (panel A) and the tail currents (panel B) obtained from the experiment shown in the preceding figure. Lidocaine induces a clear outward shift of the late current between −70 and −30 mV and little change at more positive potentials. Similar results were also obtained using longer (10-
LIDOCAINE AND QUINIDINE ON PLATEAU CURRENTS/Colatsky

TABLE 1  Comparison of Lidocaine and Quinidine Actions on Holding and Tail Currents

<table>
<thead>
<tr>
<th></th>
<th>( \Delta I_{\text{hold}} ) (nA)</th>
<th>( \Delta I_{\text{relax}} ) (nA)</th>
<th>% ( \Delta I_{\text{relax}} )</th>
<th>n</th>
</tr>
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<tbody>
<tr>
<td>Lidocaine</td>
<td>+2.3 ± 0.7</td>
<td>-1.9 ± 1.4</td>
<td>-12 ± 4</td>
<td>5</td>
</tr>
<tr>
<td>Quinidine</td>
<td>-2.1 ± 1.4</td>
<td>-5.5 ± 3.1</td>
<td>-68 ± 6</td>
<td>5</td>
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</table>

Data presented as mean ± SEM. Drug concentrations were 5 \( \mu \)g/ml.

* Holding potential averaged -43 ± 2 mV in both groups.

second) step depolarizations, as well as 0.04-Hz, 85-mV ramp voltage commands (Fig. 6), consistent with changes in steady state (or background) current. Figure 4B shows that lidocaine depresses the outward tails at all potentials. As plotted, the data have been interpreted as isochronal activation curves for the delayed rectifier current, \( i_{\text{K}} \), (cf. Kass and Tsien, 1975). The curves are sigmoidal, with half-maximal activation occurring at -23 mV in normal Tyrode's solution and -20 mV in 5 \( \mu \)g/ml lidocaine. The shift in midpoint of the curves is not significant; in four experiments, the shift averaged 0.4 ± 1.3 mV. The decrease in tail amplitude (-12 ± 4%, \( n = 5 \)), was considerably smaller than the change observed with similar concentrations of quinidine. The apparent reductions in delayed rectification are consistent with previous reports of lidocaine and quinidine action in nerve (Hille, 1966; Strichartz, 1975; Yeh and Narahashi, 1976). A similar inhibition of potassium conductance by quinidine has been described by Ducouret (1976) in frog atrial muscle, although at somewhat higher concentrations (50 \( \mu \)M).

**Steady State Sodium ("Window") Currents**

The results so far indicate that lidocaine shortens the cardiac action potential, despite a reduction in delayed rectification, by producing additional steady outward current at plateau potentials. The outward current may arise either through enhancement of steady state potassium conductance (Arnsdorf and Bigger, 1972) or through a reduction in inward background current (Weld and Bigger, 1976). One possible target for the action of lidocaine on background conductance is the component of the excitatory sodium current which is maintained during the action potential plateau. This current corresponds to the tetrodotoxin (TTX)-sensitive "window" current previously described in Purkinje fibers from sheep (Attwell et al., 1979) and dogs (Colatsky and Gadsby, 1980).

Earlier voltage clamp studies of the excitatory sodium current in rabbit Purkinje fibers (Colatsky and Tsien, 1979b; Colatsky, 1980) suggested the presence of little or no overlap between steady state sodium activation and inactivation curves, predict-
FIGURE 7 Tetrodotoxin (TTX)-sensitive steady state current in the rabbit Purkinje fiber. A: Changes in holding current at —41 following application of 10 μM TTX. Traces were filtered at 100 Hz. Fiber AS01. Apparent cylindrical surface area: 0.0031 cm². B: Current-voltage relation for currents measured 10 seconds after stepping to various potentials in the absence (○) and presence (●) of 10 μM TTX. Fiber AR01. Apparent cylindrical surface area: 0.0033 cm².

A negligible maintained component of sodium current at plateau potentials, at least under the conditions of those experiments (10-26°C, 10-20 mM external sodium concentration). Since the voltage-dependence of cardiac sodium channel gating is sensitive to temperature (Dudel and Rüdel, 1970; Colatsky, 1980), the possible existence of an appreciable steady state sodium current at 37°C was investigated in the present study. As illustrated in Figure 7, the application of 10 μM TTX produced a sizeable outward shift in steady current at membrane potentials positive to —75 mV (panel B). In nine experiments, the net change in holding current near —40 mV was 3.7 ± 0.7 nA (see Table 2). This represents a membrane current density of 0.2 ± 0.04 μA/μF (n = 4) when normalized to the effective membrane capacitance. The record in Figure 7A demonstrates that current changes of this size could be reliably measured under the present experimental conditions. The tracing shows the change in holding current at —41 mV following the addition of 10 μM TTX to the bathing solution. After an initial delay, the current becomes more outward and appears to reach a steady level within 50 seconds.

