

# Effect of Lidocaine Hydrochloride on Membrane Conductance in Mammalian Cardiac Purkinje Fibers

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**ABSTRACT** Lidocaine depresses automaticity in cardiac Purkinje fibers by decreasing the slope of slow diastolic depolarization, but the mechanisms of this effect are poorly understood. To test the proposal that the antiautomatic effect of lidocaine might be mediated by an increase in membrane potassium conductance, transmembrane voltage ( $V_m$ ) was measured in Purkinje fibers perfused with sodium-deficient Tyrode containing choline as the major cation.  $V_m$  was varied by altering the external potassium concentration,  $[K]_o$ , from 0.5 to 150 mM before and after lidocaine,  $2.14 \times 10^{-5}$  M, a concentration considered equivalent to clinical plasma antiarrhythmic levels. In Purkinje fibers, resting  $V_m$  varies linearly with  $[K]_o$  plotted on a logarithmic scale from 4 to 150 mM, approximately as predicted by the Nernst equation. At  $[K]_o$  of 0.5–2.7 mM, resting  $V_m$  diverges from the predicted potassium equilibrium potential ( $V_K$ ) resulting in an increased driving force for the outward  $K^+$  current ( $V_m - V_K$ ). In choline Tyrode at  $[K]_o$  of 2.7 mM or less, lidocaine caused a significant increase in  $V_m$ , the change being a positive linear function of ( $V_m - V_K$ ) with a  $P < 0.01$ . This effect was more striking in Purkinje fibers with a  $V_m$  reduced by stretch. These findings imply that lidocaine increased membrane chord conductance for the potassium ion (gK).

Current-voltage relationships using intracellular current pulses were performed in choline Tyrode at  $[K]_o$  of 0.5, 2.0, and 4.0 mM and, at each  $[K]_o$ , lidocaine was found to increase membrane slope conductance (GK). The increase in GK was even more apparent when the

current-voltage relationships in long Purkinje fibers was corrected for cable complications or when experiments were done in short Purkinje fibers. To minimize complications due to membrane rectifier properties, GK was measured using intracellular application of small hyperpolarizing current pulses as  $V_m$  was decreased from  $-90$  to  $-60$  mv by increasing the  $[K]_o$  from 3 to 15 mM before and after lidocaine. Lidocaine increased the GK over this range of  $V_m$ .

These results suggest that lidocaine increases membrane potassium conductance within the range of  $V_m$  where the pacemaker potential is seen, an action which can account for its ability to suppress automaticity, and, in part, for its ability to prevent reentrant arrhythmias.

## INTRODUCTION

The efficacy of lidocaine hydrochloride in the treatment of cardiac ventricular arrhythmias after open heart surgery, myocardial infarction, and digitalis has been established and recently reviewed (1). Electrophysiologic investigations have shown that in cardiac Purkinje fibers, lidocaine: (a) shortens the action potential duration and decreases the effective refractory period, and (b) exerts an antiautomatic effect by decreasing the slope of slow diastolic depolarization without affecting the maximum diastolic transmembrane voltage (2-4). These observations led Bigger and Mandel to postulate that lidocaine may increase potassium conductance in mammalian cardiac Purkinje fibers (3). The present experiments were designed to test this hypothesis in sheep Purkinje fibers using microelectrode techniques. The effects of lidocaine in a concentration equivalent to clinical plasma antiarrhythmic levels on the transmembrane voltage and on current-voltage relationships suggest that lidocaine does increase potassium conductance in Purkinje fibers.

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## METHODS

Young adult female sheep were anesthetized with sodium pentobarbital (30 mg/kg). The heart was rapidly removed and false tendon (Purkinje fiber) preparations were selected for study. Our standard techniques for stimulating and recording have been previously described (5,6). When current-voltage relationships were studied, methods similar to those of Weidmann (7) and Hall, Hutter, and Nobel (8) were employed. Fig. 1 summarizes the experimental arrangement. Long Purkinje fibers were employed which were either unbranched or were of sufficient length to permit impalement with two microelectrodes at a distance of 8-10 mm from any branches. In some experiments, the Purkinje fiber was divided into segments of less than 2 mm by ligation with 6-0 nylon thread—so-called "short" Purkinje fibers (9). A pair of extracellular silver wire electrodes, insulated to the tip, was placed on the surface of the fiber and used for stimulation. A constant current generator<sup>1</sup> provided rectangular anodal or cathodal pulses, the amplitude and duration of which could be varied. Constant current pulses of 100 msec duration were passed intracellularly through a glass microelectrode filled with either 3 M KCl or 2.5 M potassium citrate. The current was led by a chloride-coated-silver wire placed in the tissue bath to an operational amplifier.<sup>2</sup> This amplifier amplified the current a millionfold and kept the bath at virtual ground. The amplified current pulse was displayed on a dual-beam cathode ray oscilloscope.<sup>3</sup> Transmembrane voltage was recorded with a second glass microelectrode which was placed within 50-200  $\mu$  of the stimulating microelectrode. The transmembrane voltage was amplified by a high input impedance and variable capacity neutralization amplifier<sup>4</sup> and displayed on the dual-beam oscilloscope. The oscilloscopic display of transmembrane voltage and current was photographed<sup>5</sup> and the enlarged images measured.

The Tyrode solution employed contained, in mmoles per liter: NaCl, 137; MgCl<sub>2</sub>, 0.5; NaH<sub>2</sub>PO<sub>4</sub>·H<sub>2</sub>O, 1.8; CaCl<sub>2</sub>, 1.8; 1.8; NaHCO<sub>3</sub>, 12; dextrose, 5.5; potassium concentration was varied. Sodium-deficient Tyrode solution was prepared by substituting choline chloride for sodium chloride on a mole for basis. The sodium-deficient Tyrode contained 13.8 mM sodium. Sodium-deficient Tyrode in which choline is the major cation is known to produce minimal changes in resting membrane conductance and to have little effect on the membrane current-voltage relationships; the findings in choline Tyrode are comparable to those observed in a completely sodium-free solution or those in normal Tyrode at subthreshold voltages (8). In sodium-deficient Tyrode, the effect of the sodium conductance on transmembrane voltage ( $V_m$ )<sup>6</sup> is minimized and the resting membrane conductance is determined primarily by potassium

<sup>1</sup> Designed by Mr. S. Ross, Columbia University, New York.

<sup>2</sup> Philbrick/Nexus Research, Dedham, Mass. (P 25).

<sup>3</sup> Tektronix, Inc., Beaverton, Ore.

<sup>4</sup> Bioelectric Instruments Div., General Microwave Corp., Farmingdale, N. Y. (NF1).

<sup>5</sup> Grass Instrument Co., Quincy, Mass.

