The Influence of Molecular Form of Local Anesthetic-Type Antiarrhythmic Agents on Reduction of the Maximum Upstroke Velocity of Canine Cardiac Purkinje Fibers

Gary A. Gintant, Brian F. Hoffman, and Robert E. Naylor

From the Department of Pharmacology, College of Physicians and Surgeons of Columbia University, New York, New York

SUMMARY. We studied the local anesthetic effects of the quaternary lidocaine analogues QX-314, QX-572, and QX-222, the tertiary amine lidocaine, its analogues tocainide, 6603, 6211, and the neutral local anesthetic benzocaine to determine if molecular charge of antiarrhythmic agents influences their local anesthetic effects on heart fibers. We used standard microelectrode techniques and canine cardiac Purkinje fibers to compare the effects of stimulation rate, drug concentration, and K+-induced changes in resting membrane potential on the reduction of fast inward sodium current using the maximum rate of rise of the action potential upstroke, V\text{max}, as an index of changes in peak sodium current. Use-dependent block, defined as a modulation of the reduction in V\text{max} by local anesthetics due to changes in the stimulation rate, was observed with the permanently charged analogues and was most prominent for agents existing predominantly in the charged form, but was absent for the neutral local anesthetic benzocaine. The development of use-dependent block during rapid stimulation preceded by prolonged periods of quiescence was an exponential process which became more rapid with increasing drug concentration. Recovery from use-dependent block during quiescence was an exponential process that was not influenced by similar drug concentration changes. All local anesthetics caused tonic block, defined as a drug-induced reduction of V\text{max} from control that attained a constant value at slow stimulation rates (cycle length range 15 seconds to 2 minutes) and was not changed by prolonged (up to 8 minutes) periods of quiescence. These findings suggest that the charged form of lidocaine and its analogues is responsible for use-dependent block of cardiac sodium channels. (Circ Res 52: 735-746, 1983)

Many antiarrhythmic drug molecules, including lidocaine, procainamide, aprindine, and tocainide, contain a tertiary nitrogen atom and, as a result, can exist as either the uncharged tertiary amine or the positively charged ammonium cation. The local anesthetic effects of these agents, which probably contribute to their antiarrhythmic efficacy (Bigger and Hoffman, 1980), may therefore be due to interactions of either the charged, uncharged, or both molecular forms with cardiac sodium channels. If charged and uncharged forms of the same drug differ in their ability to reduce fast inward sodium current, the concentrations of both drug forms would determine the drug's local anesthetic characteristics. It has been postulated that the lower pH found within ischemic areas of the heart, which would increase the concentration of the ionized drug form, may be responsible for the enhanced local anesthetic effects of lidocaine in ischemic as compared to normal areas (Kupersmith et al., 1975). Indeed, variations in pH have been demonstrated to influence markedly the ability of lidocaine to prolong the reactivation kinetics of cardiac sodium channels measured using V\text{max} (Grant et al., 1980). Both the site of action and active form of local anesthetics have been investigated in nerve (Ritchie and Ritchie, 1968; Narahashi et al., 1970; Frazier et al., 1970; Strichartz, 1973; Yeh and Narahashi, 1976; Hille, 1977b). Their results suggested that the charged form, acting from the inner membrane surface, is primarily responsible for reducing sodium current. However, both charged and uncharged drug forms cause local anesthetic effects. Indeed, benzocaine, which is uncharged at physiological pH, as well as the permanently charged lidocaine derivatives QX-314 and QX-572, reduce fast inward sodium current in frog node of Ranvier (Hille, 1977a, 1977b).

The ability of some local anesthetics to reduce sodium current in nerve and cardiac preparations is dependent on the rate of stimulation (Johnson and McKinnon, 1957; Strichartz, 1973; Hondeghem and Katzung, 1980). This phenomenon is termed frequency-dependent, or use-dependent block (Courtney, 1975). Studies in frog skeletal muscle of the effects of external pH on use-dependent block by lidocaine suggest that the protonated drug form is responsible for use-dependent block (Schwarz et al., 1977).

Because most studies of the interactions of local anesthetics with sodium channels have employed noncardiac preparations, because sodium channels in these tissues may differ from cardiac sodium channels (Reuter et al., 1978; Hondeghem, 1981), and because...
of the possible role that use-dependent block may play in determining antiarrhythmic drug efficacy (Sasyuk and Ogilvie, 1975), we investigated the effects of charged and uncharged forms of local anesthetics on fast inward sodium current of canine cardiac Purkinje fibers, using changes in \( V_{\text{max}} \) of phase 0 as indicator of changes in peak inward sodium current. A series of lidocaine analogues with different pKa values, as well as quaternary analogues and benzocaine, were chosen to provide different proportions of both drug forms for any given total drug concentration and pH (Fig. 1). Although both charged and uncharged drug forms reduce the fast inward sodium current of cardiac fibers, only the quaternary analogues and tertiary amine analogues existing predominantly as the charged molecular form cause use-dependent block. The similar characteristics of use-dependent block for both the quaternary and tertiary amine analogues suggest that the charged form of lidocaine and its analogues are responsible for use-dependent block. These results should aid in determining the electrophysiological mechanisms responsible for the efficacy of antiarrhythmic agents.

![Chemical Structures](attachment://chemical_structures.png)

**FIGURE 1.** The chemical structures of the three classes of local anesthetics. The permanently charged lidocaine analogues QX-314, QX-222, and QX-572 (not shown) all possess a quaternary, positively charged nitrogen atom on the side chain. pKa values for the tertiary amine lidocaine and its analogues, as well as the percentage of molecules existing as the charge molecular form in a solution of pH 7.4, also are indicated. Not shown is benzocaine (ethyl-p-aminobenzoate, pKa = 2.6), and QX-572 [N,N-bis-(phenylcarbamoylmethyl)dimethylammonium chloride.]

### Experimental Procedures

Adult dogs weighing 12–30 kg were anesthetized with sodium pentobarbital, 30 mg/kg, iv. Hearts were rapidly removed through a right lateral thoracotomy and rinsed with chilled, oxygenated Tyrode’s solution. Our standard Tyrode’s solution contained (in mM): NaCl, 137; NaHCO\(_3\), 12; dextrose, 5.5; NaH\(_2\)PO\(_4\), 18; MgCl\(_2\), 0.5; KCl, 4.0; and CaCl\(_2\), 2.7. During tissue dissection, a Tyrode’s solution containing 8.0 mM KCl and 4.0 mM CaCl\(_2\) was used. During experiments in which the resting membrane potential of the preparation was varied by elevating [K\(^+\)], 1 mM KCl was added to the Tyrode’s solution without compensation for osmotic changes.

