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pK_a Does Not Predict pH Potentiation of Sodium Channel Blockade by Lidocaine and W6211 in Guinea Pig Ventricular Myocardium[1]

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ABSTRACT

During diastole, tertiary amine local anesthetic molecules may exit cardiac sodium channels quickly through the membrane if they are neutral, or more slowly through the aqueous channel pore if they are charged. Extracellular acidosis potentiates sodium channel blockade by these drugs, and drug pK_a should be a potent predictor of the degree of response of drug dissociation kinetics to changes in extracellular pH. To test this hypothesis, we measured kinetics of recovery from drug-induced channel blockade in guinea pig papillary muscle exposed to lidocaine (pK_a 7.86) and to W6211 (pK_a 6.29) using $\dot{V}_{max}$ as a measure of peak sodium current. Both compounds, which are physicochemically very similar in respects other than pK_a, delayed $\dot{V}_{max}$ recovery in a pH-dependent fashion. As pH was lowered from 7.9 to 6.5, the recovery time constant rose from 86 to 230 msec for lidocaine and from 53 to 154 msec for W6211. We revised an earlier kinetic scheme of drug-channel interaction to incorporate newer concepts of drug trapping and ionization within the channel, and the resulting analytic expressions fit the data well. Important implications of the new scheme are that the pK_a of the drug-receptor complex may differ from the drug pK_a, and that deprotonation of channel-bound charged drug molecules may be a rate-limiting process.

Extracellular acidosis slows the dissociation of tertiary amine local anesthetics from Na channels of nerve (Khodorov *et al.*, 1977), skeletal muscle (Schwarz *et al.*, 1977) and heart (Betancourt and Dresel, 1979; Grant *et al.*, 1980). These compounds enter and leave the channel during the upstroke and plateau of the action potential under the control of voltage-sensitive gating mechanisms (Hille, 1977a,b; Hondeghem and Katzung, 1977; Grant *et al.*, 1984; Starmer *et al.*, 1984). The charged drug molecule is confined in its transit to the aqueous inner pore of the channel protein, whereas the neutral form may also enter and exit *via* the lipid membrane (Hille, 1977b). Both forms block the channel, but the effect of uncharged drug (*e.g.*, benzocaine, pK_a 2.6) dissipates about 5 to 6 orders of magnitude more quickly than that of charged drug (*e.g.*, the permanently charged quaternary forms). This is the basis for previous explanations of the potentiation of block in extracellular acidosis, which provides a higher proportion of the more tenacious charged form.

An earlier study from this laboratory by Broughton *et al.* (1984) of lidocaine and its two desethylation metabolites showed that lipid solubility influences the degree of slowing of recovery from block in acidosis. Lidocaine, the most lipid-soluble of the three compounds, showed a 73% slowing of $\dot{V}_{max}$ recovery at pH 6.95 when compared with pH 7.4, and glycinexylidide, which is two orders of magnitude less lipid-soluble than lidocaine, had only a 13% slowing over the same pH range. Broughton *et al.* (1984) proposed a kinetic scheme in which drug molecules within cardiac Na channels rapidly distributed themselves between neutral and charged forms according to the difference between the drug pK_a and the extracellular pH (fig. 1). Their analytic expressions predicted that little pH-induced change in the rage of drug dissociation would occur once neutral molecules predominated, *i.e.*, once pH exceeded pK_a (fig. 2).

These observations figure critically in the study of ischemic cardiac arrhythmias and their treatment, but are limited in that they were generated using lidocaine (pK_a 7.86) at pH values where charged drug molecules predominate. Our major purpose in this study was to evaluate this scheme over a wide range of proportions of neutral and charged drug molecules. To achieve this goal, we measured the rate of Na channel recovery from block by lidocaine over a wider pH range and studied another drug with a pK_a less than physiologic pH in order to

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ABBREVIATION: Na, sodium.