<sup>6</sup> Abbreviations used in this paper:  $C_m$ , membrane capacitance; GK, membrane slope conductance for potassium ion; GM, membrane slope conductance; gK, membrane chord conductance for potassium ion; gM, membrane chord conductance; I, applied current;  $I_i$ , ionic current;  $I_K$ , membrane potassium current;  $i_m$ , membrane current density;  $[K]_i$ , intracellular potassium concentration;  $[K]_o$ , external potassium concentration;  $P_K$ , membrane permeability for potassium ion;  $V_K$ , potassium equilibrium potential;  $V_m$ , transmembrane voltage;  $\tau_D$ , membrane time constant determined with depolarizing current pulses.

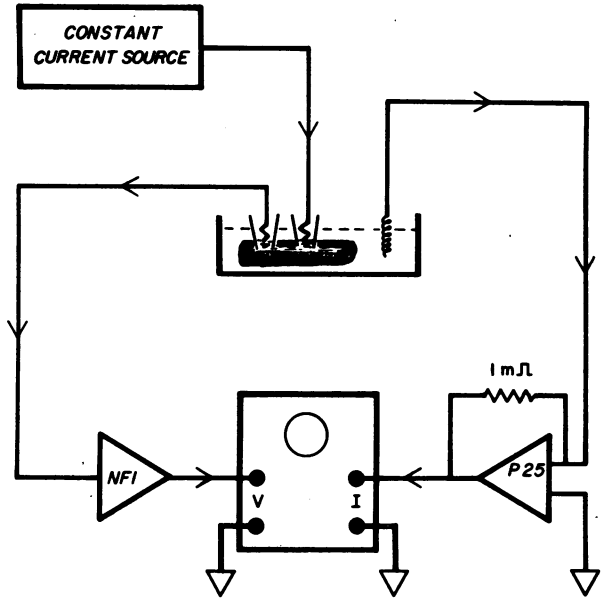


FIGURE 1 Experimental arrangement used for stimulating and for recording both the applied intracellular current and resultant transmembrane voltage change during the study of current-voltage relationships in Purkinje fibers. See Methods.

conductance with other ions playing a minor role (10-14). When high external potassium concentrations  $[K]_o$  of 10-120 mM were used, the solutions were made isomolar by decreasing choline.

The Tyrode solution was equilibrated with 95% oxygen and 5% carbon dioxide in a reservoir and infused into the tissue bath at a constant rate of 8 ml/min. In the tissue bath temperature was maintained between 35.5 and 36.5°C and the pH was 7.36.

Lidocaine hydrochloride was used in a concentration of  $2.14 \times 10^{-5}$  M (5  $\mu$ g/ml). In cardiac Purkinje fibers, this concentration both abbreviates the action potential duration and exerts a strong antiautomatic effect by decreasing the slope of slow diastolic depolarization (2-4). For the reasons discussed by Bigger and Mandel, this concentration is considered equivalent to clinical antiarrhythmic plasma levels (3).

The statistical significance of changes before and after lidocaine was determined by the *t* test for paired samples (15). Correlation coefficients and levels of statistical significance were determined by standard methods (15).

## RESULTS

*Effect of lidocaine on the transmembrane voltage characteristics of the Purkinje fiber in a sodium-deficient Tyrode solution at various external potassium concentrations.* In our experiments, the mole for mole substitution of choline chloride for sodium chloride produced either no change or a small increase in the resting transmembrane voltage ( $V_m$ ) similar to that seen in previous investigations (8, 16). Spontaneous diastolic depolarization did not occur in the sodium-deficient Tyrode solution nor could electrically induced regenerative depolarization be elicited in the Purkinje fibers studied.

TABLE I  
Effect of Lidocaine on Resting Transmembrane Voltage Characteristics\* and Potassium Permeability in Sodium-Deficient Tyrode at Various  $[K]_o$ .

Control		Lidocaine			
$[K]_o$	<i>n</i>	Resting $V_m$	Resting $V_m$	<i>P</i>	$P_{K_{lidocaine}}/P_{K_{control}}$
<i>mM</i>		<i>mv</i>	<i>mv</i>		
0.5	4	-51.7 ± 6.17	-64.8 ± 8.08	<0.001	1.89
1.0	4	-64.4 ± 4.18	-75.6 ± 4.93	<0.005	1.65
1.5	4	-73.8 ± 8.30	-84.2 ± 8.30	<0.001	1.68
2.0	4	-78.0 ± 5.45	-84.8 ± 5.82	<0.005	1.45
2.7	4	-87.8 ± 4.04	-90.0 ± 2.35	<0.010	1.27

No significant change in  $V_m$  was noted in 12 experiments at  $[K]_o$  of 4.0, 10.0, 60.0, 90.0, and 120.0 mM.

\* Mean ± SD *n* = number of preparations; resting  $V_m$  = resting transmembrane voltage (mv); *P* = probability based on *t* test for paired samples;  $P_{K_{control}}$  = potassium permeability, control;  $P_{K_{lidocaine}}$  = potassium permeability after lidocaine.

In each experiment, multiple impalements were employed to determine the resting  $V_m$  before and after the application of lidocaine. Results obtained in 32 different preparations appear in Table I. In Fig. 2, the mean resting  $V_m$  from these experiments is plotted

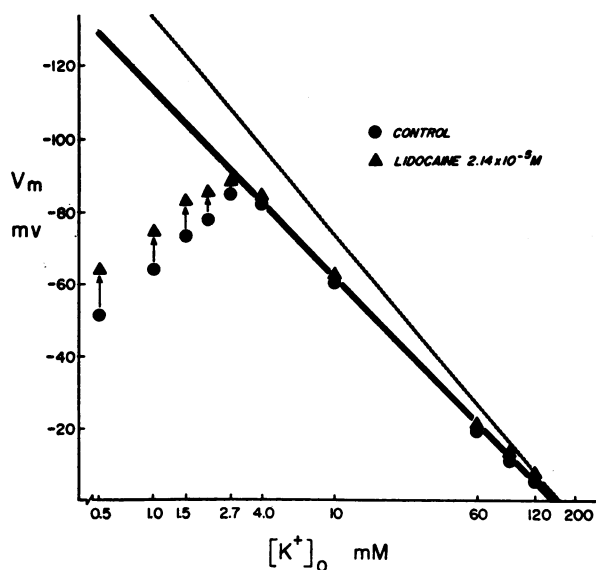


FIGURE 2 The resting transmembrane voltage ( $V_m$ ) in sodium-deficient Tyrode during the control period (circles) and after lidocaine (triangles) is plotted as a function of  $[K]_o$  on a logarithmic scale. The arrows show the direction of change. The heavy line is a regression line determined from experimental results at  $[K]_o$  of 10, 60, 90, and 120 mmole/liter which has been extrapolated both to the abscissa and towards the ordinate. The thin line represents  $V_K$  as calculated from the Nernst equation. Significant hyperpolarization occurs after the application of lidocaine at  $[K]_o$  of 2.7 mM and less. See text for discussion.