Despite its small size, the TTX-sensitive background current plays an important role in maintaining the action potential plateau. Figure 8 illustrates the effects of increasing TTX concentration on action potential duration during repetitive stimulation at 1 Hz. In normal Tyrode's solution, the action potential requires 882 msec to repolarize to —60 mV. Addition of 0.1-5 μM TTX progressively shortens the action potential and depresses the plateau, with no obvious change in resting potential. Changes in action potential overshoot and maximum rate of rise could not be determined in these experiments because the stimulus artifact overlapped the action potential upstroke. Panel B presents the data, normalized to the duration of the action potential in normal Tyrode's solution, as a function of log TTX concentration. Half-maximal shortening of the action potential is estimated to occur at a concentration of 1.6 μM TTX. This value is very close to that obtained for half-maximal block of the excitatory sodium current in rabbit Purkinje fibers (0.82 μM) by C.J. Cohen et al. (1981), as well as for half-maximal inhibition of background sodium channels in dog Purkinje fibers (1.2 μM) by Colatsky and Gadsby (1980).

Changes in Holding Currents with Lidocaine and TTX

An increase in potassium conductance has been generally regarded as the primary mechanism mediating the effect of lidocaine on action potential shape (Arnsdorf and Bigger, 1972). The following experiments were designed to test this explanation and to evaluate the importance of steady state sodium channels as a target for lidocaine's action.

Changes in holding current at —40 mV were

<table>
<thead>
<tr>
<th></th>
<th>+Lidocaine</th>
<th>+TTX</th>
<th>Lidocaine +TTX</th>
<th>Δ</th>
<th>%Δ</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Summary of all data (n)</td>
<td>1.8 ± 0.3 (7)</td>
<td>3.7 ± 0.7 (9)</td>
<td>3.9 ± 1.2 (7)</td>
<td></td>
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<tr>
<td>B. Lidocaine (n = 4)</td>
<td>2.1 ± 0.4</td>
<td>4.3 ± 0.7</td>
<td>2.2 ± 0.6</td>
<td>112 ± 30</td>
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<tr>
<td>C. TTX (n = 5)</td>
<td>4.9 ± 1.2</td>
<td>5.1 ± 1.3</td>
<td>0.3 ± 0.2</td>
<td>4.5 ± 3.8</td>
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Lidocaine concentration was 5 μg/ml; TTX concentration was 10 μM. In two experiments, a single exposure to TTX and lidocaine in combination was used for comparing the effects of each agent alone in bracketing runs. The data summarized in group A also include results from other experiments in which only a single agent was tested. Membrane potential was held near —40 mV in all experiments. Data presented as mean ± SEM. Percent (%Δ) was calculated for each experiment, then averaged.
Figure 8 Concentration dependence of TTX action on the cardiac action potential plateau. A: Changes in action potential duration recorded from a rabbit Purkinje fiber during repetitive stimulation at 1 Hz. Records were obtained in normal Tyrode's solution (longest action potential), 0.01, 1, and 5 μM TTX. The sampling rate (8 msec/point) did not permit resolution of the action potential upstroke. B: Analysis of the changes in action potential duration, measured as the time taken to repolarize to −60 mV, plotted as a function of log TTX concentration. Duration of the action potential in TTX was normalized to that measured in normal Tyrode’s. Fiber AP05.

measured on application of either 5 μg/ml lidocaine or 10 μM TTX alone, or when both agents were present at the same time. Table 2 summarizes the results obtained in these experiments. As noted above, both lidocaine and TTX shift the holding current to more outward levels, although 10 μM TTX appears to be considerably more effective in doing so. The net change with TTX alone averaged 3.7 ± 0.7 nA (n = 9), nearly double that seen with 5 μg/ml lidocaine alone (1.8 ± 0.3 nA, n = 7). When TTX was applied in the presence of lidocaine, a substantial further outward shift occurred, representing an increase of 112 ± 6% (n = 4) over the change with lidocaine alone. In contrast, the complementary experiment of adding lidocaine in the presence of 10 μM TTX reveals only a small amount of additional outward current (4.5 ± 3.8%, n = 5). The size of the outward shift produced by lidocaine may, in fact, be slightly reduced by a concomitant suppression of the delayed rectifier current, but this effect should be rather small, near −40 mV, where little delayed rectification is activated (cf. Fig. 5), as well as at more positive potentials if the delayed rectifier channel shows inward-going rectification, as suggested by Noble and Tsien, (1968).