$$U + D_c \underset{\ell_c}{\overset{k_c}{\rightleftharpoons}} B_c$$

$$k_p \uparrow\downarrow \ell_p$$

$$U + D_n \underset{\ell_n}{\overset{k_n}{\rightleftharpoons}} H^+ + B_n$$

$$\tau = \frac{1+R}{\ell_n R + \ell_c}$$

Fig. 1. Earlier scheme of local anesthetic interaction with cardiac sodium channels (adapted from Broughton *et al.*, 1984). Both neutral and charged drug molecules enter and block the channel during the upstroke and plateau phases of the transmembrane action potential, and exit during diastole. The time constant of recovery from block is determined by the relative proportion of neutral and charged molecules, which in turn is determined by the pH and the drug pK_a. Abbreviations: U, unblocked channel; D_c, D_n, charged, neutral drug molecules; B_c, B_n, channels blocked by charged, neutral drug molecules, and $R = 10^{pH-pK_a}$, or ratio of neutral to charged drug molecules. Rate constants are given to the processes of protonation (k_p) and deprotonation (ℓ_p) of drug molecules within the channel.

AGGREGATE τ_r (ms)

0, 100, 200, 300, 400, 500

LIDOCAINE

0, 1, 2, 3

b^n / b^c

(RATIO OF NEUTRAL TO CHARGED DRUG)

Fig. 2. Theoretical relationship of aggregate time constant of $\dot{V}_{max}$ recovery after local anesthetic block, τ_r, to extracellular pH using analytic expressions generated by kinetic scheme in figure 1 (adapted from Broughton *et al.*, 1984). No further decline should occur once neutral drug molecules predominate.

evaluate the rate of recovery from block by primarily neutral molecules. We used W6211, an experimental tertiary amine local anesthetic drug with pK_a of 6.29. Its electrophysiologic effects in heart have not been reported previously. To test these drugs at higher pH required a nonbicarbonate buffer to avoid precipitation of calcium carbonate. A secondary goal was to establish the acceptability of such a buffer in this model of recovery of cardiac Na channels from local anesthetic blockade.

Methods

Experimental procedures. The hearts of stunned 250 to 350 g guinea pigs (Charles River Breeding Labs., Wilmington, MA) were dissected in oxygenated Krebs-Henseleit solution at room temperature. Papillary muscles of 0.5 to 1.0 mm diameter were removed from the right ventricle and were mounted in a three-compartment single sucrose gap chamber with 0.5 to 1.0 mm of uncut muscle in the anterior or test chamber.

The posterior or current-injection chamber was perfused with a high-potassium Krebs-Henseleit solution containing (millimolar): $NaHCO_3$, 21; KCl, 122.3; $CaCl_2$, 1.8; $MgSO_4$, 1.2; glucose, 11; and bubbled with 95% O_2-5% CO_2. The middle compartment was perfused with isotonic sucrose solution containing 0.01 mM $CaCl_2$ and bubbled with 100% O_2.

The control solution for the anterior chamber contained (millimolar): NaCl, 120; KCl, 4.3; $CaCl_2$, 1.8; $MgSO_4$, 1.2; glucose, 11; and was buffered with 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid 15 mM. The pH was adjusted to 6.5, 6.9, 7.4 and 7.9 using HCl 1.2 M or NaOH 1.0 M using a pH analyzer (Instrumentation Laboratory, Inc., Lexington, MA, model 113). The ionized calcium concentration over this pH range was 1.61 (pH 6.5) to 1.46 mM (pH 7.9). The temperature in the test chamber was monitored using a probe thermistor (Yellow Springs Instrument Co., Yellow Springs, OH, Model 511) and maintained at 37 ± 0.5°C. The flow rate was 3 ml/min and the chamber capacity 0.7 ml. Lidocaine and W6211 (2-[N-(2-methoxyethyl)-methylamino]-2′,6′-acetoxylidide, the gracious gift of Dr. Bertil Takman, Astra Pharmaceutical Products, Inc., Worcester, MA) were added in a concentration of 15 μM.