as a function of  $[K]_o$  on a logarithmic scale. Under control conditions (circles), the resting  $V_m$  was determined at  $[K]_o$  of 10, 60, 90, and 120 mM to calculate from experimental observations a regression line (the heavy line). Extrapolation of the regression line intersects the abscissa at  $[K]_o$  of 150 mM giving an estimate of the intracellular potassium concentration (13). The thin line represents the potassium equilibrium potential ( $V_K$ ) as predicted from the Nernst equation were the membrane of the Purkinje fiber acting as a perfect potassium electrode, assuming  $[K]_i$  to be 150 mM. The regression line was also extrapolated towards the ordinate and represents the relationship between  $V_m$  and  $[K]_o$  plotted on a logarithmic scale were the membrane conductance constant over the entire range of  $[K]_o$  employed in these experiments.

According to the Nernst equation, at a temperature of 36°C, a 61 mv change in resting  $V_m$  is expected for every decade change in  $[K]_o$ . The regression line derived from our experimental observations showed a 51 mv change in resting  $V_m$  for every decade change in  $[K]_o$ . Approximately the same divergence between the predictions of the Nernst equation and the experimental observations has been previously noted in normal saline Tyrode (17). In the present experiments, the approximation of  $V_K$  as derived from the experimental observations has been employed in the calculations. The interpretation of our results is unaffected whether one or the other  $V_m$  is used in the calculations.

Since a sodium-deficient Tyrode solution was employed and chloride ions are known to play only a minor role in determining the resting ionic current ( $I_i$ ) of the resting Purkinje fiber (10, 11), resting  $I_i$  can be expressed as:

$$I_i \approx I_K = gK(V_m - V_K), \quad (1)$$

where  $I_K$  is potassium current and  $gK$  is membrane chord conductance for potassium ion. Note that under control conditions in Fig. 2,  $V_m$  approximates  $V_K$  above a  $[K]_o$  of 4.0 mM. As  $[K]_o$  is further lowered, increasing divergence is noted between  $V_m$  and  $V_K$  due to a decrease in the membrane conductance to the outward flow of potassium ions (8, 17). These results agree well with the radioactive potassium efflux studies of Carmeliet which were conducted over a wide range of  $[K]_o$  (10, 11). From Fig. 2, it is clear that the driving force for the potassium ion ( $V_m - V_K$ ) increases as  $[K]_o$  is progressively lowered below 4.0 mM (18).

After the application of lidocaine (Table I and Fig. 2), statistically significant hyperpolarization occurred when ( $V_m - V_K$ ) was significantly greater than zero, i.e., at  $[K]_o$  of 2.7 mM or less. The magnitude of the hyperpolarization induced by lidocaine was positively correlated with the magnitude of the driving force

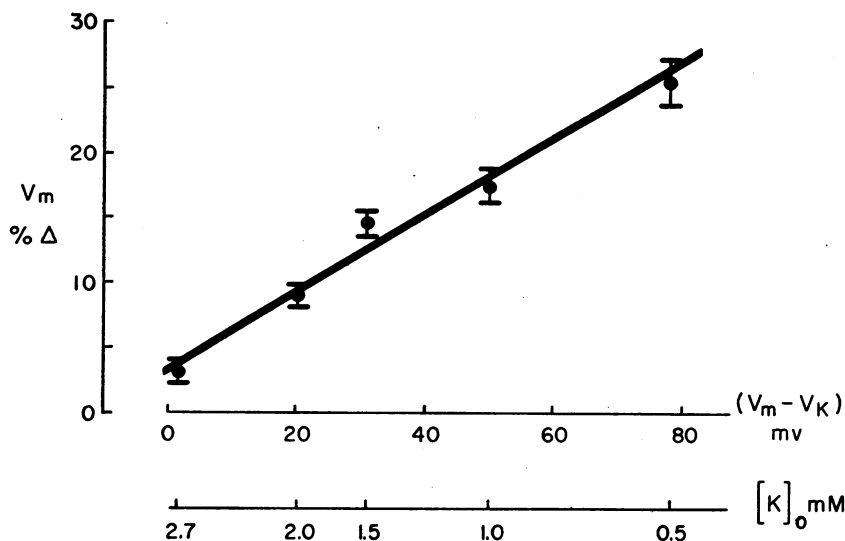


FIGURE 3 The per cent change in resting transmembrane voltage ( $V_m$ ) caused by application of lidocaine [ $(V_{m\text{lidocaine}} - V_{m\text{control}}) \times 100$ ] is plotted as a function of the outward driving force for the potassium ion ( $V_{m\text{control}} - V_K$ ).  $[K]_o$  is also indicated on the abscissa. A strong linear positive correlation exists between the per cent change in  $V_m$  and  $(V_m - V_K)$  ( $r = 0.99$ ,  $P < 0.01$ ).

( $V_m - V_K$ ) for the potassium ion. This is seen in Fig. 3 which shows the mean per cent change in resting  $V_m$  to be a linear function of  $(V_m - V_K)$  ( $r = 0.99$ ,  $P < 0.01$ ). As predicted from equation 1, no significant change in the resting  $V_m$  was noted after the application of lidocaine (Table I and Fig. 2) when  $(V_m - V_K)$  approached zero at  $[K]_o$  of 4.0 mM or above.

It is possible to calculate the permeability for the potassium ion ( $P_K$ ) of the resting membrane with the Goldman equation using the change in  $V_m$  caused by lidocaine at  $[K]_o$  of 2.7 mM or less (19-21). These calculations showed clearly that  $P_K$  increased after the application of lidocaine (Table I).

