pH-Dependent Electrophysiological Effects of Quinidine and Lidocaine on Canine Cardiac Purkinje Fibers

STANLEY NATTEL, VICTOR ELHARRAR, DOUGLAS P. ZIPES, AND JOHN C. BAILEY

SUMMARY We used standard microelectrode techniques to evaluate the effects of lidocaine and quinidine on canine Purkinje fibers at normal pH (7.3) and in the presence of acidosis (pH 6.9). Acidosis alone reduced resting potential, action potential amplitude, and $\dot{V}_{max}$, while increasing APD_{90} and conduction time. Lidocaine concentrations of 6×10^{-6} to 1.5×10^{-5} M had minimal effect on resting potential, action potential amplitude, and $\dot{V}_{max}$ at pH 7.3. At pH 6.9, the same lidocaine concentrations significantly reduced resting potential (3-10%), action potential amplitude (3-8%) and $\dot{V}_{max}$ (14-22%). Quinidine (6×10^{-6} to 1.5×10^{-5} M) reduced resting potential (3-5%), action potential amplitude (4-9%), and $\dot{V}_{max}$ (19-34%) at pH 7.3. At pH 6.9, quinidine produced significantly greater reductions in resting potential (4-15%), action potential amplitude (5-18%), and $\dot{V}_{max}$ (22-49%). These changes were associated with much more quinidine- and lidocaine-induced prolongation of interelectrode conduction time at acidic than at normal pH. Inexcitability occurred at pH 6.9 in four of 14 experiments with 1.5×10^{-5} M quinidine and in two of 10 with 1.5×10^{-5} M lidocaine, and was reversed at the same drug concentration by normalizing pH. Acidosis did not alter the $\dot{V}_{max}$-resting potential relationship in either the absence or presence of antiarrhythmic agents. Furthermore, changes in ionization did not account for the alterations in electrophysiological effects of quinidine and lidocaine produced by acidosis. Our data suggest that extracellular pH changes may modify importantly the effects of antiarrhythmic agents.
Circ Res 48: 55-61, 1981

CONSIDERABLE information is available regarding the cellular electrophysiological actions of antiarrhythmic agents. The vast majority of this knowledge has been obtained from studies using normal cardiac tissues exposed to drugs in solutions that simulate the normal extracellular environment. Much less is known about the effects of antiarrhythmic drugs on diseased tissues or in the presence of an abnormal extracellular milieu. Such knowledge is important to understanding the actions of antiarrhythmic agents in patients with organic heart disease.

Lidocaine has been shown to slow conduction velocity in acutely ischemic myocardium (Kupersmith et al., 1975) and to depress membrane responsiveness and the maximum slope of phase 0 ($\dot{V}_{max}$) in subendocardial Purkinje fibers of 24-hour-old myocardial infarcts (Sasyniuk and Kus, 1974; Allen et al., 1978; Wang et al., 1979). Epicardial tissue from 24- to 72-hour-old infarctions develops depressed excitability and membrane responsiveness when exposed to lidocaine (Lazzara et al., 1978), as does hypoxic normal endocardial tissue (Hondeghem et al., 1974; Lazzara et al., 1978). Lidocaine depresses membrane responsiveness increasingly as extracellular potassium concentration is increased above 3 mM (Singh and Vaughan Williams, 1971; Rosen et al., 1973; Weld and Bigger, 1975). Increases in extracellular potassium concentration also enhance the depressant effects of quinidine (Watanabe et al., 1963; Dreifus et al., 1974); and disopyramide (Kus and Sasyniuk, 1978) on $\dot{V}_{max}$.

Little published information exists regarding the effects of pH changes on the cellular electrophysiological actions of antiarrhythmic agents. The long-recognized efficacy of alkalinizing solutions in treating patients with procainamide and quinidine toxicity (Wasserman et al., 1958), supported by experimental evidence from in vivo studies on dogs (Bellet et al., 1959a, 1959b), suggests the potential clinical importance of such interactions. Furthermore, the effects of pH changes may be important during ischemia since tissue pH in acutely ischemic myocardium may be as low as 6.8 (Neely et al., 1975). The effects of lidocaine on acutely ischemic myocardium (Kupersmith et al., 1975) could therefore be due, at least in part, to alterations of lidocaine's actions in the presence of acidosis. Grant and Strauss (1979) have published preliminary data showing that acidosis enhances lidocaine's slowing effect on the recovery time constant of $\dot{V}_{max}$ in guinea pig papillary muscles. Our study was de-

From the Krannert Institute of Cardiology, and the Department of Medicine, Indiana University School of Medicine, Indianapolis, Indiana.

This study was supported in part by the Herman C. Krannert Fund; by Grants HL 06308, HL 07182, and HL 18795 from the National Heart, Lung, and Blood Institute of the National Institutes of Health, Bethesda, Maryland; by the American Heart Association, Indiana Affiliate, Inc., Indianapolis, Indiana; and by the Medical Research Council of Canada.

Dr. Nattel was a Research Fellow of the Medical Research Council of Canada; Postdoctoral Fellow, Cardiology, Indiana University School of Medicine.

Address for reprints: John C. Bailey, M.D., Indiana University School of Medicine, Krannert Institute of Cardiology, 1100 West Michigan Street, Indianapolis, Indiana 46223.

Received March 28, 1980; accepted for publication August 11, 1980.

signed to evaluate the ways in which changes in extracellular pH alter the cellular electrophysiological actions of lidocaine and quinidine.