We employed small, free-running intertrabecular Purkinje fiber bundles (length < 5mm) with attached ventricular muscle, and larger, free-running Purkinje fiber bundles excised from either ventricle. As compared to the larger bundles, the smaller preparations displayed minimal diastolic depolarization and provided stable membrane potentials more negative than ~80 mV during prolonged periods of quiescence.

Preparations were mounted in a Lucite chamber (Aronson et al., 1973) maintained at 35–37°C and perfused with solution equilibrated with 5% CO\(_2\)/95% O\(_2\). The preparations were stimulated through Teflon-coated bipolar silver wire electrodes (stimuli < 2 msec in duration and 1.3 to 2 times threshold). Transmembrane potentials were measured through machine-pulled fiber-filled borosilicate glass capillary microelectrodes filled with 3 M KCl (resistances 8–30 M\(\Omega\), tip potentials < 5 mV) connected through 3 M KCl-Ag/AgCl junctions to amplifiers with high input resistance and variable input capacitance neutralization. The transmembrane potentials were displayed on a Tektronix 565 oscilloscope and a Gould chart recorder. These signals were also differentiated with an operational amplifier whose output was linear between 50 and 800 V/sec, and which was calibrated with a sawtooth pulse of variable duration (Bigger et al., 1968). This provided the first time derivative of phase 0 of the action potential, which was displayed on the oscilloscope at a faster sweep speed. We also used a peak hold circuit to record the maximum rate of rise of the upstroke (\(V_{\text{max}}\)) on the chart recorder. All reported results were obtained from single impalements maintained throughout the experiment. Measurements were rejected if either the microelectrode resistance varied by greater than 5 M\(\Omega\) during an experiment, or if the measured potential differed by more than 3 mV from the original zero reference upon withdrawal of the microelectrode from the preparation.

Our supply of lidocaine and lidocaine analogues was generously supplied by Astra Pharmaceuticals. Benzocaine was obtained from Sigma Chemical Co. Drugs were added to Tyrode’s solution from refrigerated stock solutions. Concentrations refer to the concentration of salt (with the exception of benzocaine).

Where applicable, data is expressed as mean ± SD. Exponential functions were analyzed by a least squares regression method. Differences between paired samples were analyzed by paired t-test (Snedecor and Cochran, 1967). Inactivation curves were fitted by eye.

We define “use-dependent block” as the modulation of the drug-induced reduction of \(V_{\text{max}}\) caused by changes in

<table>
<thead>
<tr>
<th>DRUG</th>
<th>-R GROUP</th>
</tr>
</thead>
<tbody>
<tr>
<td>LIDOCAINE</td>
<td>(\text{CH}_2-\text{N}-\text{C}_2\text{H}_5)</td>
</tr>
<tr>
<td>pKa = 7.88</td>
<td>75% charged</td>
</tr>
<tr>
<td>TOCAINIDE</td>
<td>(\text{CH}_2-\text{N}-\text{C}_2\text{H}_5)</td>
</tr>
<tr>
<td>pKa = 7.8</td>
<td>75% charged</td>
</tr>
<tr>
<td>6603</td>
<td>(\text{CH}_2-\text{N}-\text{C}_2\text{H}_5)</td>
</tr>
<tr>
<td>pKa = 9.8</td>
<td>95% charged</td>
</tr>
<tr>
<td>62II</td>
<td>(\text{CH}_2-\text{N}-\text{CH}_2\text{CH}_3)</td>
</tr>
<tr>
<td>pKa = 6.3</td>
<td>7% charged</td>
</tr>
</tbody>
</table>
the rate of electrical activity. The magnitude of use-dependent block is dependent on the stimulation basic cycle length (BCL), and is therefore frequency-dependent. We employ the term "tonic block" to describe the drug-induced reduction of $V_{\text{max}}$ from control values which is present at low frequencies of stimulation (BCL range, 15 seconds to 2 minutes) and is not altered by periods of quiescence (up to 8 minutes duration). The expression "rest recovery" is used to describe recovery from use-dependent block which occurs during periods of infrequent stimulation or quiescence. All three terms refer to changes of $V_{\text{max}}$ of upstrokes elicited from similar membrane potentials just prior to the upstroke, the membrane activation voltage (MAV), in order to eliminate changes in $V_{\text{max}}$ due to normal voltage-dependent sodium channel inactivation (Hodgkin and Huxley, 1952; Weidmann, 1955). For this reason, both MAV and $V_{\text{max}}$ values are reported when comparing $V_{\text{max}}$ values.

Representative examples of cardiac action potential changes caused by QX-314, QX-222, tetracaine, and benzocaine are displayed as figure insets; examples for some of these lidocaine analogues have previously been published (Gliklich and Hoffman, 1978).

On the Use of $V_{\text{max}}$ and Its Relation to Channel Block

We used changes in $V_{\text{max}}$ as an indicator of changes of fast inward sodium current. For a uniformly propagating action potential in a linear cable $V_{\text{max}}$ is linearly proportional to the total ionic current crossing the membrane at the time of $V_{\text{max}}$ (Hodgkin and Huxley, 1952; Narashashi et al., 1970). There is evidence that during a fast upstroke, the majority of ionic current crossing the cardiac membrane at the time of $V_{\text{max}}$ is sodium current, and that other membrane currents do not make any significant contributions. Reduction of the outward background potassium conductance with CsCl has no effect on $V_{\text{max}}$ of fast upstrokes of normally polarized and partially depolarized cardiac Purkinje fibers bathed in elevated [K+] solutions (Gintant and Hoffman, 1981), and block of early outward current with tetrathyammonium ions does not significantly reduce $V_{\text{max}}$ (Kenyon and Gibbons, 1979). Slow inward current apparently does not contribute to the fast upstroke due to the slower activation kinetics of calcium channels compared with sodium channels (Isenberg and Klöckner, 1980, 1982; Cohen et al., 1983). Potassium currents would be expected to contribute little to the total ionic current (Hondeghem, 1978). Assuming uniform propagation of the cardiac action potential down the Purkinje fiber surface, changes in $V_{\text{max}}$ should be a reasonable indicator of changes of fast inward sodium current.