*Effect of lidocaine on transmembrane voltage characteristics of stretched Purkinje fibers.* Lidocaine had a remarkable restorative effect on  $V_m$  of fibers with a decreased resting or maximum diastolic transmembrane voltage due to stretch. In Fig. 4, panel A shows a spontaneous action potential of a stretched fiber in normal Tyrode solution at a  $[K]_o$  of 2.7 mM. The preparation had been stimulated electrically through external electrodes at a cycle length of 800 msec for 1 hr after which spontaneous depolarization was allowed to stabilize for an additional 30 min. The maximum diastolic transmembrane voltage was  $-70$  mv. Panel B of Fig. 4 was recorded after 20 min of quiescence caused by the substitution of sodium-deficient Tyrode for normal Tyrode at the same  $[K]_o$ . At this time,  $V_m$  was  $-61$  mv, essentially unchanged from the maximum diastolic transmembrane voltage in normal Tyrode. Panel C of Fig. 4 was recorded in sodium-deficient Tyrode at the

same  $[K]_o$  30 min after the application of lidocaine at which time the resting  $V_m$  was  $-93$  mv. Similarly, lidocaine produced a significant hyperpolarization in five other fibers which showed a decreased resting  $V_m$  or maximum diastolic transmembrane voltage due to stretch; two of these experiments were performed at

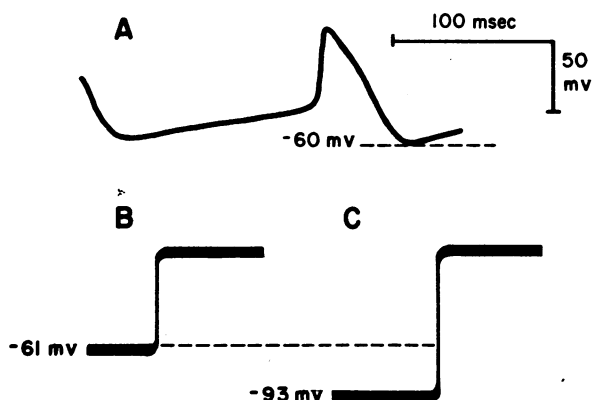


FIGURE 4 The effect of lidocaine on a sheep Purkinje fiber injured by stretching ( $[K]_o = 2.7$  mM in each panel).

(A) Transmembrane voltage ( $V_m$ ) recorded in normal Tyrode shows markedly reduced maximum diastolic  $V_m$  and automaticity. (B) Shows the resting transmembrane voltage recorded in sodium-deficient Tyrode. Spontaneous activity ceased when sodium was reduced. The figure shows withdrawal of the microelectrode to determine resting  $V_m$ . The resting transmembrane voltage was  $-61$  mv. (C) 30 min after adding lidocaine the resting transmembrane voltage was  $-93$  mv a 32 mv hyperpolarization.

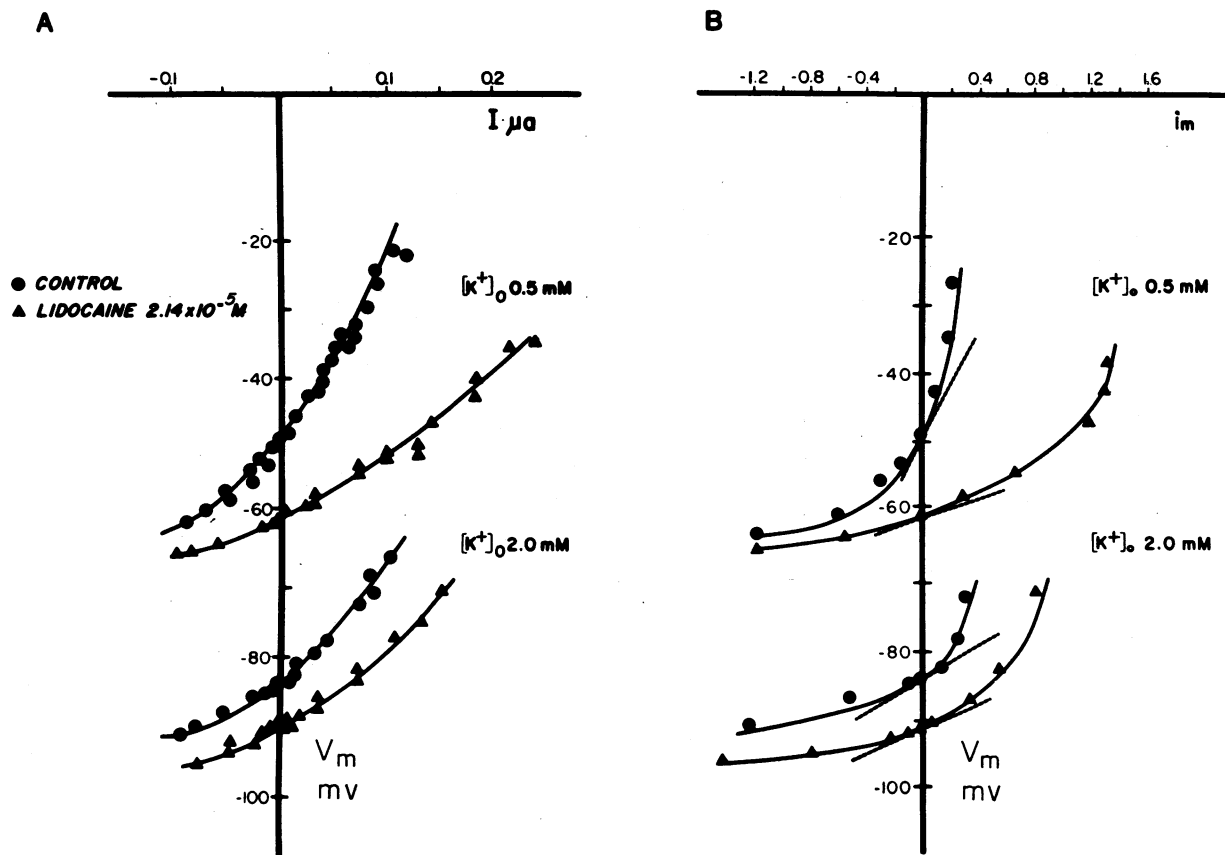


FIGURE 5 Current-voltage relationships in long Purkinje fibers perfused with sodium-deficient Tyrode at  $[K]_o$  of 0.5 mM (upper curves, experiment 25, Table II) and 2.0 mM (lower curves, experiment 20, Table II) before (circles) and after (triangles) the application of lidocaine. In panel A, the observed transmembrane voltage ( $V_m$ ) is plotted as a function of the applied intracellular current ( $I$ ) in  $\mu$ a. In Panel B, the membrane current density ( $i_m$ ) was calculated to correct for cable complications; the observed  $V_m$  is plotted as a function of  $i_m$  in arbitrary units. Slope conductance (GM) was found to increase after lidocaine for both depolarizing and hyperpolarizing pulses over the entire range of the curves at both values of  $[K]_o$ . For depolarizing currents, the increased GM was manifested by the curve becoming less steep and shifting to the right after lidocaine. Since the GM for both depolarizing and hyperpolarizing pulses increases after lidocaine, the curves might be expected to cross-over (23) but this does not occur due to the increase in resting  $V_m$ . Chord conductance ( $g_M$ ) was estimated from tangents drawn to the curves in panel B (the interrupted lines) at  $i_m = 0$ . The ratio of resting membrane conductance after lidocaine as compared to the control value was 3.79 at  $[K]_o = 0.5$  mM and 1.30 at  $[K]_o = 2.0$  mM. See text for further discussion.