Methods

Adult mongrel dogs of either sex, weighing 10–20 kg, were anesthetized with secobarbital (30 mg/kg, iv). Their hearts were removed rapidly through a right thoracotomy and immersed in cool, oxygenated Tyrode's solution. Free-running false tendons were excised from either ventricle and pinned to the paraffin floor of a 10-ml Lucite muscle chamber. The tissue was superfused at a rate of 8 ml/min with Tyrode's solution maintained at 37°C and gassed with 95% O_2–5% CO_2. A Teflon-coated, silver bipolar electrode was used to stimulate the tissue at a frequency of 2 Hz, with a pulse duration of 1–2 msec and current intensity 1.25–1.5 times diastolic threshold.

Glass microelectrodes filled with 3 M KCl and with a tip resistance of 10 to 30 MΩ were used to record the transmembrane potential from single cells. The microelectrode was connected through an Ag-AgCl junction to the input of a high-impedance, capacitance-neutralizing amplifier. The first time derivative of the transmembrane potential was obtained using an electronic differentiator which was linear between 100 to 1000 V/sec. A bipolar Teflon-coated silver electrode was positioned on the false tendon approximately 1 cm distal to the impaled cell to obtain an electrogram. The recordings were displayed on a storage oscilloscope (Tektronix D-15) and photographed with Polaroid film. The following measurements were made from the Polaroid pictures: resting potential (transmembrane potential at the onset of phase 0), action potential amplitude, $\dot{V}_{max}$, and action potential duration measured at 50% and 90% of repolarization. Conduction time was measured between the onset of the differentiated signal and the first rapid deflection in the bipolar electrogram.

The composition of the Tyrode's solution was (in mM): Na^+, 147; K^+, 4; Cl^-, 133.4; HCO_3^-, 22; $H_2PO_4^-$, 0.9; Mg^{2+}, 0.7; Ca^{2+}, 2; and dextrose, 5. The pH of this solution was 7.3 ± 0.05 after 1 hour of oxygenation. Solutions with modified pH were prepared by changing NaCl and $NaHCO_3$ concentrations reciprocally to keep the Na^+ concentration constant. An acid solution was prepared with a Cl^- concentration of 145.4 mM, a HCO_3^- concentration of 10 mM, and a pH of 6.9 ± 0.05. All solutions were gassed with 95% O_2–5% CO_2 for 1 hour prior to use, so that pH changes due to changing CO_2 concentration did not occur during the experiment. Superfusate pH was measured using a pH analyzer (Orion Research, model 601A) accurate to ±0.01 pH units connected to a pH electrode (Microelectrodes, model MI 412) located in the tissue bath.

In the first series of experiments, we evaluated the effects on action potential characteristics of pH change alone, of lidocaine at pH 7.3 and pH 6.9, and of quinidine at pH 7.3 and 6.9. In each experiment, a fiber was impaled and superfused for 30 minutes with drug-free solution at either pH 7.3 or 6.9. The superfusate then was changed to drug-free solution at the pH that had not been used during the first superfusion period for a further 30 minutes of superfusion. We varied the sequence of pH change to exclude any systematic time-related bias in the results. The fiber then was superfused with a solution containing either lidocaine hydrochloride or quinidine gluconate, at the same initial pH as had been used in the control period. After 30 minutes of superfusion with lidocaine or 60 minutes with quinidine, the pH of the superfusate was changed while the drug concentration was kept constant, for a further 30 minutes. Each fiber was therefore studied at pH 7.3 and pH 6.9 in random order without drugs, and then at pH 7.3 and pH 6.9 in the same order as used for the control period but in the presence of lidocaine or quinidine. Steady state effects always were achieved within 30 minutes after changing pH or adding lidocaine, and 60 minutes after adding quinidine.

In experiments studying lidocaine, washout was obtained by superfusing for 30 minutes at pH 7.3 with drug-free solution. We did not attempt to return to drug-free solution after quinidine because we found in preliminary studies that the effects of higher quinidine concentrations were not completely reversible even after 2 hours of superfusion with drug-free solution. To ensure that loss of impalement was not responsible for loss of resting potential and $\dot{V}_{max}$ in the presence of quinidine and acidosis, the tissue was re-exposed to quinidine at normal pH if quinidine at pH 6.9 was the last experimental condition studied. Continuous impalement was maintained throughout each experiment. Action potential characteristics were measured three times during the last 5 minutes of each superfusion period, and varied by less than 2% over this time interval. The mean of these values was taken to be characteristic of the steady state and was used for statistical analysis.

Drug concentrations of 6×10^{-6} M, 1×10^{-5} M, and 1.5×10^{-5} M were studied for both quinidine and lidocaine. Inexcitability occurred in several experiments during drug superfusion at pH 6.9. When this occurred, stimulus intensity and duration were increased in an attempt to produce propagated responses. In all preparations developing inexcitability, this progressed so that maximum extracellular stimulation failed to elicit all-or-none depolarization. Superfusion then was altered to solution at pH 7.3 with the same drug concentration to determine whether normalizing pH reversed the inexcitability. The resting potential of the last propagated impulse before inexcitability occurred was taken to represent the resting potential at steady state during that superfusion period. Resting potential was the only variable evaluated in experiments in which inexcitability occurred. Six experiments uncomplicated by

inexcitability were performed at each drug concentration.