The use of changes in $V_{\text{max}}$ to quantify the extent of drug-blocked, nonconducting cardiac sodium channels is open to criticism, because $V_{\text{max}}$ may not be directly proportional to available sodium conductance as has been shown for myelinated nerve (Ulbricht and Wagner, 1975) and cooled rabbit Purkinje fibers (Bean et al., 1982). For a uniform ("membrane") action potential, changes in $V_{\text{max}}$ will be proportional to changes in the available sodium conductance if the sodium conductance is greater than nonsodium conductances, and sodium channel activation is much faster than channel inactivation (Cohen et al., 1981). Measurements of cardiac sodium channel kinetics (presently available for cooled single cells and embryonic chick heart myocytes) provide some indication of the relative rates of activation and inactivation at different potentials (Brown et al., 1981; Ebihara and Johnson, 1980). However, even if the rates of these two processes are sufficiently different under drug-free conditions to satisfy the requirements for a proportional relationship between $V_{\text{max}}$ and available sodium conductance, there is presently no way to be certain that the relationship between activation and inactivation kinetics in canine cardiac fibers remain similar after exposure to local anesthetics.

We chose to study the effects of lidocaine analogues on canine cardiac Purkinje fibers in order to allow the direct application of our findings toward understanding the antiarrhythmic efficacy of lidocaine in various canine arrhythmia models. We chose to use changes in $V_{\text{max}}$ as an indicator of changes in fast inward sodium current (and to infer changes in the number of drug-blocked sodium channels) because direct measurement of fast inward sodium current in any cardiac preparation under physiological conditions is not yet possible (Colatsky and Tsien, 1979; Lee et al., 1979; Colatsky, 1980), and because changes in $V_{\text{max}}$ have been employed consistently to characterize the effects of antiarrhythmic drugs on cardiac sodium channels (Gliklich and Hoffman, 1978; Grant et al., 1980; Weld et al., 1982). We recognize that we have not quantified changes in available sodium conductance, but believe that our data are adequate to demonstrate the approximate magnitude of changes in fast inward sodium current for the various experimental conditions.

Results

Effects of Quaternary Local Anesthetics

Characteristics of the Development of Use-Dependent Block

We first determined whether the drug-induced reduction of $V_{\text{max}}$ observed during superfusion with the permanently charged analogue QX-314 was modified by changing the stimulation rate (Fig. 2). Equilibration of the preparation for 1 hour with $2 \times 10^{-5}$ M QX-314 (BCL = 2 sec) resulted in the reduction of $V_{\text{max}}$ from 570 to 500 V/sec. Shortening the BCL to 700 msec resulted in an additional, gradual reduction of $V_{\text{max}}$.

![Figure 2](http://circres.ahajournals.org/)

**Figure 2.** The effect of changes in stimulation rate on the reduction of $V_{\text{max}}$ during superfusion with QX-314. Changes in $V_{\text{max}}$ (●) and MAV (□) for cooled single canine Purkinje fibers were examined as the stimulation rate was varied. Similar stimulation rate changes produced minimal changes of $V_{\text{max}}$ prior to drug exposure. The slight changes in MAV represent real changes in membrane potential which occur following changes in stimulation rate.
from 500 to 420 V/sec. After attainment of this new steady state \( V_{\text{max}} \) value, the BCL was lengthened from 700 msec to 20 seconds, for a total of 18 minutes. During this recovery period, \( V_{\text{max}} \) increased to its drug-free control value. When the BCL was again shortened to 700 msec, \( V_{\text{max}} \) declined toward its earlier reduced value.

The magnitude and rate of development of use-dependent block following prolonged recovery periods is dependent on the quaternary local anesthetic concentration (Fig. 3). As illustrated in Figure 3A, 1 hour of superfusion with \( 4 \times 10^{-5} \) M QX-314 (BCL = 500 msec) resulted in a 42% reduction of \( V_{\text{max}} \) (–92 mV to 380 V/sec). Regular stimulation then was terminated for 15.5 minutes to allow maximal rest recovery, as determined by observing increasing \( V_{\text{max}} \) values of intermittent, infrequent stimulated upstrokes. Upon resumption of stimulation, \( V_{\text{max}} \) slowly decreased from 560 to 390 V/sec. Subsequent superfusion for 1 hour with \( 8 \times 10^{-5} \) M QX-314 (500 msec BCL) resulted in the further reduction of \( V_{\text{max}} \) (–92 mV to 285 V/sec, 56% compared with control, Fig. 3B). Again, stimulation was interrupted for 16 minutes to allow maximal rest recovery. Upon resumption of rapid stimulation, \( V_{\text{max}} \) declined rapidly from 520 to 290 V/sec.

In Figure 3C, the time course of the fall of \( V_{\text{max}} \), expressed as the difference between \( V_{\text{max}} \) of any particular upstroke and the final, reduced \( V_{\text{max}} \) value, is plotted on a logarithmic scale against the beat number of the particular upstroke during the stimulus train. With both drug concentrations, the rate of development of use-dependent block is well characterized as a monoexponential process. However, the 2-fold increase in drug concentration resulted in a decrease in the time constant characterizing the development of use-dependent block from 142 beats to 62 beats. From four similar experiments, the time constant for the fall of \( V_{\text{max}} \) with the higher concentration of QX-314 was twice as rapid as the time constant obtained during superfusion with the lower drug concentration (143.20 \( \pm \) 50 beats for \( 4 \times 10^{-5} \) M vs. 70.0 \( \pm \) 50 for \( 8 \times 10^{-5} \) M, difference \( p < 0.05 \), Table 1). Qualitatively similar effects of increased drug concentration on the rate of development of use-dependent block have been obtained with repeated intracellular injections of QX-314 by this laboratory (Frame et al., 1981).