$[K]_o$  of 2.7 mM and one each at  $[K]_o$  of 0.5, 1.5, and 4.0 mM. These results suggest that stretch decreases membrane potassium conductance thus increasing or creating a difference between  $V_m$  and  $V_K$ . Lidocaine, by increasing membrane potassium conductance, causes  $V_m$  to approach  $V_K$  and hyperpolarizes the membrane.

*Current-voltage relationships in long Purkinje fibers.* Membrane current-voltage relationships of long Purkinje fibers were measured in sodium-deficient Tyrode before and after the application in a total of nine experiments. Three experiments were performed at each

of three  $[K]_o$ : 0.5, 1.5, and 4.0 mM. Representative experiments of the effect of lidocaine on membrane current-voltage relationships are shown in Fig. 5 ( $[K]_o = 0.5$  and 2.0 mM) and Fig. 6 ( $[K]_o = 4.0$  mM). In panel A of Figs. 5 and 6, the observed  $V_m$  is plotted as a function of the amplitude of the applied current ( $I$ ). In panel B of Figs. 5 and 6, the fiber is considered an infinite cable and the relationship suggested by Cole and Curtis (22) to correct for cable complications was used to estimate the membrane current density ( $i_m$ ):

$$i_m \approx I \cdot (dI/dV). \quad (2)$$

I was measured directly and tangents were constructed to the curves in panel A of Figs. 5 and 6 to obtain  $dI/dV$ ; these two values were used to solve equation 2 for  $i_m$ . The observed  $V_m$  was then plotted as a function of  $i_m$  (panels B of Figs. 5 and 6). The cable properties of the Purkinje fiber tend to "smooth-out" nonlinearities in the membrane current-voltage relationship, but by mathematically correcting for cable complications using equation 2, one can approximate the relationship for a uniformly depolarized membrane which accentuates the curvatures in the membrane current-voltage relationship (8, 22).

The membrane slope conductance (GM) can be defined by the following:

$$\text{Panel A of Figs. 5 and 6} \quad GM = dI/dV, \quad (3)$$

$$\text{Panel B of Figs. 5 and 6} \quad GM = di_m/dV. \quad (4)$$

GM was determined by constructing tangents to the curves in Figs. 5 and 6. Lidocaine was found to increase GM over the entire curve at each of the three  $[K]_o$  studied. For depolarizing currents, the curve became less steep and shifted to the right after lidocaine in all experiments. Since the GM for both depolarizing and hyperpolarizing pulses increases after lidocaine, the curves might be expected to "cross-over" (23), and, indeed, this occurs at a  $[K]_o$  of 4.0 mM where the resting  $V_m$  is unaffected (Fig. 6). Crossing-over did not occur

at either a  $[K]_o$  of 0.5 or 2.0 mM due to the increase in resting  $V_m$  after lidocaine (Fig. 5). The increase in  $V_m$  after application of lidocaine at  $[K]_o$  of 0.5 and 2.0 mM might in itself produce a voltage-dependent increase in membrane conductance (23). Further experiments, to be discussed below, were designed to determine whether the increase in GM was due to lidocaine or to the increased  $V_m$  per se.

The steady-state membrane current-voltage relationship gives an estimate of the slope conductance (GM) rather than the chord conductance ( $gM$ ) of the resting membrane. The relationship between GM and GK can be described by the following when equation 1 pertains:

$$GM \approx GK = dI_K/dV = gK + (V_m - V_K) \frac{dgK}{dV}, \quad (5)$$

when  $I$  or  $i_m = 0$ , GK approximates  $gK$ .

Table II lists the  $gK$  at  $I$  or  $i_m = 0$ , the polarization resistance, and the ratio of  $gK$  after lidocaine as compared to the control for three experiments each at  $[K]_o$  of 0.5, 2.0, and 4.0 mM. In every experiment, the  $gK$  increased and the polarization resistance decreased after the application of lidocaine.

*Current-voltage relationships in short Purkinje fibers.* In two experiments, current-voltage relationships were determined in short Purkinje fibers in sodium-deficient Tyrode at a  $[K]_o$  of 4.0 mM. The fibers were shortened

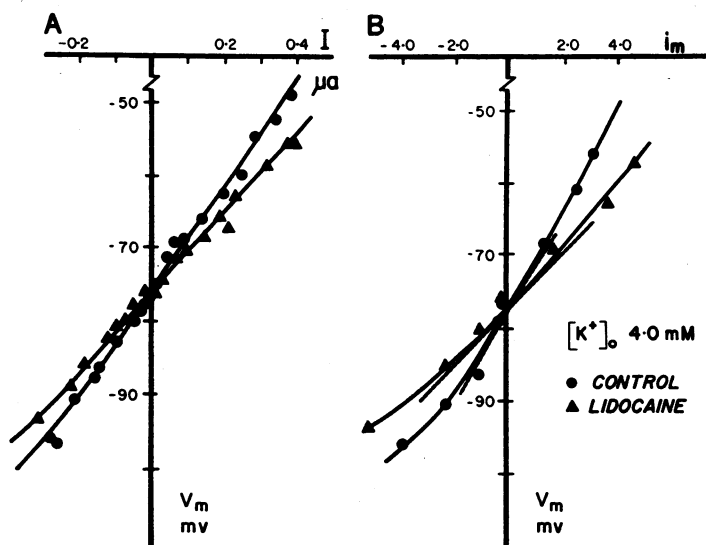


FIGURE 6 Current-voltage relationships in a long Purkinje fiber at  $[K]_o = 4.0$  mM (experiment 30A, Table II). The symbols and calculations are as in Figure 7. The slope conductance (GM) for both depolarizing and hyperpolarizing intracellular currents increased after lidocaine, and since no shift in the resting  $V_m$  occurred, crossing-over of the curves (23) was noted at  $I$  or  $i_m = 0$ . For depolarizing currents, the slope became less steep and the curve was shifted to the right. The ratio of membrane conductance at the resting  $V_m$  after lidocaine as compared to the control was 1.74. See text for further discussion.

TABLE II  
*Effect of Lidocaine on Membrane Conductance and Polarization Resistance as Determined from Current-Voltage Relationships in Long and Short Sheep Purkinje Fibers at Various  $[K]_o$  in Sodium-Deficient Tyrode*

Control				Lidocaine		
$[K]_o$	Exp.	gK	Polarization resistance	gK	Polarization resistance	$gK_{\text{lidocaine}}/gK_{\text{control}}$
		$\mu\text{mho}$	$k\Omega$	$\mu\text{mho}$	$k\Omega$	
Long Purkinje fibers						
0.5	50	2.08	480	2.65	377	1.27
	24	4.35	230	5.26	190	1.21
	25	2.74	364	10.20	98	3.72
2.0	16	1.89	530	1.96	510	1.04
	20	3.52	284	4.59	218	1.30
	14	2.00	500	6.58	152	3.29
	37	3.21	312	3.93	254	1.22
	40	4.18	239	5.37	186	1.28
	30A	5.95	168	7.46	134	1.25
Short Purkinje fibers*						
4.0	51A	1.77	567	2.77	361	1.56
	51B	2.12	471	2.99	334	1.41

gK = membrane potassium chord conductance from GK at  $I = 0$  or for small hyperpolarizing pulses (see text and equations 8 and 9);  $gK_{\text{lidocaine}}/gK_{\text{control}}$  = ratio of membrane conductance after lidocaine to that of the control.