An additional series of experiments was performed to determine whether acidosis has any direct effect on the sodium channel independent of effects on resting potential. The $\dot{V}_{max}$-resting potential relationship was studied by measuring $\dot{V}_{max}$ at various levels of resting potential as $[K^+]_o$ was increased from 4 to 12 mM and returned again to 4 mM. Each cell was studied with continuous impalement at both pH 7.3 and 6.9. Five experiments were performed in the absence of antiarrhythmic drugs, four in the presence of lidocaine and four in the presence of quinidine. The preparation was stimulated at a cycle length of 500 msec for all experiments.

Statistical analysis of drug-induced changes in action potential characteristics was performed for each drug concentration using a two-way analysis of variance. Duncan's multiple range test was applied to evaluate differences between variables at pH 7.3, pH 6.9, a given drug concentration at pH 7.3 and the same drug concentration at pH 6.9 (Steel and Torrie, 1960). We assessed the pH dependency of drug responses by calculating an F statistic for interaction effects from the analysis of variance (Steel and Torrie, 1960). The effects of acidosis alone were evaluated by comparing variables in the absence of drugs at pH 6.9 with those at pH 7.3 using Student's paired *t*-test. The significance of linear correlation was determined by applying Student's *t*-test to the correlation coefficient. Two-tailed tests were used for all statistical comparisons, and null hypotheses were rejected if their probability was less than 5%. All analyses were performed using raw data. Group averages are expressed in this paper as the mean ± the standard error of the mean.

Results

Acidosis reduced resting potential, action potential amplitude, and $\dot{V}_{max}$, while prolonging the terminal phase of repolarization without significantly affecting the plateau (Table 1). An increase in the interelectrode conduction time indicated that acidosis slowed conduction. Acidosis did not alter the $\dot{V}_{max}$-resting potential relationship of cells studied in the absence of antiarrhythmic drugs (Fig. 1).

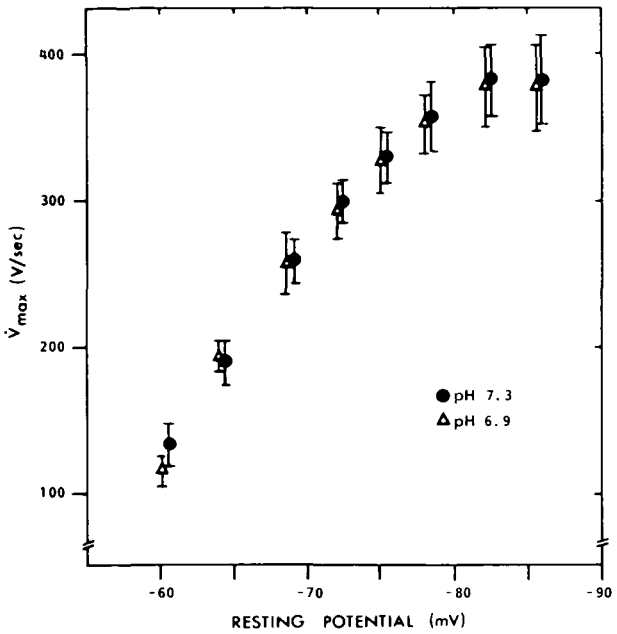

FIGURE 1 *Effect of changing pH on the $\dot{V}_{max}$-resting potential relationship. Each point represents the mean ± SE of $\dot{V}_{max}$ for five Purkinje fibers, each of which was studied at both pH 7.3 (circles) and 6.9 (triangles) in the absence of antiarrhythmic drugs.*

At pH 7.3, quinidine reduced resting potential slightly and action potential amplitude and $\dot{V}_{max}$ significantly (Table 2). These changes were associated with modest, statistically insignificant prolongation of interelectrode conduction time. At pH 6.9, quinidine produced greater reductions in resting potential, action potential amplitude, and $\dot{V}_{max}$, and significantly prolonged conduction time. Quinidine prolonged APD_{90} more at pH 6.9 than at pH 7.3, while shortening APD_{50} at both pH 6.9 and 7.3. The effects of quinidine on resting potential, action potential amplitude, $\dot{V}_{max}$ and APD_{90} showed statistically significant pH dependency. Whereas $\dot{V}_{max}$ was much more depressed by quinidine at pH 6.9 than at pH 7.3, changing pH from 7.3 to 6.9 did not alter the relationship between resting potential and $\dot{V}_{max}$ in fibers exposed to quinidine (Fig. 2).

Lidocaine at pH 7.3 had negligible effects on resting potential and action potential amplitude, while reducing $\dot{V}_{max}$ and prolonging conduction time

TABLE 1 *Effects of Acidosis on Action Potential Characteristics of Purkinje Fibers (n = 36)*

	Value at pH 7.3	Value at pH 6.9	% Change	*P*
RP (mV)	−92.9 ± 0.7	−89.2 ± 0.7	−4.0 ± 0.4	<0.001
APA (mV)	129 ± 0.7	126 ± 0.7	−2.4 ± 0.2	<0.001
APD_{50} (msec)	181 ± 4.3	184 ± 4.3	+1.8 ± 0.8	N.S.
APD_{90} (msec)	248 ± 4.6	265 ± 5.1	+7.0 ± 0.7	<0.001
$\dot{V}_{max}$ (V/sec)	539 ± 18	508 ± 18	−6.0 ± 0.9	<0.001
CT (msec)	4.9 ± 0.4	5.2 ± 0.4	+7.2 ± 1.3	<0.001

RP = membrane resting potential; APA = action potential amplitude; APD_{50} = action potential duration measured at 50% of repolarization; APD_{90} = action potential duration measured at 90% repolarization; $\dot{V}_{max}$ = maximum rate of change of voltage during phase 0; CT = conduction time; N.S. = not significant.