Figure 9 shows the results of an experiment in which a wider range of membrane potentials was explored in evaluating the interactions of TTX and lidocaine on steady membrane currents. The addition of 10 μM TTX to normal Tyrode’s solution produced a strong outward shift of the current-voltage relation for potentials positive to −85 mV. When lidocaine was subsequently added in the continued presence of TTX, no additional outward current developed over the voltage range of −100 to −10 mV. These findings were confirmed in six other experiments. Saikawa and Carmeliet (in press) have recently reported a similar occlusion of lidocaine’s action by TTX in sheep Purkinje fibers.

Discussion

The results indicate that lidocaine and quinidine, at concentrations believed to be clinically relevant, exert strikingly different effects on the membrane currents underlying the cardiac action potential plateau. The predominant effect of lidocaine appears to be an inhibition of steady state TTX-sensitive sodium channels (cf. Saikawa and Carmeliet, in press), while quinidine strongly suppresses delayed rectification. These actions are consistent with the observed changes in the configuration of the rabbit Purkinje fiber action potential.

Figure 9 Combined effects of lidocaine and TTX on the background membrane current-voltage relation. Currents were measured 10 seconds after voltage steps to differentiate potentials. Application of 10 μM TTX alone (△) produced the usual outward shift of steady current over the plateau range of potential compared to control (○). Addition of 5 μg/ml lidocaine to the TTX-containing Tyrode’s solution (●) produced no additional outward shift in the current-voltage relation. Fiber AQ02.
Steady State Sodium Currents in Rabbit Purkinje Fibers

Previous work in Purkinje fibers from sheep (Attwell et al., 1979) and dogs (Colatsky and Gadsby, 1980) suggested that a fraction of excitatory sodium channels remains open in the steady state and contributes current which helps to support the action potential plateau (Coraboeuf et al., 1979). This article reports the presence of a similar current in the rabbit Purkinje fiber. This component of time-independent membrane current is differentiated from an ohmic sodium “leak” current (cf. McAllister et al., 1975), both by its TTX sensitivity and by its absence at potentials more negative than -90 mV (Colatsky and Gadsby, 1980). Current-voltage relations obtained in the presence and absence of 10 μM TTX reveal a small “window” of TTX-sensitive inward current which averaged 0.2 μA/μF referred to the effective membrane capacitance. This magnitude of steady current is only a small fraction of the maximum sodium current required for generating the action potential upstroke. Previous experiments in rabbit Purkinje fibers (Colatsky and Tsien, 1979a; 1979b; Colatsky, 1980) predict a maximal peak sodium current density at 37°C of about 400 μA/μF. Assuming that 1.6 μM TTX produces half-maximal inhibition of the steady state sodium channels (Fig. 7), 10 μM TTX should block 86% of the channels. The ratio of steady state to peak sodium current is thus calculated to be 0.06%. For comparison, the steady state sodium current is 3% of the peak value in squid giant axon (Fitzhugh, 1960), and 0.1% in vertebrate nerve (Dodge and Frankenhauser, 1958). Nevertheless, because of the marked inward-going rectification exhibited by the cardiac cell membrane (Noble, 1965), the maintained sodium current remains an appreciable fraction of the total membrane current during the action potential plateau as evidenced by the dramatic effects of even low (0.1 μM, 6% occupancy) concentrations of TTX on the shape of the action potential (see Fig. 8). Similar effects of TTX on cardiac action potential duration have been described in rabbit (Okada, 1975), dog (Coraboeuf et al., 1979), and sheep Purkinje fibers (Dudel et al., 1967).

The existence of a steady state sodium “window” current was not predicted by earlier measurements of excitatory sodium channel properties in the rabbit Purkinje fiber. The apparent degree of overlap in the voltage-dependence of steady-state inactivation (hω) and peak sodium permeability (PNa) appeared to be negligible when measured directly in voltage clamp experiments at 10-26°C (Colatsky and Tsien, 1979b; Colatsky, 1980). The present results suggest that the extent of overlap required to produce the amount of maintained sodium current observed experimentally is small enough to be easily missed, and would represent an unresolvable fraction of the total membrane current in those earlier experiments. In addition, displacement of the hω (V) curve to more negative potentials with decreasing temperature could have further reduced the apparent degree of overlap and, consequently, the size of the steady state current, as discussed previously (Colatsky, 1980). The amount of maintained excitatory sodium current at 37°C can be estimated roughly from earlier voltage clamp data, assuming that warming shifts the hω (V) curve toward more positive potentials by 13.6 mV [i.e., 8 mV/10°C (cf. Colatsky, 1980)]. This correction places the midpoint of the hω (V) relation at -61 mV for this analysis. The calculation uses the Goldman-Hodgkin-Katz equation (cf. Hodgkin and Katz, 1949),