\* Segments less than 2 mm in length.

by a ligature to lengths of less than 2.0 mm. In the short preparation, cable complications are minimized and applied current produces fairly uniform membrane polarization throughout the preparation (9). In this experimental arrangement, the intracellularly applied current ( $I$ ) approximates the membrane current density ( $i_m$ ) which had to be calculated in experiments on long Purkinje fibers (see equation 2 and the discussion of  $i_m$  above). Fig. 7 shows the current-voltage relationship obtained in a short fiber before and after the application of lidocaine. Resting  $V_m$  was unchanged and the polarization resistance of the preparation decreased after lidocaine (Table II). Over the entire current-voltage relationship, the slope became less steep and the curve was shifted to the right after lidocaine, i.e., crossing-over (23) of the curves occurred. These changes all indicate an increase in GM (GK). Both GM and gK as determined from tangents constructed to the curves in Fig. 7 were increased. The magnitude of change is equivalent to that noted in long Purkinje fibers and directly confirms the validity of mathematically correcting for cable properties. Lidocaine, therefore, increases membrane gK at a  $[K]_o$  of 4.0 mM, a normal value for plasma or extracellular fluid  $K^+$ .

*Membrane time constant in long Purkinje fibers.* The membrane time constant was calculated from the voltage decay curve after offset of depolarizing current

pulses ( $\tau_D$ ). Further:

$$\tau_D = C_m/GM, \quad (6)$$

where  $C_m$  represents the membrane capacitance and GM the membrane slope conductance. Since membrane capacitance is thought to remain constant (24, 25), it would be anticipated that if lidocaine increased GM,  $\tau_D$  should decrease. It is clear from Table III that lidocaine did cause a significant decrease in  $\tau_D$  at  $[K]_o$  of 0.5, 2.0, and 4.0 mM. Time constants calculated from hyperpolarizing pulses, showed essentially the same change as  $\tau_D$ .

*Membrane conductance changes before and after lidocaine while varying the resting  $V_m$  by altering  $[K]_o$ .* In three experiments, the resting  $V_m$  of the Purkinje fiber was gradually decreased by increasing the  $[K]_o$  from 3.0 to 15.0 mM in sodium-deficient Tyrode. Studies in this  $[K]_o$  range obviate the voltage-dependent conductance changes caused by a lidocaine-induced shift in resting  $V_m$  as occurred in experiments at a  $[K]_o$  of 2.7 mM or less. Depolarization of the membrane by increasing  $[K]_o$  increases membrane potassium conductance (8, 25, 26) unlike depolarization by applied current pulses which results in decreased membrane potassium conductance (8, 23, 26-30).

Membrane slope conductance (GM) was calculated from small intracellular hyperpolarizing pulses of con-

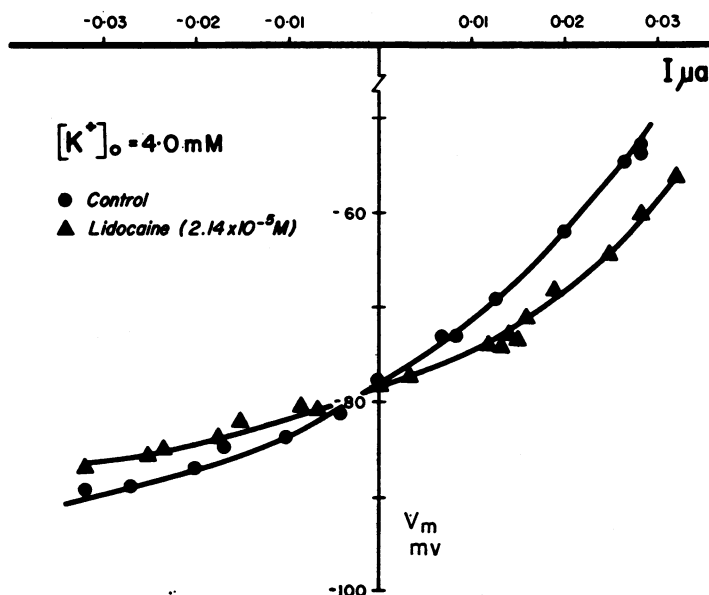


FIGURE 7 Current-voltage relationships determined in a short Purkinje fiber (1.6 mm) at  $[K^+]_o = 4.0$  mM before (circles) and after the application of lidocaine (triangles). The use of a short fiber minimizes cable complications, and much less applied current is required to produce a change in the transmembrane voltage ( $V_m$ ) than in long Purkinje fibers (see Fig. 8). After lidocaine, depolarizing currents result in a less steep curve which is shifted to the right, and with no change in the resting  $V_m$ , hyperpolarizing pulses result in crossing-over at  $I = 0$ . These results indicate that lidocaine increased membrane slope conductance for both depolarizing and hyperpolarizing pulses. At  $I = 0$ , i.e., at the resting  $V_m$ , the ratio of membrane conductance after lidocaine as compared to the control was 1.56. See text for further discussion.

stant amplitude which resulted in small membrane voltage changes ( $< -7$  mv). For the reasons discussed above, GM in sodium-deficient Tyrode is determined primarily by GK; GM measured by small hyper-

polarizing pulses approximates resting membrane chord conductance ( $gK$ ).

In Fig. 8, GM is plotted as a function of the resting  $V_m$  as the  $V_m$  was gradually decreased by increasing the  $[K^+]_o$  from 3.0 to 15.0 mM. Both the control data and the data after lidocaine showed a good fit (least squares method) for the exponential regression of GM on  $V_m$  ( $P < 0.01$ ). The exponential character of the curve may be accounted for by the exponential relationship between  $V_m$  and  $[K^+]_o$  (see Fig. 2). Clearly, lidocaine produces an increase in GM over the range of  $V_m$  studied. The ratio of  $GM_{\text{lidocaine}}/GM_{\text{control}}$  varied between 1.14 and 1.20 which is very similar to the ratios found in both long and short Purkinje fibers as calculated from current-voltage relationships (Table II) and to the permeability ratios calculated with the Goldman equation from the change in  $V_m$  lidocaine was applied to fibers perfused with  $[K^+]_o \leq 2.7$  mM.