TABLE 2 *pH-Dependent Effects of Quinidine on Action Potential Characteristics of Purkinje Fibers**

	pH 7.3			pH 6.9			Significance of interaction†
	Control	Quinidine	(% Change)	Control	Quinidine	(% Change)	
				6×10^{-6} M Quinidine			
RP (mV)	−91.4 ± 1.2	−89.1 ± 1.1‡	(−2.5%)	−86.9 ± 1.7	−83.6 ± 1.5§	(−3.8%)	N.S.
APA (mV)	129 ± 1.7	124 ± 1.3§	(−3.9%)	126 ± 1.7	120 ± 1.4§	(−5.3%)	N.S.
APD_{50} (msec)	200 ± 7.4	156 ± 7.6‡	(−22%)	202 ± 6.8	175 ± 9.1‡	(−15%)	N.S.
APD_{90} (msec)	273 ± 7.5	274 ± 3.7	(0%)	289 ± 9.1	315 ± 3.8§	(+8.9%)	$P < 0.02$
$\dot{V}_{max}$ (V/sec)	546 ± 33	440 ± 23§	(−19%)	504 ± 32	395 ± 18.9§	(−22%)	N.S.
CT (msec)	5.2 ± 0.8	6.1 ± 1.0	(+17%)	5.5 ± 0.9	6.4 ± 1.1	(+16%)	N.S.
				1×10^{-5} M Quinidine			
RP (mV)	−91.3 ± 1.3	−87.2 ± 1.3	(−4.5%)	−86.2 ± 1.5	−76.1 ± 3.2§	(−12%)	$P < 0.02$
APA (mV)	127 ± 1.3	117 ± 2.5‡	(−7.7%)	124 ± 1.6	105 ± 6.5§	(−16%)	N.S.
APD_{50} (msec)	151 ± 11.2	125 ± 6.4§	(−17%)	154 ± 11.1	146 ± 5.8	(−5%)	N.S.
APD_{90} (msec)	222 ± 13	245 ± 13§	(+10%)	239 ± 13	295 ± 17§	(+23%)	$P < 0.01$
$\dot{V}_{max}$ (V/sec)	488 ± 37	359 ± 53§	(−26%)	459 ± 31	279 ± 71§	(−39%)	N.S.
CT (msec)	5.1 ± 1.0	6.5 ± 1.4	(+27%)	5.3 ± 1.1	7.8 ± 1.7§	(+47%)	N.S.
				1.5×10^{-5} M Quinidine			
RP (mV)*	−94.3 ± 0.5	−89.7 ± 0.4	(−4.9%)	−89.7 ± 0.4	−76.7 ± 3.8§	(−15%)	$P < 0.005$
APA (mV)	132 ± 1.4	120 ± 2.2§	(−9.0%)	129 ± 1.7	105 ± 5.5§	(−18%)	$P < 0.002$
APD_{50} (msec)	198 ± 8.5	145 ± 8.2§	(−27%)	203 ± 9.9	171 ± 8.9§	(−16%)	N.S.
APD_{90} (msec)	269 ± 5.5	284 ± 2.7	(+5.4%)	290 ± 10.6	344 ± 19.7§	(+19%)	$P < 0.02$
$\dot{V}_{max}$ (V/sec)	597 ± 40	397 ± 23§	(−34%)	581 ± 36	294 ± 37§	(−49%)	$P < 0.05$
CT (msec)	4.0 ± 0.5	4.9 ± 0.8	(+23%)	4.3 ± 0.8	6.0 ± 1.0§	(+42%)	N.S.

* $n = 6$ for all comparisons except RP for 1.5×10^{-5} M quinidine ($n = 7$), which includes one experiment in which inexcitability occurred. For definition of abbreviations, see Table 1.

† A statistically significant interaction indicates that quinidine's effect on an action potential characteristic is pH-dependent.

‡ $P < 0.05$ and § $P < 0.01$ = significance of difference between action potential characteristic at a given quinidine concentration and pH to drug-free control at the same pH.

slightly (Table 3). At pH 6.9, however, lidocaine reduced resting potential and action potential amplitude significantly. Furthermore, lidocaine reduced $\dot{V}_{max}$ and prolonged conduction time more at pH 6.9 than at pH 7.3. APD_{50} and APD_{90} were shortened by all lidocaine concentrations at both pH 7.3 and 6.9. Lidocaine's effects on resting potential, action potential amplitude, $\dot{V}_{max}$, and conduction time showed statistically significant dependence on extracellular pH. The relationship between resting potential and $\dot{V}_{max}$, however, was not affected by changing extracellular pH from 7.3 to 6.9 in preparations treated with lidocaine (Fig. 3).