\[ I_{Na} = P_{Na} \frac{F^2 V N_{ao} - N_{ai} \exp(VF/RT)}{RT - 1 - \exp(VF/RT)} \]

where \( P_{Na} \) is the relative peak sodium permeability at V, hω the level of steady state inactivation, V the membrane potential, Nao and Naï the external and internal sodium concentrations, respectively, and R, T, and F have their usual meanings. For hω = 0.01, V = -40 mV, Nao = 150 mM, Naï = 8 mM, and \( P_{Na} = 0.123 \times 10^{-5} \text{ cm/sec} \) (cf. Colatsky, 1980), the predicted “window” current at 37°C is 0.3 μA/cm², in surprisingly good agreement with the experimental results.

Lidocaine Blocks Steady State Sodium Channels

The ability of lidocaine to produce additional outward (repolarizing) current in the plateau range of potential appears to be independent of an increase in potassium conductance. Earlier investigations of the antiarrhythmic action of lidocaine suggested that lidocaine’s effectiveness lay in its ability to enhance potassium conductance (Arnsdorf and Bigger, 1972), which served to reduce the rate of pacemaker depolarization, shorten the action potential, raise threshold, and repolarize partially depolarized cells (Bigger and Mandel, 1970; Arnsdorf and Bigger, 1972, 1975; Arnsdorf and Mehlman, 1978). The present results show that lidocaine fails to increase steady outward current in the presence of high concentrations of TTX, suggesting that the principle action of lidocaine is an inhibition of TTX-sensitive sodium channels. No evidence for an increase of potassium conductance was found, even when membrane potential was held at -110 mV. Similar conclusions recently were reached by Saikawa and Carmeliet (in press) in their study of sheep Purkinje fibers. Lidocaine was found to slightly depress delayed rectification in the rabbit Purkinje fiber, similar to its effect in myelinated nerve (Hille, 1966; Strichartz, 1973). This effect, however, was relatively small compared to the change in TTX-sensitive current. In this respect, the present findings complement those of Weld and Bigger (1976), who also reported increases in steady outward current, coupled with a reduction
in the pacemaker current, \( i_{K2} \), at diastolic potentials. The apparent discrepancy between the voltage clamp data reported here and previous results using constant current techniques (Arnsdorf and Bigger, 1972, 1975) might be attributable to difficulties in the interpretation of slope conductance measurements, particularly when a negative slope occurs in the steady state. Net membrane current-voltage characteristic. The analysis of slope conductance data in terms of chord conductances assumes that the membrane current is carried by only a single ion species (in this case, potassium). Both of these assumptions may be invalid. The current-voltage relations presented by Arnsdorf and Bigger (1972) exhibit considerable nonlinearity near the resting potential, making both the measurement of slope conductance and its interpretation somewhat uncertain. In addition, the existence of a lidocaine-sensitive sodium current at these potentials would present additional problems. Several studies have indicated that a TTX-sensitive steady state current may flow near the normal diastolic potential in both sheep and dog Purkinje fibers (Attwell et al., 1979; Colatsky and Gadsby, 1980). Such results are consistent with the 5- to 8-mV hyperpolarization produced by 1 \( \mu \)M TTX in fully polarized dog Purkinje fibers (Coraboeuf et al., 1979). Pharmacological block of an overlapping steady inward current might appear to enhance potassium conductance in two ways: first, by reducing or abolishing the negative slope in the steady state membrane current-voltage characteristic, and, second, by producing a hyperpolarization, which might itself augment potassium conductance. Similar considerations may also explain the small (10%) lidocaine-induced increase in radiolabeled potassium efflux reported by Kabela (1973). The use of sodium-deficient solutions to remove the contribution of sodium current in measurements of slope conductance may not be completely effective in preparations like the sheep Purkinje fiber, in which diffusional ion exchange with the bulk solution is restricted by the presence of narrow intercellular clefts.