TABLE III  
Membrane Time Constant in Long Purkinje Fibers

$[K^+]_o$	$\tau_{D\text{control}}$	$\tau_{D\text{lidocaine}}$	% $\Delta$	$\tau_{D\text{control}}/\tau_{D\text{lidocaine}}$
mM	msec	msec		
0.5	42.0	13.0	66.7	3.23
	24.6	14.8	39.8	1.78
2.0	16.6	12.4	25.3	1.34
	27.0	21.1	22.0	1.28
	24.4	18.4	24.6	1.32
4.0	22.7	14.9	34.4	1.56
	20.5	13.7	33.2	1.49
	18.0	15.3	15.0	1.18

$\tau_{D\text{control}}$  = membrane time constant, control;  $\tau_{D\text{lidocaine}}$  = membrane time constant after lidocaine; % $\Delta$  = per cent decrease in  $\tau_D$  after lidocaine.

## DISCUSSION

Previous electrophysiologic investigations have shown that lidocaine in concentrations equivalent to clinical antiarrhythmic plasma levels shortens the action po-



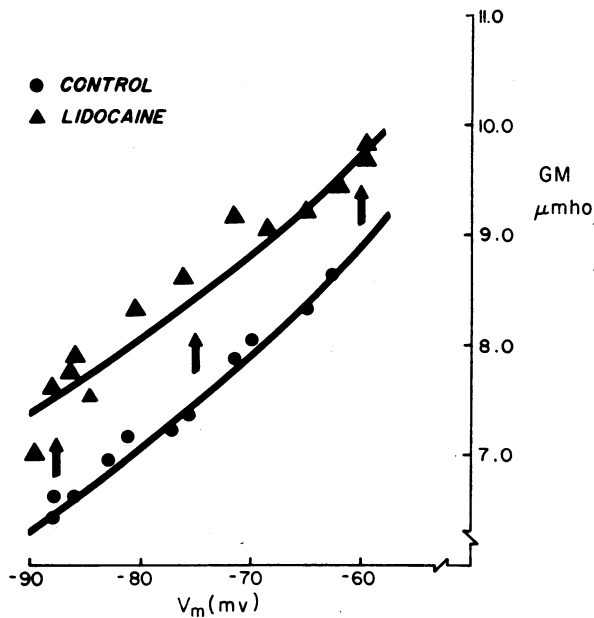


FIGURE 8 Membrane conductance (GM) in  $\mu\text{mho}$  calculated from changes in transmembrane voltage produced by small hyperpolarizing constant current pulses plotted as a function of the resting  $V_m$  which was gradually decreased by increasing  $[\text{K}]_o$  from 3 to 15 mM. Control data is represented by the circles; data after lidocaine by the triangles. The arrows show the direction of change. GM is increased after lidocaine over the range of  $V_m$  and  $[\text{K}]_o$  studied.

tential duration and effective refractory period in both Purkinje and ventricular muscle fibers, and exerts an antiautomatic effect in Purkinje pacemaker cells by decreasing the slope of slow diastolic depolarization without affecting the maximum diastolic transmembrane voltage at  $[\text{K}]_o$  of 2.7–3.0 mM (2–3). On the basis of these observations, Bigger and Mandel postulated that lidocaine may increase potassium conductance in cardiac Purkinje fibers (3).

The present study has shown by a variety of techniques that lidocaine increases membrane conductance, by increasing membrane potassium conductance, over a wide range of  $[\text{K}]_o$  (0.5–15 mM). The increase in membrane potassium slope (GK) or chord (gK) conductance as determined from current-voltage relationships is of comparable magnitude at the various  $[\text{K}]_o$  employed and is of the same magnitude as the increase in potassium permeability ( $P_K$ ) calculated from the change in resting  $V_m$  in the presence of a significant outward driving force for the potassium ion (Tables I and II). The current-voltage relationships also demonstrate that lidocaine increases membrane potassium conductance over the range of the pacemaker potential at external potassium concentrations seen clinically.

Kabela has recently shown that lidocaine enhances the efflux of  $^{42}\text{K}$  from canine Purkinje fibers at a  $[\text{K}]_o$

of 5.0 mM which he attributes to an increased membrane potassium conductance.<sup>7</sup> He further noted that lidocaine did not have this effect in atrial tissues. It is perhaps significant that lidocaine is efficacious in the treatment of ventricular arrhythmias but is rather ineffective in the treatment of atrial arrhythmias (1, 31, 32). Lidocaine has been thought to have minimal influence on the electrophysiologic characteristics of the atrial fiber (33, 34).

In considering our findings, it seems appropriate to discuss the possible role of ions other than  $\text{K}^+$ . Resting membrane conductance in normal saline Tyrode solution is determined primarily by potassium conductance with other ions having been shown to play a minor role (8–14, 35). The accepted ratio of resting  $g_{\text{Na}}$  to  $g_{\text{K}}$  is 1/19 (9). The contribution of the sodium ion was further minimized in our study by the use of sodium-deficient Tyrode in which the external and internal sodium concentrations were essentially the same, thus the effect of a change in  $g_{\text{Na}}$  on resting  $V_m$  must be minimal. In normal saline Tyrode solution, an increased membrane conductance due to an increase in either  $g_{\text{Na}}$  or  $g_{\text{Ca}}$  would result in depolarization of the resting membrane rather than in the observed hyperpolarization, and would increase rather than decrease the slope of slow diastolic depolarization (36). It is difficult, therefore, to assign a major role to the sodium, chloride, or calcium ion which in the presence of an increased membrane conductance could account for the effect of lidocaine on slow diastolic depolarization. If the increased GM after lidocaine does result in part from an increased sodium, chloride, or calcium conductance, or if it occurs despite an actual decrease in sodium conductance, the influence of these conductance changes in the range of the pacemaker potential must be masked by a more profound increase in potassium conductance.

The effect of lidocaine on an automatic Purkinje pacemaker cell in normal Tyrode at a  $[\text{K}]_o$  of 2.7 mM is seen in Fig. 9. The decreased spontaneous rate can clearly be attributed to a decrease in the slope of slow diastolic depolarization since neither the maximum diastolic transmembrane voltage nor the apparent threshold voltage had changed. The probably mechanism is illustrated in Fig. 10. Before lidocaine (panel A), slow diastolic depolarization is present. The cause of diastolic depolarization in Purkinje fibers is thought to be a gradual decrease in the outward potassium current due to a decreasing  $g_{\text{K}}$  which is voltage and time dependent (23, 37–39). This view is supported by the radioactive tracer studies of Haas and Kern who determined potassium fluxes in voltage-clamped Purkinje fibers (30). The subthreshold potassium current-

<sup>7</sup> Kabela, E. L. 1972. The effects of lidocaine on potassium efflux from various tissues of dog heart. Manuscript submitted for publication.