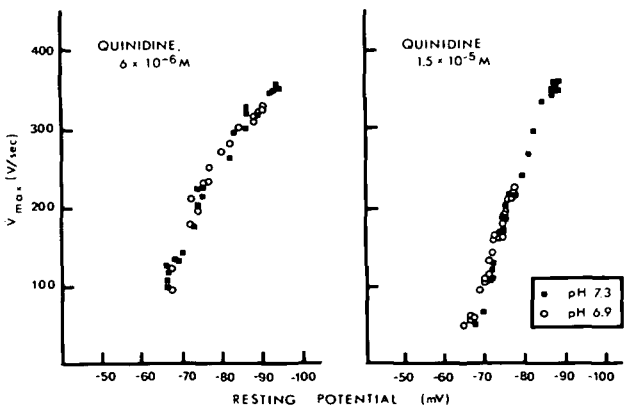

FIGURE 2 *Effect of changing pH on the $\dot{V}_{max}$-resting potential relationship in quinidine-treated tissues. Studies were performed on Purkinje fibers exposed for 1 hour to quinidine at 6×10^{-6} M (left) and 1.5×10^{-5} M (right). The $\dot{V}_{max}$-resting potential relationship in the presence of a constant quinidine concentration was evaluated at both pH 7.3 and pH 6.9 for each fiber.*

In four of 14 experiments with 1.5×10^{-5} M quinidine and two of 10 with 1.5×10^{-5} M lidocaine, these drugs at pH 6.9 produced inexcitability to maximal extracellular current stimulation (approximately 5 mA) with a stimulus duration of up to 10 msec. These were the only experiments in which changes in stimulating current strength or duration were made. Changing the pH of the superfusate to 7.3 while maintaining the same drug concentration reversed inexcitability in each case. In all of these experiments, inexcitability was associated with a marked decrease in membrane resting potential to less than −65 mV.

Since the degree of ionization of lidocaine and quinidine changes over the pH range 7.3 to 6.9, we examined the correlation between various molecular forms of the drugs and drug effects. Concentrations of ionized and non-ionized drug at pH 7.3 and 6.9 were calculated from the pKa (7.9 for lidocaine, 8.4 for quinidine) (Avery, 1976) and total concentration of the drug used in each experiment (Avery, 1976). Log (concentration)-response relationships then were examined for total drug, ionized drug, and non-ionized drug concentrations and each of the action potential characteristics studied. Figure 4 is an example of such a series of graphs relating changes in $\dot{V}_{max}$ produced by quinidine to the con-

TABLE 3 *pH-Dependent Effects of Lidocaine on Action Potential Characteristics of Purkinje Fibers**

	pH 7.3			pH 6.9			Significance of interaction†
	Control	Lidocaine	(% Change)	Control	Lidocaine	(% Change)	
				6 × 10⁻⁶ M Lidocaine			
RP (mV)	−92.0 ± 2.2	−91.2 ± 2.2	(−0.9%)	−89.4 ± 1.8	−86.1 ± 2.7§	(−3.7%)	N.S.
APA (mV)	127 ± 2.1	125 ± 2.6	(−1.6%)	124 ± 1.6	121 ± 2.4§	(−2.5%)	N.S.
APD_{50} (msec)	178 ± 6.7	151 ± 7.6§	(−15%)	178 ± 5.8	151 ± 8.5§	(−15%)	N.S.
APD_{90} (msec)	244 ± 7.1	218 ± 5.2§	(−11%)	260 ± 9.5	237 ± 5.9§	(−9%)	N.S.
$\dot{V}_{max}$ (V/sec)	557 ± 63	530 ± 73	(−5%)	532 ± 62	460 ± 59§	(−14%)	$P < 0.05$
CT (msec)	5.3 ± 1.0	5.9 ± 1.2	(+11%)	5.7 ± 1.1	7.0 ± 2	(+22%)	N.S.
				1 × 10⁻⁵ M Lidocaine			
RP (mV)	−97.0 ± 1.3	−96.6 ± 1.1	(−0.5%)	−93.5 ± 1.7	−90.4 ± 1.2§	(−3.3%)	$P < 0.01$
APA (mV)	131 ± 1.7	129 ± 0.8	(−1.5%)	127 ± 1.8	122 ± 0.9§	(−4%)	$P < 0.02$
APD_{50} (msec)	191 ± 3.4	149 ± 6.9§	(−22%)	192 ± 7.1	153 ± 4.0§	(−20%)	N.S.
APD_{90} (msec)	251 ± 5.7	221 ± 7.4§	(−12%)	267 ± 8.4	241 ± 8.7§	(−10%)	N.S.
$\dot{V}_{max}$ (V/sec)	575 ± 53	520 ± 49‡	(−10%)	525 ± 56	434 ± 44‡	(−17%)	$P < 0.05$
CT (msec)	5.7 ± 1.7	6.2 ± 1.7	(+9%)	6.1 ± 1.7	7.1 ± 2§	(+16%)	N.S.
				1.5 × 10⁻⁵ M Lidocaine			
RP (mV)*	−91.4 ± 1.7	−90.9 ± 1.5	(−0.5%)	−89.2 ± 1.9	−80.7 ± 3.9§	(−9.5%)	$P < 0.002$
APA (mV)	126 ± 2.0	124 ± 1.2	(−1.6%)	124 ± 1.7	116 ± 2.4§	(−7.5%)	$P < 0.05$
APD_{50} (msec)	167 ± 9.4	130 ± 5.6§	(−22%)	173 ± 7.3	126 ± 5.8§	(−27%)	N.S.
APD_{90} (msec)	229 ± 10	195 ± 7.8§	(−15%)	245 ± 10	215 ± 11§	(−12%)	N.S.
$\dot{V}_{max}$ (V/sec)	475 ± 37	465 ± 46	(−2.5%)	449 ± 38	349 ± 42§	(−22%)	$P < 0.05$
CT (msec)	4.2 ± 0.7	4.8 ± 0.7‡	(+14%)	4.6 ± 0.8	5.6 ± 0.6§	(+22%)	$P < 0.02$

* $n = 6$ for all comparisons except RP for 1.5×10^{-5} M lidocaine ($n = 8$) which includes two experiments in which inexcitability occurred. For definition of abbreviations, see Table 1.