The development of use-dependent block with the quaternary analogues QX-572 and QX-222 was also well characterized as a monoeponential process whose rate was doubled by a similar 2-fold increase of drug concentration (Table 1). For either the lower or higher drug concentration, the development of use-dependent block was most rapid with QX-572, intermediate for QX-222, and slowest for QX-314. Because of the long time required for equilibration of the local anesthetic effects of these quaternary analogues with our preparations (\( \geq 1 \) hour, see Fig. 2), the time required to achieve maximal rest recovery, and the inability to reverse the effects of these agents even after prolonged drug-washout periods (\( \geq 2 \) hours duration), we studied a maximum of two different drug concentrations in any individual preparation. However, we were able to compare the effects of a
TABLE 1
Effect of Increasing Drug Concentrations of Quaternary Lidocaine Analogues on the Rate of Development and Recovery from Use-Dependent Block

<table>
<thead>
<tr>
<th>Drug</th>
<th>MAV (-mV)</th>
<th>$\tau_{on}$ (bits)</th>
<th>$\tau_{off}$ (min)</th>
<th>MAV (-mV)</th>
<th>$\tau_{on}$ (bits)</th>
<th>$\tau_{off}$ (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>QX-314</td>
<td>85-90</td>
<td>166</td>
<td>5.2</td>
<td>86-92</td>
<td>82</td>
<td>4.8</td>
</tr>
<tr>
<td></td>
<td>85-91</td>
<td>191</td>
<td>4.9</td>
<td>87-94</td>
<td>82</td>
<td>4.7</td>
</tr>
<tr>
<td></td>
<td>87-91</td>
<td>142</td>
<td>4.7</td>
<td>86-91</td>
<td>62</td>
<td>4.7</td>
</tr>
<tr>
<td></td>
<td>81-83</td>
<td>74</td>
<td></td>
<td>81-83</td>
<td>54</td>
<td></td>
</tr>
<tr>
<td>QX-222</td>
<td>83-85</td>
<td>59</td>
<td>2.0</td>
<td>83-85</td>
<td>29</td>
<td>1.9</td>
</tr>
<tr>
<td></td>
<td>84-88</td>
<td>71</td>
<td>1.6</td>
<td>84-88</td>
<td>32</td>
<td>1.9</td>
</tr>
<tr>
<td></td>
<td>71-80</td>
<td>62</td>
<td>1.1</td>
<td>83-86</td>
<td>42</td>
<td>1.6</td>
</tr>
<tr>
<td>QX-572</td>
<td>80-82</td>
<td>29</td>
<td>0.46</td>
<td>81-82</td>
<td>19</td>
<td>0.32</td>
</tr>
<tr>
<td></td>
<td>81-83</td>
<td>32</td>
<td>0.85</td>
<td>80-82</td>
<td>34</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>84-87</td>
<td>36</td>
<td>0.67</td>
<td>85-87</td>
<td>19</td>
<td>0.65</td>
</tr>
</tbody>
</table>

For each maintained impalement, the effect of a 2-fold increase in drug concentration on the rate of development and recovery from use-dependent block for the three quaternary local anesthetics is presented. For each drug, the time constant characterizing the exponential decline of $V_{\text{m}}$, ($\tau_{\text{on}}$) is significantly different for the two drug concentrations ($P < 0.05$), whereas the time constant characterizing the exponential increase of $V_{\text{m}}$ during rest recovery ($\tau_{\text{off}}$) is unaffected ($P > 0.05$). $R_{\text{on}}$, the ratio of $\tau_{\text{on}}$ for the lower vs. higher drug concentration, approximated 2.0 for each drug; $R_{\text{off}}$, the ratio of $\tau_{\text{off}}$ for these same drug concentrations, approximated a value of 1.0. $[K^+]_0 = 5$ min, BCL = 500 msec.

reasonably wide concentration range on the rate of development of use-dependent block with QX-222, exposing some preparations to only one drug concentration. In Figure 4, the reciprocal values of the time constants used to characterize the development of use-dependent block have been plotted for various QX-222 concentrations. For the range of concentrations tested, a linear relationship appears to adequately describe changes in the rate of development of use-dependent block associated with different QX-222 concentrations. Depolarization and conduction failure associated with greater QX-222 concentrations precluded their further study.

Characteristics of Recovery from Use-Dependent Block

We also investigated the effect of drug concentration on recovery from use-dependent block. A typical example is illustrated in Figure 5. After a steady reduction of $V_{\text{max}}$ had been obtained with $4 \times 10^{-5}$ M QX-314 (500 msec BCL), rapid stimulation was terminated, and $V_{\text{max}}$ values of seven single action potential upstrokes elicited at various times during the ensuing rest recovery period were recorded. The fiber then was equilibrated with $8 \times 10^{-5}$ M QX-314 and the protocol repeated. During the 15.5-minute rest recovery period with $4 \times 10^{-5}$ M QX-314, $V_{\text{max}}$ increased by 180 V/sec ($-90$ mV MAV-$380$ V/sec to $-88$ mV-$560$ V/sec), whereas, during a 15-minute rest recovery period with $8 \times 10^{-5}$ M QX-314, $V_{\text{max}}$ increased by 235 V/sec ($-93$ mV-$285$ V/sec to $-88$ mV-$520$ V/sec). In Figure 5, the increasing $V_{\text{max}}$ values obtained during the recovery periods are plotted against the elapsed recovery time. The time course of $V_{\text{max}}$ recovery for both drug concentrations has been fitted to an exponential curve with a time constant of 4.6 minutes. In three experiments utilizing this protocol, the time constants characterizing the recovery processes for either concentration of QX-314 were...
not significantly different (4.93 ± 0.21 minutes for 4 × 10⁻⁵ M vs. 4.73 ± 0.04 minutes for 8 × 10⁻⁵ M, P < 0.05). For both QX-572 and QX-222, the increase of V_max during rest recovery periods was also well approximated as an exponential process whose rate was not influenced by similar changes of drug concentration (Table 1). The fastest recovery kinetics were observed with QX-572, and the slowest with QX-314. The effects of higher concentrations of quaternary analogues on recovery kinetics were not studied due to their tendency to cause fiber depolarization during recovery periods.

Characteristics of Tonic Block

As demonstrated in Figure 3, prolonged rest recovery periods do not necessarily result in full recovery of V_max to pre-drug control values. The magnitude of tonic block depends upon the resting membrane potential as well as the drug and drug concentration. Figure 6A demonstrates the effect of membrane potential on tonic block. During the drug-free control period, MAV-V_max values were obtained during superfusion with Tyrode’s solution containing [K⁺]o = 4, 5, 6, 8, and 10 mM. From this data, a control “h” inactivation curve was constructed (Weidmann, 1955). The fiber was exposed for 1.5 hours to Tyrode’s solution containing 4 × 10⁻⁵ M QX-314 ([K⁺]o = 4 mM, BCL = 1 sec), which resulted in a 50% reduction of V_max. To determine what proportion of the reduction of V_max was due to tonic as compared to use-dependent block, the BCL was prolonged from 1 second to 5 seconds, and subsequently to 90 seconds, which resulted in a gradual increase of V_max from 300 to 380 to 470 V/sec for each BCL. To ensure that only tonic block remained, the BCL was doubled in duration to 3 minutes, for a total of 12 minutes, which resulted in no further recovery of V_max. Subsequent rapid stimulation (BCL = 1 sec) resulted in the exponential decline of V_max to 285 V/sec (~90 mV MAV).