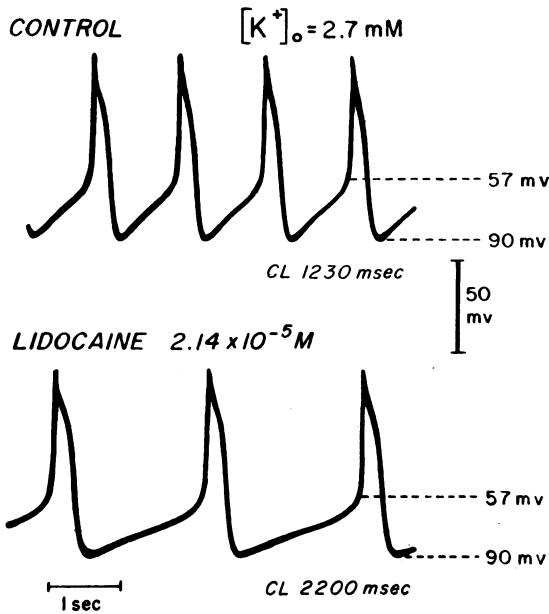


FIGURE 9 The effect of lidocaine on automaticity in sheep Purkinje fiber (normal Tyrode,  $[K^+]_o = 2.7$  mM). In the control period (upper panel), the automatic cell has a maximum diastolic transmembrane voltage of  $-90$  mV, and apparent threshold voltage of  $-57$  mV, and a spontaneous cycle length (CL) of 1,230 msec (49 depolarizations/min). 5 min after lidocaine perfusion was begun (lower panel), maximum diastolic transmembrane voltage and apparent threshold voltage were unchanged, but the spontaneous CL had increased to 2,200 msec (27 depolarizations/min) due to a decrease in the slope of slow diastolic depolarization. Shortly thereafter, spontaneous activity ceased.

voltage relationship was found to be similar to the previously described subthreshold intracellularly applied current-voltage relationship (30). A small inward sodium current remains relatively constant until the threshold voltage (Th) is approached at which time a voltage-dependent increase in sodium conductance occurs resulting in the spontaneous action potential (9, 40–42). The postulated situation after lidocaine is seen in panel B. The increased  $g_K$  after lidocaine leads to an increased outward potassium current which decreases the slope of slow diastolic depolarization.

Ventricular arrhythmias arise not only from increased automaticity, but from reentry which involves conduction delay, block, summation, and inhibition (43–49) from altered temporal dispersion (50, 51), and from boundary currents (46, 52, 53). Many of the postulated antiarrhythmic actions of lidocaine on reentry and altered temporal dispersion have been discussed in detail recently (1,3,4). The present study further shows that lidocaine, by increasing  $g_K$ , increases the resting  $V_m$  of stretched fibers. This would tend to restore conduction velocity towards normal thus abolishing condi-

tions favorable to reentry and prevent activation by premature beats until higher transmembrane voltages are attained. This effect would also decrease temporal dispersion of excitability. Lidocaine may render boundary currents ineffective by eliminating the source of the electromotive potential difference and by decreasing the distance of electronic current spread since increased membrane conductance would decrease the space constant. Several of these arrhythmogenic situations may coexist. Experimentally, Deck has shown

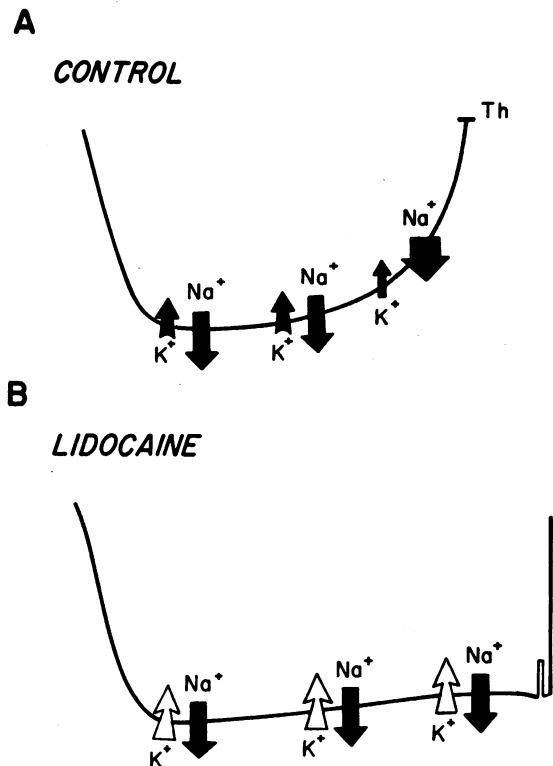


FIGURE 10 Diagrammatic representation of the postulated mechanism by which lidocaine depresses automaticity. Slow diastolic depolarization in the control situation (panel A) is attributed to a gradual decrease in the outward potassium current due to a decreasing  $g_K$  which is voltage dependent and possibly time dependent (see text). This is represented by the black arrows labeled  $K^+$  which gradually decrease in size as phase 4 proceeds. The inward sodium current remains constant (black arrows labeled  $Na^+$  of equal size) until the threshold voltage (Th) is approached at which time a voltage-dependent increase in sodium conductance occurs resulting in a marked increase in the inward sodium current (large  $Na^+$  arrow) which provokes the spontaneous action potential. The situation after lidocaine is shown in panel B. The increased  $g_K$  after lidocaine results in an increased outward potassium current (the larger white  $K^+$  arrows) which decreases the slope of slow diastolic depolarization. The inward sodium current remains constant (black  $Na^+$  arrows) and, in this illustration, the transmembrane voltage required for the marked increase in sodium conductance is not attained since spontaneous depolarization does not occur.

that in Purkinje fibers, a modest stretch results in a decreased maximum diastolic transmembrane voltage with an increased space constant and specific membrane resistance, as well as, in some preparations, an increase in spontaneous depolarization (54, 55). Trautwein and Kassebaum demonstrated that in the sheep Purkinje fiber, the application of weak depolarizing currents, which would be comparable to boundary currents, increases the slope of slow diastolic depolarization resulting in the production or enhancement of spontaneous rhythmicity (56). Enhanced automaticity due to an increase in slow diastolic depolarization will not only in itself produce cardiac arrhythmias, but may also decrease the membrane activation voltage thus decreasing conductivity in the Purkinje fiber, a situation conducive to the development of reentrant arrhythmias (57). These phenomena are presumed to underly many arrhythmias encountered clinically; in each, the ability of lidocaine to increase gK would have an antiarrhythmic effect.

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