† A statistically significant interaction indicates that quinidine's effects on an action potential characteristic is pH-dependent.

‡ $P < 0.05$ and § $P < 0.01$ = significance of difference between action potential characteristic at a given lidocaine concentration and pH to drug-free control at the same pH.

centrations of total, ionized, and non-ionized quinidine in each experiment. Although there was a linear relationship between the log of total or ionized quinidine concentrations and quinidine-induced changes in $\dot{V}_{max}$, non-ionized quinidine concentrations correlated very poorly with quinidine's effects. These results suggest that quinidine's effects on $\dot{V}_{max}$ are related to the concentrations of total or ionized quinidine, but not to concentrations of non-ionized quinidine. Total and ionized drug concentrations showed a positive log-linear relationship with changes in each of the action potential characteristics studied. In contrast, non-ionized drug concentrations correlated much more poorly with drug effects. In addition to the effect of quinidine on $\dot{V}_{max}$ (discussed above), quinidine's effects

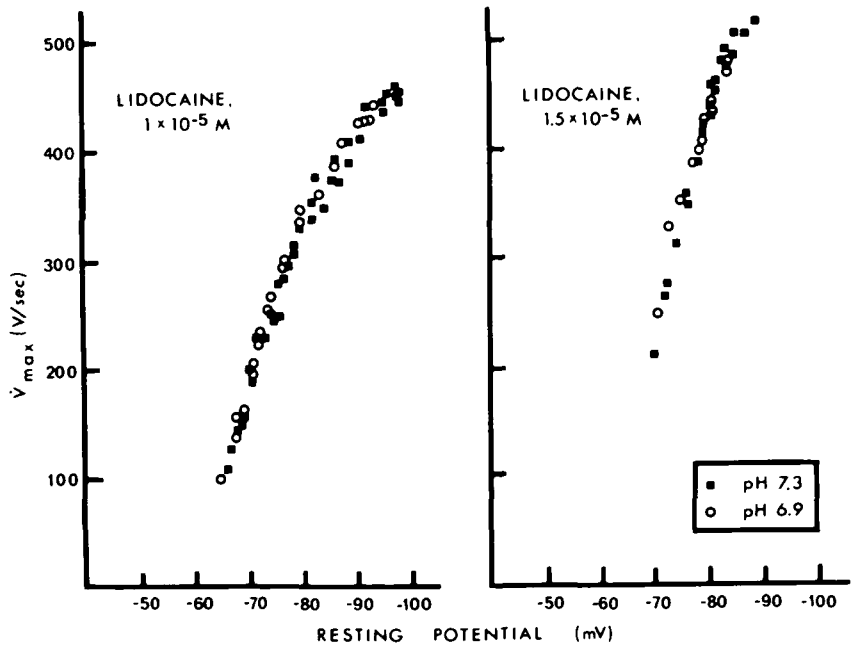

FIGURE 3 *Effect of changing pH on the $\dot{V}_{max}$-resting potential relationship in lidocaine-treated tissues. Studies were performed on Purkinje fibers exposed for 30 minutes to lidocaine at 1 × 10⁻⁵ M (left) and 1.5 × 10⁻⁵ M (right). The $\dot{V}_{max}$-resting potential relationship in the presence of a constant lidocaine concentration was evaluated at both pH 7.3 and pH 6.9 for each fiber.*