The effect of membrane potential on tonic block was assessed during superfusion with drug-containing Tyrode’s solution with [K⁺]o = 2.7, 5, 7, and 9 mM. For each value of [K⁺]o, after determining V_max values during stimulation with a 1-second BCL, the BCL was repeatedly doubled until further prolongation resulted in no further V_max recovery. In this manner, both the tonic and use-dependent blocking effects of the drug were determined for different MAV’s. We define an inactivation curve in which the drug-induced reduction of V_max depends solely on tonic block as a steady state inactivation curve.

![Figure 5](image1)

**Figure 5.** Effect of QX-314 concentration on the rate of recovery from use-dependent block. The percent difference between V_max of the last upstrokes of the stimulus train and V_max values during the rest recovery period have been plotted for the various times the preparation was stimulated with either 4 × 10⁻⁵ M (A) or 8 × 10⁻⁵ M (○) QX-314. The data was fit to an exponential curve (τ_max = 4.6 minutes) for both drug concentrations. Data obtained from same preparation as Figure 3. Inset: representative changes in action potential configuration caused by QX-314. Left, control; right, 4 × 10⁻⁵ M QX-314 (500 msec BCL). Square pulse on differentiated trace represents 200 V/sec calibration; other calibrations as in Figure 4 inset.

![Figure 6](image2)

**Figure 6.** Modulation of tonic- and use-dependent block by resting membrane potential. Panel A: tonic block was determined by varying [K⁺]o in a step-wise manner and recording V_max during infrequent stimulation. Tonic block is demonstrated by a 7-mV displacement of the steady state inactivation curve in a hyperpolarizing direction (A) compared to control (○). During rapid stimulation, use-dependent block accounted for a progressively smaller fraction of the drug-induced reduction of V_max as the fiber was depolarized (△). Panel B: the effect of drug concentration on tonic block by QX-222. Protocol similar to Panel A: tonic block is characterized by a 2.5-mV and 5-mV hyperpolarized shift of the steady state inactivation curve with 4 × 10⁻⁵ M and 1.2 × 10⁻⁴ M QX-222, respectively.
In Figure 6A, the steady state inactivation curve representing tonic block with QX-314 (solid triangles) is parallel to the control inactivation curve and displaced 7 mV in a hyperpolarizing direction. Tonic block by QX-314 is, therefore, voltage dependent. For example, at -80 mV, $V_{\text{max}}$ was reduced from control by 28% (500 to 360 V/sec), whereas, at -70 mV, $V_{\text{max}}$ was decreased by 61% (285 to 110 V/sec). The hyperpolarizing shift of the steady state inactivation curve was dose-dependent for all concentrations of quaternary analogues tested (two expts. with QX-314 [range 2-8 X 10^-5 M], two expts. with QX-222 [range 4 X 10^-5 to 1.2 X 10^-4 M]). An example of the dose-dependent shift of the steady state inactivation curve with QX-222 is illustrated in Figure 6B. A slight reduction in the slope of the steady state inactivation curve measured at its midpoint was sometimes observed.

The reduction of $V_{\text{max}}$ during rapid stimulation is due to the sum of the tonic and use-dependent blocking effects of these agents. The contribution of use-dependent block to the reduction of $V_{\text{max}}$ was also modulated by MAV. Use-dependent block was much less pronounced for K+-depolarized fibers as compared to normally polarized fibers. For example, in Figure 6A, 85% of the reduction of $V_{\text{max}}$ (compared to drug-free control) caused by QX-314 was due to use-dependent block when the MAV was -88 mV, whereas only 28% of the reduction of $V_{\text{max}}$ was due to use-dependent block when the MAV was -74 mV (BCL = 1 sec). A decline in the contribution of use-dependent block to the reduction of $V_{\text{max}}$ of K+-depolarized fibers was observed with QX-314 (n = 3) and QX-222 (n = 2). We did not systematically investigate the effect of MAV on the reduction of $V_{\text{max}}$ by QX-572.

**Effects of Tertiary Amine and Neutral Local Anesthetics**

The characteristics of use-dependent block observed with tocainide were qualitatively similar to those of the quaternary analogues. However, the kinetics of the development and recovery from use-dependent block with tocainide were much faster than the quaternary local anesthetics (Table 2). Furthermore, higher concentrations of tocainide usually were necessary to obtain use-dependent reduction of $V_{\text{max}}$. Of sufficient magnitude to be reliably characterized. Figure 7 illustrates the effects of increasing tocainide concentrations on the rate of development of use-dependent block. Due to the smaller change of $V_{\text{max}}$ caused by rapid stimulation with tocainide compared to the quaternary analogues, data from three stimulation sequences for each drug concentration were pooled to characterize the decline of $V_{\text{max}}$ during the stimulus trains. In Figure 7A, the difference between $V_{\text{max}}$ of upstrokes during the stimulus train and the final, reduced $V_{\text{max}}$ value upon termination of stimulation is plotted. Rapid stimulation with 4 X 10^-5 M tocainide following maximal rest recovery resulted in the exponential decline of $V_{\text{max}}$ of 75 V/ sec ($X$ of 3, MAV range -86 to -88 mV) characterized by a time constant of 6.1 beats, whereas with 1 X 10^-4 M tocainide $V_{\text{max}}$ declined by 110 V/sec ($X$ of 3, MAV range -87 to -89 mV) with a shorter time constant of 3.4 beats. The more rapid kinetics of use-dependent block with tocainide as compared to the quaternary local anesthetics was not due to the higher tocainide concentrations used, since similar rapid kinetics were observed with tocainide concentrations which provided concentrations of the charged drug form equal to those employed with the quaternary analogues (Table 2). For all tocainide concentrations tested, the time constant characterizing the decline of $V_{\text{max}}$ decreased with increasing drug concentrations. Qualitatively similar results were also obtained with the tertiary amine analogue 6603 (n = 2). The rapid kinetics of use-dependent block with lidocaine prevented their further quantitation with our experimental methods.