Comparison with Previous \( V_{\text{max}} \) Measurements

The results with lidocaine appear to be at odds with previous reports in another respect, since the observed block of background sodium channels occurs at lidocaine concentrations believed to have little effect on the excitatory sodium current as measured by action potential upstroke velocity \( (V_{\text{up}}) \) (Davis and Tatem, 1969; Bigger and Mandel, 1970; Weld and Bigger, 1975; Chen et al., 1975), although a recent voltage clamp study reports substantial inhibition of sodium current with 10-20 \( \mu \)M lidocaine (Lee et al., 1981). This apparent discrepancy can be understood if \( V_{\text{up}} \) were a nonlinear measure of maximal sodium conductance. The reliability with which \( V_{\text{max}} \) measurements report sodium channel properties has been the subject of much recent debate (Cohen and Strichartz, 1977; Hodeghem, 1978; Strichartz and Cohen, 1978; Walton and Fozzard, 1979). Calculations using a variety of models for excitation predict that \( V_{\text{max}} \) will be a poor measure of maximal sodium conductance if the rate of sodium channel activation (\( \tau_{\text{m}^-} \)) is slow relative to the rate of voltage change during the action potential upstroke (Hunter et al., 1975; I.S. Cohen et al., in press). In this case, a given reduction in maximal sodium conductance will produce smaller changes in \( V_{\text{max}} \), since the accompanying decrease in the rate of depolarization permits more complete activation of the sodium channel and, consequently, a higher effective conductance during the upstroke. These considerations are less important for measures involving steady state characteristics of the sodium channel. Action potential duration, for example, may be expected to mirror a change in maximal sodium conductance more reliably than \( V_{\text{max}} \). It is thus not unreasonable to find that the action potential plateau appears more sensitive to lidocaine than does \( V_{\text{max}} \). Coraboeuf et al. (1979) report a roughly 6-fold difference in the TTX concentrations required to reduce \( V_{\text{max}} \) and action potential duration to 50% of their control values. The half-maximal concentration of 1.6 \( \mu \)M found here for TTX shortening of the action potential is in reasonable agreement with the dissociation constant for TTX inhibition of the excitatory sodium channel measured directly in voltage clamp experiments near 20°C (C.J. Cohen et al., 1981).

Effects of Quinidine

Quinidine has been assumed to exert its antiarrhythmic effect by blocking the excitatory sodium channel and delaying its reactivation (Chen et al., 1975; Chen and Gettes, 1976; Lee et al., 1981). A similar inhibition of background sodium channels might therefore be expected. However, the absence of a significant outward shift in steady current with quinidine at plateau potentials reported here may reflect the marked reduction in delayed rectification produced by quinidine, which could offset an inhibition of the steady state sodium channels. A second possibility is that block of cardiac sodium channels by quinidine has an important voltage-dependent component. Action potential studies indicate that quinidine inhibits \( V_{\text{max}} \) in a frequency-dependent manner. \( V_{\text{max}} \) is virtually unchanged at long (>10-second) cycle lengths, but decreases dramatically as the cycle length is shortened (Chen et al., 1975). Such would be the case if quinidine bound preferentially to open (activated) channels. This specific type of interaction has been proposed for quinidine by Hodeghem and Katzung (1977) in their model for antiarrhythmic drug action. The protocols used in the present experiments (i.e., 1- to 10-second
voltage steps from a holding potential of $-40 \text{ mV}$, or slow ramp depolarizations) will activate only a small fraction of the total sodium channel population, and leave the remainder in the resting or inactivated state. Consequently, little block of the sodium channel may occur in these experiments. It is conceivable that higher quinidine concentrations or longer exposures to the drug could produce a measurable inhibition of the steady state sodium conductance, even with few channels in the open state. The exact nature of the interaction of quinidine with the various states of the sodium channel remains to be elucidated, using appropriate voltage clamp protocols (Hille, 1977). The small net inward shift in steady membrane current reported here is consistent, nevertheless, with the observed prolongation of the rabbit Purkinje fiber action potential, which itself may be antiarrhythmic (cf. Hauswirth and Singh, 1979). These data are also consistent with the reported failure of quinidine and quinidine-like compounds to repolarize Purkinje fibers arrested at the low level of resting potential (Arnsdorf and Mehlman, 1978), in contrast to the ability of lidocaine and TTX to repolarize such fibers rapidly (Gadsby and Cranefield, 1977).

In conclusion, the present study provides direct evidence for the importance of steady state sodium channels, both in shaping the normal cardiac action potential and in serving as a target for antiarrhythmic drug action. The data suggest that lidocaine’s therapeutic effects are mediated primarily by inhibition of TTX-sensitive sodium channels, rather than by an increase in potassium conductance. In contrast, quinidine appears to prolong the action potential plateau by depressing delayed rectification.

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T J Colatsky

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