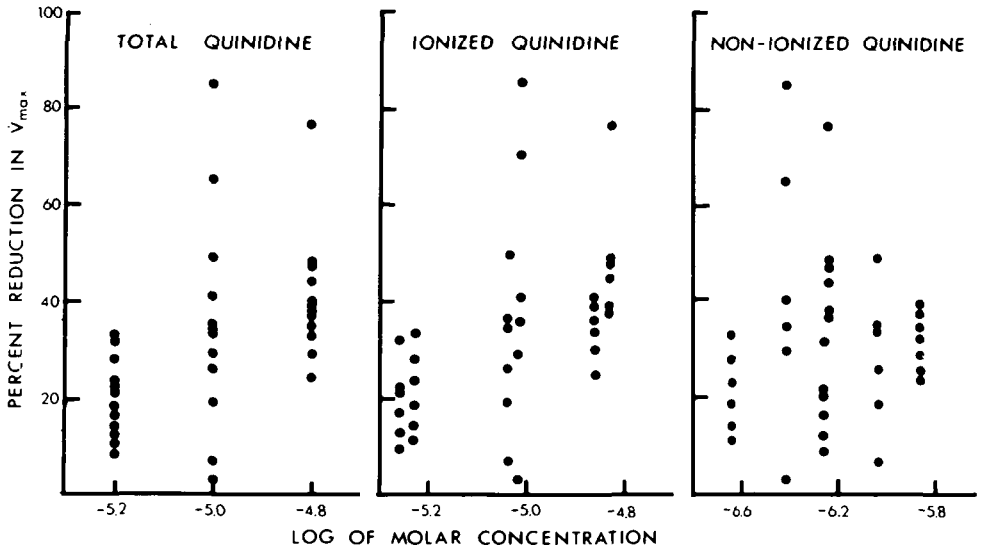

FIGURE 4 *Relationship between percent reduction in $\dot{V}_{max}$ produced by quinidine and the logarithm of the concentrations of non-ionized quinidine (right), ionized quinidine (middle), and non-ionized plus ionized quinidine (left). There was a positive linear correlation between quinidine's effect on $\dot{V}_{max}$ and concentrations of total ($r = 0.48$, $P < 0.01$) and ionized ($r = 0.51$, $P < 0.01$) quinidine concentrations, but no relationship between quinidine's effects and non-ionized quinidine concentrations ($r = 0.09$, N.S.).*

on APD_{90}, action potential amplitude, and resting potential, as well as lidocaine's effects on $\dot{V}_{max}$, APD_{50}, action potential amplitude, and resting potential, correlated much better with total drug concentrations than with non-ionized drug concentrations.

Discussion

We have demonstrated that the depressant effects of quinidine and lidocaine on resting potential, action potential amplitude and $\dot{V}_{max}$ are pH dependent, increasing at more acid extracellular pH. Quinidine and lidocaine reduce the $\dot{V}_{max}$ initiated at any given resting potential in cells whose resting potential is varied by exposure to changing concentrations of potassium (Chen et al., 1975). We did not find any change in the relationship between $\dot{V}_{max}$ and resting potential in cells exposed to quinidine or lidocaine when pH was changed from 7.3 to 6.9. The pH-dependent depression of $\dot{V}_{max}$ by quinidine and lidocaine therefore is accounted for by alterations in resting potential. Depolarization reduces $\dot{V}_{max}$ by increasing the fraction of sodium channels that are inactivated, and may enhance the binding of antiarrhythmic agents to sodium channels by making the complex of drug with the inactivated channel a favored state (Hille, 1977; Hondeghem and Katzung, 1977). We found no evidence to suggest that pH changes altered the interaction between quinidine or lidocaine and the sodium channel at any given resting potential.

The mechanism by which lidocaine and quinidine substantially reduce resting potential at pH 6.9 is unclear, but possibilities include inhibition of Na^+, K^+-ATPase, changes in passive ion fluxes, decreased membrane permeability to potassium or increased membrane permeability to sodium. Quinidine is capable of binding to Na^+,K^+-ATPase and inhibiting its activity (Besch and Watanabe, 1977), but the pH dependency of this effect is unknown. The available evidence suggests that lidocaine increases potassium permeability (Arnsdorf and Bigger, 1972). Weld and Bigger (1975) have shown that hyperpolarization eliminates lidocaine's depression of $\dot{V}_{max}$, a finding that would be unlikely if lidocaine altered the transmembrane sodium gradient.

We found that total or ionized lidocaine and quinidine concentrations correlated better with the effects of these drugs than did non-ionized drug concentrations. These findings support the conclusion of Gliklich and Hoffman (1978), from work with quaternary derivatives of lidocaine, that the ionized form of antiarrhythmic agents may enter the cell from the extracellular space and produce important electrophysiological effects. These results are not consistent with the hypothesis that only non-ionized molecules of local anesthetic agents are able to penetrate cell membranes and affect depolarization-induced changes in ionic conductances, for which there is some evidence in studies of nerve tissues (Narahashi et al., 1970). Furthermore, these results suggest that changes in ionization do not account for the differences between the effects of lidocaine and quinidine at pH 6.9 compared to their effects at pH 7.3.

Skinner and Kunze (1976) showed in rabbit atrium that acidosis caused a loss of intracellular potassium which then accumulated in the extracellular space. This resulted in a net decrease of 5 mV in the calculated potassium equilibrium potential when pH was changed from 7.5 to 6.8. The average decrease of 3.7 mV in resting potential which occurred when pH was lowered from 7.3 to 6.9 in our studies probably is due to the same phenomenon. Since acidosis did not alter the overshoot or the relationship between $\dot{V}_{max}$ and resting potential, the

reduction in resting potential produced by acidosis probably accounts for the concomitant reductions in action potential amplitude and $\dot{V}_{max}$. Van Bogaert and Carmeliet (1972) have reported that acidosis reduced $\dot{V}_{max}$ in bovine Purkinje fibers when resting potential was kept constant by injecting intracellular current. This effect was present, however, only for values of pH less than 6. We have shown that moderate changes in pH within the physiological range do not alter the steady state $\dot{V}_{max}$-resting potential relationship, and presumably do not affect the voltage dependency of sodium channel activation and inactivation.

We altered extracellular pH by changing biocarbonate concentration. Since P_{CO_2} was unaltered, intracellular buffering systems may have been particularly effective in preventing changes in intracellular pH. Caution therefore must be exercised in extrapolating our results to the effects of antiarrhythmic agents in the presence of myocardial ischemia, which causes profound intracellular as well as extracellular acidosis.

Acknowledgments

We are grateful to David Mendel, Darlene Massad, and Connie Nurnberger for technical assistance, and to Marsha Meece for secretarial aid.