Due to the rapid recovery kinetics with tocainide, an experimental protocol different from that described for Figure 5 was employed to investigate the rest recovery process. For these experiments, the fiber was stimulated at either a 400 or 500 msec BCL until a steady $V_{\text{max}}$ value was achieved. Stimulation then was interrupted to allow $V_{\text{max}}$ recovery. Upon resumption of stimulation, $V_{\text{max}}$ of the first upstroke was recorded, and the sequence of stimulus train/pause was repeated with a recovery period of different duration. The difference between the $V_{\text{max}}$ value of the last upstroke of the stimulus train and the upstroke following the recovery period was compared

<table>
<thead>
<tr>
<th>Tocainide concentration (M)</th>
<th>BCL (msec)</th>
<th>MAV (-mV)</th>
<th>$\tau_{\text{on}}$ (beats)</th>
<th>$\tau_{\text{off}}$ (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0 x 10^-4</td>
<td>400</td>
<td>87-88</td>
<td>5.3</td>
<td>0.87</td>
</tr>
<tr>
<td>3.0 x 10^-4</td>
<td>87-88</td>
<td>1.8</td>
<td>0.75</td>
<td></td>
</tr>
<tr>
<td>8.0 x 10^-5</td>
<td>500</td>
<td>88-89</td>
<td>3.3</td>
<td>1.58</td>
</tr>
<tr>
<td>1.6 x 10^-4</td>
<td>88-89</td>
<td>2.6</td>
<td>2.03</td>
<td></td>
</tr>
<tr>
<td>2.4 x 10^-4</td>
<td>90-91</td>
<td>1.7</td>
<td>1.07</td>
<td></td>
</tr>
<tr>
<td>8.0 x 10^-5</td>
<td>500</td>
<td>83-85</td>
<td>3.0</td>
<td>1.77</td>
</tr>
<tr>
<td>1.6 x 10^-4</td>
<td>83-85</td>
<td>2.6</td>
<td>2.03</td>
<td></td>
</tr>
<tr>
<td>3.6 x 10^-4</td>
<td>83-85</td>
<td>2.1</td>
<td>1.05</td>
<td></td>
</tr>
<tr>
<td>4.0 x 10^-5</td>
<td>400</td>
<td>91-93</td>
<td>3.4</td>
<td>1.30</td>
</tr>
<tr>
<td>1.0 x 10^-4</td>
<td>91-93</td>
<td>2.6</td>
<td>1.35</td>
<td></td>
</tr>
<tr>
<td>4.0 x 10^-5</td>
<td>400</td>
<td>86-88</td>
<td>6.1</td>
<td>1.20</td>
</tr>
<tr>
<td>1.0 x 10^-4</td>
<td>87-89</td>
<td>3.4</td>
<td>1.21</td>
<td></td>
</tr>
</tbody>
</table>

For each maintained impalement, the effect of tocainide concentration on the rate of development and recovery from use-dependent block is presented. The BCL and drug concentrations employed in each experiment are indicated. For the same concentration changes, $\tau_{\text{on}}$ was consistently reduced, with no consistent change of $\tau_{\text{off}}$. See text for further discussion.
with the maximal rest recovery following prolonged rest recovery periods, and plotted as the percent of maximal recovery vs. rest recovery duration. The rest recovery duration was measured from the time of the first absent (expected) upstroke during the recovery period to the upstroke which ended the recovery period. If the MAV varied by more than 3 mV during an experiment, the experiment was discarded. Results from one experiment are illustrated in Figure 7B, obtained from the same fiber as in Figure 7A. For all concentrations tested, tocainide did not appreciably influence the rate of recovery of $V_{\text{max}}$.

The tertiary amine analogues lidocaine, tocainide, and 6603 also caused tonic block, as evidenced by a dose-dependent shift of the steady state inactivation curve in a hyperpolarizing direction ($n = 2$ for lidocaine, 6603, $n = 1$ for tocainide). The contribution of use-dependent block to the overall reduction of $V_{\text{max}}$ during rapid stimulation decreased as the fibers were depolarized by elevating $[K^+]_o$.

Of the non-quaternary local anesthetics tested, only 6211 and benzocaine have pKa values lower than 7.4. With 6211 (93% uncharged at physiological pH) the dose-dependent reduction of $V_{\text{max}}$ was predominantly due to the tonic blocking effects of the drug, with very rapid stimulation ($\text{BCL} \leq 300 \text{ msec}$) causing minimal use-dependent block. The neutral local anesthetic benzocaine caused only tonic block. Figure 8A depicts the effect of benzocaine on the reduction of $V_{\text{max}}$ for any MAV. Increasing benzocaine concentrations caused a dose-dependent displacement of the inactivation curve in a hyperpolarizing direction. For any given benzocaine concentration and MAV, lengthening the BCL from 1 second to as long as 20 seconds caused no change of $V_{\text{max}}$. The local anesthetic effects of benzocaine were reversible, as demonstrated by the return of the inactivation curve to its control position following 70 minutes of superfusion with drug-free Tyrode’s solution. Figure 8B demonstrates further the lack of use-dependent block by benzocaine. The effect of rapid stimulation (BCL range of 1 sec to 250 msec) on $V_{\text{max}}$ was first determined during superfusion with drug-free solutions containing $[K^+]_o = 2.7, 5, 7.5, 10, \text{ and } 12 \text{ mM}$. This protocol then was repeated after equilibration with $2 \times 10^{-4} \text{ M benzocaine}$. For any MAV, any further reduction of $V_{\text{max}}$ during rapid stimulation with benzocaine was equivalent to that observed during drug-free control periods. Benzocaine also caused no changes in the reactivation kinetics of sodium channels ($2 \times 10^{-4} \text{ M, } n = 2$) as assessed using $V_{\text{max}}$ and standard membrane responsiveness protocols (Mandel and Bigger, 1971; Gettes and Reuter, 1974).