References

Allen JD, Brennan FJ, Wit AL (1978) Actions of lidocaine on transmembrane potentials of subendocardial Purkinje fibers surviving in infarcted canine hearts: Circ Res **43:** 470–481

Arnsdorf MF, Bigger JT (1972) Effect of lidocaine hydrochloride on membrane conductance in mammalian cardiac Purkinje fibers. J Clin Invest **51:** 2252–2263

Avery GS (ed) (1976) Drug Treatment, Sydney, A.D.I.S. Press, p 891

Bellet S, Hamdan G, Somlyo A, Lara R (1959a) A reversal of the cardiotoxic effects of quinidine by molar sodium lactate. Am J Med Sci **237:** 165–176

Bellet S, Hamdan G, Somlyo A, Lara R (1959b) A reversal of the cardiotoxic effects of procaineamide by molar sodium lactate. Am J Med Sci **237:** 177–189

Besch HR Jr, Watanabe AM (1977) Binding and effect of tritiated quinidine on cardiac subcellular enzyme systems: Sarcoplasmic reticulum vesicles, mitochondria and Na^+,K^+-adenosine triphosphatase. J Pharmacol Exp Ther **202:** 354–363

Chen CM, Gettes LS, Katzung BG (1975) Effect of lidocaine and quinidine on steady-state characteristics and recovery kinetics of $(dV/dt)_{max}$ in guinea pig ventricular myocardium. Circ Res **37:** 20–29

Dreifus SD, de Azevedo IM, Watanabe Y (1974) Electrolyte and antiarrhythmic drug interaction. Am Heart J **88:** 95–107

Gliklich JI, Hoffman BF (1978) Sites of action and active forms of lidocaine and some derivatives on cardiac Purkinje fibers. Circ Res **43:** 638–651

Grant AO, Strauss HC (1979) The influence of pH on the electrophysiological effects of lidocaine in guinea pig ventricular myocardium (abstr). Am J Cardiol **43:** 475

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

Hondeghem LM, Katzung BG (1977) Time- and voltage-dependent interactions of antiarrhythmic drugs with cardiac sodium channels. Biochim Biophys Acta **472:** 373–398

Hondeghem LM, Grant AO, Jensen RA (1974) Antiarrhythmic drug action: Selective depression of hypoxic cardiac cells. Am Heart J **87:** 602–605

Kupersmith J, Antman EM, Hoffman BF (1975) In vivo electrophysiological effects of lidocaine in canine acute myocardial infarction. Circ Res **36:** 84–91

Kus T, Sasyniuk BI (1978) The electrophysiological effects of disopyramide phosphate on canine ventricular muscle and Purkinje fibers in normal and low potassium. Can J Physiol Pharmacol **56:** 139–149

Lazzara R, Hope RR, El-Sherif N, Scherlag BJ (1978) Effects of lidocaine on hypoxic and ischemic cardiac cells. Circ Res **41:** 872–879

Narahashi T, Frazier DT, Yamada M (1970) The site of action and active form of local anesthetics. I. Theory and pH experiments with tertiary compounds. J Pharmacol Exp Ther **171:** 32–44

Neely JR, Whitmer JT, Rovetto MJ (1975) Effect of coronary blood flow on glycolytic flux and intracellular pH in isolated rat hearts. Circ Res **37:** 733–741

Rosen MR, Merker C, Pippenger CE (1973) Effects of clinically relevant plasma lidocaine concentrations on electrophysiologic properties of canine Purkinje fibers (abstr). Circulation **48** (suppl IV): 209

Sasyniuk BI, Kus T (1974) Comparison of the effects of lidocaine on electrophysiological properties of normal Purkinje fibers and those surviving acute myocardial infarction (abstr). Fed Proc **33:** 476

Singh BN, Vaughan Williams EM (1971) Effect of altering potassium concentration on the action of lidocaine and diphenylhydantoin on rabbit atrial and ventricular muscle. Circ Res **29:** 286–295

Skinner RB, Kunze DL (1976) Changes in extracellular potassium activity in response to decreased pH in rabbit atrial muscle. Circ Res **39:** 678–683

Steel RGD, Torrie JH (1960) Principles and Procedures of Statistics. New York, McGraw-Hill, pp 194–231

van Bogaert PP, Carmeliet E (1972) Sodium inactivation and pH in cardiac Purkinje fibers (abstr). Arch Int Physiol Biochem **80:** 833–835

Wang, CM, James CA, Maxwell RA (1979) Effects of lidocaine on the electrophysiological properties of subendocardial Purkinje fibers surviving acute myocardial infarction. J Mol Cell Cardiol **11:** 669–681

Wasserman F, Brodsky L, Dick MM, Kathe JH, Rodensky PL (1958) Successful treatment of quinidine and procaine amide intoxication. N Engl J Med **259:** 797–802

Watanabe Y, Dreifus LS, Likoff W (1963) Electrophysiologic antagonism and synergism of potassium and antiarrhythmic agents. Am J Cardiol **12:** 702–710

Weld FM, Bigger JT Jr (1975) Effect of lidocaine on the early inward transient current in sheep cardiac Purkinje fibers. Circ Res **37:** 630–639

Circulation Research
JOURNAL OF THE AMERICAN HEART ASSOCIATION

pH-dependent electrophysiological effects of quinidine and lidocaine on canine cardiac purkinje fibers.

S Nattel, V Elharrar, D P Zipes and J C Bailey

Circ Res. 1981;48:55-61
doi: 10.1161/01.RES.48.1.55
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 0009-7330. Online ISSN: 1524-4571