Discussion

Effect of Molecular Charge on Block of Sodium Channels

Studies on squid giant axons (Narahashi et al., 1970; Frazier et al., 1970) and frog skeletal muscle (Schwarz et al., 1977) investigating the reduction of fast inward sodium current by charged and uncharged molecular forms of local anesthetics concluded that the reduction of sodium current was due mainly to the charged form and that use-dependent block also depended on

Figure 7. The effect of tocainide concentration on the rate of development and recovery from use-dependent block. Panel A: following maximal rest recovery with either $4 \times 10^{-5} \text{ M (})$ or $1 \times 10^{-4} \text{ M (})$ tocainide, the decline of $V_{\text{max}}$ during stimulation has been plotted on a logarithmic scale as the difference between $V_{\text{max}}$ obtained at the beginning of the stimulus train and the final, reduced value of $V_{\text{max}}$. $\tau_{\text{on}}$ decreased from 6.1 to 3.4 beats following the increase in drug concentration. BCL = 400 msec. Inset: representative action potential configuration changes caused by tocainide. Upper, control; lower, $1 \times 10^{-4} \text{ M tocainide}$. Calibrations same as Figure 4, inset. Panel B: the time course of $V_{\text{max}}$ recovery has been plotted as the percent of maximum rest recovery. Increasing the drug concentration did not influence the rate of recovery of $V_{\text{max}}$. Same fiber as in panel A, $[K^+]_o = 5 \text{ mM}$. Inset: chart recording from a similar experiment illustrating protocol. Upper trace represents $V_{\text{max}}$ signal from peak-hold circuit; lower trace displays transmembrane potential. Calibrations: 100 V/sec and 50 mV vertical, 5 sec horizontal.
The local anesthetic effects of benzocaine. Panel A: a control "h" inactivation curve was first determined by varying $[K^+]_o$ in steps from 2.7 to 12 mm, noting $V_{\text{max}}$ and corresponding MAV values (○). This sequence then was repeated after equilibration with increasing benzocaine concentrations (△, $1 \times 10^{-4}$ M, □, $4 \times 10^{-4}$ M, ×, $8 \times 10^{-4}$ M) which caused a reversible, dose-dependent shift of the inactivation curve in a hyperpolarizing direction compared to drug-free control and drug washout (○).

Inset: an example of action potential changes caused by $2 \times 10^{-4}$ M benzocaine (1 sec BCL, $[K^+]_o = 2.7$ mm). Voltage and $V_{\text{max}}$ traces following benzocaine indicated by asterisk. Calibrations: 100 msec and 20 mV for voltage trace, 0.5 msec and 200 V/sec for differentiated signal. Panel B: shortening the BCL from >1 second (○, □) to 250 msec (△, △) results in slight reductions of $V_{\text{max}}$ of similar magnitude during superfusion with either benzocaine (open symbols) or drug-free Tyrode’s solution (closed symbols).

Use-dependent block was observed with the quaternary analogues and the tertiary amine analogues existing predominantly as the charged molecular form. In general, use-dependent block decreased as the pKa (and hence, proportion of tertiary amine existing as charged molecular form) decreased. For the quaternary and tertiary amine analogues, the development of use-dependent block following prolonged rest recovery periods, as well as recovery from use-dependent block during periods of quiescence, was well characterized by an exponential process (Figs. 3, 5, 7; Tables 1, 2). The rate of development of use-dependent block was accelerated with increasing drug concentrations, whereas the rate of recovery from use-dependent block for the same changes of drug concentrations was unaffected (Figs. 3, 4, 5, 7; Tables 1 and 2). No use-dependent block was observed with greater concentrations of the neutral local anesthetic benzocaine (Fig. 8B). The similar characteristics of use-dependent block for the quaternary and tertiary amine analogues and the lack of use-dependent block with benzocaine suggest that the charged form of lidocaine and these lidocaine analogues is responsible for use-dependent block of cardiac sodium channels.

Other investigations of the effects of antiarrhythmic agents on cardiac electrical activity have found characteristics of use-dependent block similar to those reported here. Courtney (1980) used quinea pig papillary muscles and reported that the exponential development of use-dependent block by both alprenolol and tocainide was accelerated with increased drug concentrations, but that the exponential process characterizing recovery from use-dependent block was insensitive to drug concentration. Heistracher (1971) reported that recovery from use-dependent block after rapid stimulation with quinidine was an exponential process not influenced by drug concentration. Similar results have been reported for procainamide (Sada et al., 1979). Development and recovery from use-dependent block with the β-blocker propafenone is an exponential process (Kohlhardt and Seifert, 1980). The similarities in the characteristics of use-dependent block for lidocaine analogues and for structurally dissimilar drugs lead us to speculate that all local anesthetic antiarrhythmic agents with net positive charge may demonstrate similar use-dependent blocking characteristics in cardiac preparations.

Since the kinetics of use-dependent block for the quaternary analogues differ, it is apparent that factors other than net positive charge determine the kinetics of use-dependent block. Molecular weight and size, as well as lipid solubility of local anesthetics, have been implicated as factors influencing use-dependent block (Courtney, 1980; Sada and Ban, 1980, 1981). Further studies are necessary to ascertain how these factors act to modify use-dependent block in different preparations.

We assume that use-dependent block results from the accumulation of drug-associated, nonconducting channels when the rate of drug association exceeds...
the rate of drug dissociation during rapid stimulation. It is possible that the association of benzocaine to sodium channels is enhanced during rapid stimulation, but that drug dissociation is so rapid that use-dependent block does not occur even at the most rapid stimulation rates possible for these preparations. If benzocaine was rapidly associating and later dissociating from sodium channels during the action potential, one might expect a delay in the recovery of drug-associated sodium channels during or immediately after the repolarization phase of the action potential. However, the rate of sodium channel "reactivation" of benzocaine-treated fibers, as assessed using membrane responsiveness protocols, was indistinguishable from that of drug-free fibers. This result suggests that benzocaine does not dissociate from sodium channels during repolarization, and supports the conclusion that activity does not modulate sodium channel block by benzocaine in cardiac fibers.

**Effect of Resting Membrane Potential on Block of Sodium Channels**

All local anesthetics studied reduced $V_{\text{max}}$ during slow stimulation. This local anesthetic effect, which we called tonic block, was shown as a displacement of the steady state inactivation curve in a hyperpolarizing direction (Figs. 6, 8). Similar effects have been reported for guinea pig fibers and isolated rat ventricular cells exposed to lidocaine (Chen et al., 1975; Lee et al., 1981). For all concentrations tested, the extent of the displacement increased with increasing drug concentrations (Figs. 6 and 8A). We did not intensively investigate possible changes in the slope of the steady state inactivation curve due to the limited number of data points acquired with the experimental protocol, as well as the time required to obtain each point. Further investigations are necessary to determine to what extent shifts in the voltage-dependence of our steady state inactivation curves represent changes in the voltage dependence of the inactivation gating mechanism of sodium channels.

Tonic block was predominantly responsible for the drug-induced reduction of $V_{\text{max}}$ of $K^+$-depolarized fibers, with rapid stimulation producing only a slight further reduction of $V_{\text{max}}$ (Fig. 6A). Although the difference between $V_{\text{max}}$ values obtained during slow and rapid stimulation was greatest for normally polarized fibers, the percent decrease of $V_{\text{max}}$ above the level of tonic block observed during rapid stimulation of $K^+$-depolarized fibers was sometimes greater than that observed for normally polarized fibers. The diminution of the contribution of use-dependent block to the reduction of $V_{\text{max}}$ of $K^+$-depolarized fibers was not due to the $K^+$-induced shortening of the action potential duration (and concomitant prolongation of the diastolic interval), since stimulation rates chosen to maintain the diastolic interval at approximately equal lengths for different $K^+$ concentration did not dramatically alter the magnitude of use-dependent block with tocinamide, and since abbreviation of the action potential duration using voltage clamp tech-

niques did not affect use-dependent block with QX-222 (unpublished observations). As a consequence of the modulation of use-dependent block by resting membrane potential, both stimulation rate and resting membrane potential must be considered when assessing the use-dependent blocking characteristics of local anesthetics.

Our conclusion that the charged form of lidocaine and its analogues is responsible for use-dependent block of cardiac sodium channels is consistent with the modulated receptor hypothesis proposed by Hille (1977b) and Hondeghem and Katzung (1977) to explain the interaction of local anesthetics with sodium channels. According to this model, the reduction of fast inward sodium current is due to the accumulation of drug-associated, nonconducting channels. Accumulation results from the binding of drug to a channel receptor which changes states or conformations as the channel cycles through the resting, open, and inactivated state, as determined by Hodgkin-Huxley kinetics. The affinity of the receptor for drug differs for the three different receptor conformations. During rapid stimulation, sodium channels spend a greater proportion of time in the open state (during the upstroke) and inactivated state (during the plateau and repolarization phases of the action potential), as compared to periods of electrical quiescence. If the affinity for charged drug of the receptor of an open or inactivated channel is greater than the affinity of a resting channel receptor, accumulation of drug-associated, nonconducting channels during rapid stimulation would be expected. For any given stimulation rate, increasing the drug concentration would, by increasing the probability of drug combining with receptor during an action potential, increase the rate of development and final magnitude of use-dependent block. If the affinity for drug of the inactivated channel receptor were greater than that of a channel in the resting state, one would expect more drug-blocked channels for depolarized fibers, such as occurs with tonic block. Further experiments are necessary to determine whether increased affinity of either open or inactivated sodium channels for charged drug forms can account for use-dependent block, as well as whether tonic and use-dependent block is due to drug association with the same channel receptor.

Conditions within the heart undoubtedly play an important role in determining the local anesthetic effects of antiarrhythmic agents. Changes in intracellular and extracellular pH, which would modify the proportion of charged and uncharged tertiary amine drug forms, have been demonstrated in the ischemic myocardium (Gerbert et al., 1971). Membrane depolarization, due to the elevation of [K$^+$], (Harris et al., 1954, Hill and Gettes, 1980), and perhaps other factors (Corr et al., 1979) within and around injured areas may occur. Changes in cardiac rate and rhythm also are likely to occur. Such changes would be expected to modulate the local anesthetic effects of antiarrhythmic agents by modifying the magnitude of tonic
and use-dependent block. This modulation could account for the different antiarrhythmic drug effects on impulse conduction observed within normal compared to ischemic or injured myocardial tissue (Bigger and Hoffman, 1980). Further studies are necessary to determine the effects of these agents and modulating factors in vivo. The results reported here should aid in determining which electrophysiological mechanisms may be responsible for antiarrhythmic drug efficacy.

We would like to thank Drs. H. G. Vassallo and R. A. Ronfeld of Astra Pharmaceutical Products, Worcester, Massachusetts, for the generous supply of lidocaine and lidocaine analogues. We would also like to thank Dr. David Gadsby for comments on the manuscript. Dr. Ira Cohen for his criticisms and hospitality, and Maryann Gintant for secretarial assistance. This work was presented as part of Young Investigators Awards Competition at the 31st Scientific Session of the American College of Cardiology. Supported in part by grants HL-08508-18 and HL-12738-13 from the NHLBI.

Address for reprints: Dr. Brian F. Hoffman, Department of Pharmacology, College of Physicians and Surgeons, Columbia University, 630 West 168th Street, New York, New York 10032. Received December 28, 1981; accepted for publication April 29, 1983.

References


Frazier DT, Narahashi T, Yamada M (1970) The site of action and active form of local anesthetics. II. Experiments with quaternary compounds. J Pharmacol Exp Ther 171: 45-51


Hille B (1977a) The pH-dependent rate of action of local anesthetics on the node of Ranvier. J Gen Physiol 69: 475-496

Hille B (1977b) Local anesthetics: Hydrophilic and hydrophobic pathways for the drug-receptor reaction. J Gen Physiol 69: 497-515


Hodgkem LM (1975) Validity of Vmax as a measure of the sodium current in cardiac and nervous tissues (Letter to the Editor). Biophys J 23: 147-152, 1978

Hodgkem LM (1981) Cardiac sodium channels are different from nerve and skeletal muscle sodium channels (abstr). Biophys J 33: 211a


Isenberg G (1976) Cardiac Purkinje fibers: Cesium as a tool to block inward rectifying potassium currents. Pfluegers Arch 365: 99-106


Isenberg G, Klickvern V (1982) Calcium currents of isolated bovine ventricular myocytes are fast and of large amplitude. Pfluegers Arch 395: 30-41

Johnson EA, McKinnon MG (1957) The differential effect of quinidine and pyrilamine on the myocardial action potential at various rates of stimulation. J Pharmacol Exp Ther 120: 460-468


Kupersmith J, Antman EM, Hoffman BF (1975) In vivo electro-

Downloaded from http://circres.ahajournals.org/ by guest on October 18, 2017
Sada H, Kojima M, Ban T (1979) Effect of procarainamide on transmembrane action potentials in guinea pig papillary muscles as affected by external potassium concentration. Naunyn Schmiedebergs Arch Pharmacol 309: 179-190
Weidmann S (1955) Effect of the cardiac membrane potential on the rapid availability of the sodium-carrying system. J Physiol (Lond) 127: 213-224
INDEX TERMS: Antiarrhythmic drugs • Local anesthetics • Cardiac sodium channels • Use-dependent block • Lidocaine
The influence of molecular form of local anesthetic-type antiarrhythmic agents on reduction of the maximum upstroke velocity of canine cardiac Purkinje fibers.

G A Gintant, B F Hoffman and R E Naylor

Circ Res. 1983;52:735-746
doi: 10.1161/01.RES.52.6.735

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1983 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/52/6/735

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/