The muscle was stimulated at 1 Hz with constant current pulses of 2 msec duration. The strength of the test stimulus was adjusted to maintain latency (time from stimulus to peak upstroke velocity of action potential) constant at 0.5 to 1.0 msec. Preparations with high thresholds for stimulation or in which a short and constant latency was not possible were discarded. A conventional glass microelectrode containing 3 M KCl and of 10 to 30 megohms resistance was inserted into a cell, and another was placed in close proximity to the muscle. The transmembrane potential, recorded as the difference signal of the two microelectrodes, was displayed on a Tektronix RM-565 oscilloscope after having passed through Ag/AgCl half-cells (WPI EH-3FS), matched voltage followers with high input impedance (10^{10} ohms) and input capacitance neutralization and the 3A9 differential amplifier of the oscilloscope. $\dot{V}_{max}$, the maximum slope of the upstroke of the action potential, was obtained by a differentiator circuit with linear output over 10 to 1000 V/sec. Thirty-five-millimeter photographs of action potentials and $\dot{V}_{max}$ were taken with a Grass C4 camera and enlarged (8 ×) for digitizing (graf/pen, DEC PDP 11/03).

After positioning of the extracellular electrode and impalement of a cell with the intracellular electrode, we allowed a 1-hr equilibration period before recording. The preparation was stimulated by a train of 10 pulses with an interstimulus interval of 1000 msec within the train and 2000 msec between trains. Pulses within the train are defined as steady-state pulses. Test pulses were introduced at varying coupling intervals after the last beat of the train. After drug-free conditions had been recorded, a drug solution at pH 7.4 was infused for 1 hr, while the preparation was stimulated at 1 Hz. We then measured action potential characteristics and $\dot{V}_{max}$ of the test pulse and the steady-state pulse immediately preceding it. An equilibration period of 25 to 30 min was allowed after each exchange of solution pH. The sequence of solution changes was: drug-free solution (pH 7.4), drug solution (lidocaine 15 μM or W6211 15 μM, pH 7.4), drug solution of test pH, drug solution (pH 7.4). Each preparation was exposed to a single drug. Because of the difficulty in maintaining stable impalements in actively contracting muscles, some experiments recorded data for the second drug solution at pH 7.4 in a cell different from the one used for the first drug solution at pH 7.4 and the drug solution of test pH.

Data analysis. Oscilloscope records included the action potential and $\dot{V}_{max}$ of the last steady-state impulse and, after it, the test impulse. Measurements were made of the resting membrane potential (milli-

volts), action potential duration (interval in msec from the onset of the upstroke to the point 1 mV positive to the resting potential) and $\dot{V}_{max}$ (in volts per sec from the output of the differentiator circuit). The coupling interval was defined as the time elapsed between the 1 mV repolarization point of the final steady-state action potential and the onset of the upstroke of the test action potential.

The value of $\dot{V}_{max}$ of the test pulse was measured five times and averaged for each coupling interval. The resulting experimental data points were fit to the exponential function

$$\dot{V}_{max}(t) = \dot{V}_{max}(\infty) + [\dot{V}_{max}(0) - \dot{V}_{max}(\infty)]\exp(-t/\tau_r) \quad (1)$$

using a nonlinear least-squares procedure (Marquardt, 1963). τ_r is the overall time constant for $\dot{V}_{max}$ recovery. $\dot{V}_{max}(0)$ and $\dot{V}_{max}(\infty)$ are the values of $\dot{V}_{max}$ at a diastolic interval of 0 msec and after attainment of steady-state value.

To analyze the effect of the drugs at pH 7.4, we compared action potentials and $\dot{V}_{max}$ under drug-free conditions with those after the initial 1 hr drug equilibration period. In comparing the effect of pH under drug conditions, we compared action potentials and $\dot{V}_{max}$ during infusion of the drug solution at test pH with those during the infusions of drug solution of pH 7.4, which immediately preceded and followed the test solution. Experiments in which recovery kinetics of $\dot{V}_{max}$ in the two drug solutions at pH 7.4 differed by more than 15% were not used in the data analysis. Otherwise, they were averaged and analyzed as a single data point.

Determination of lipid solubility. Lipid solubility of W6211 was determined by octanol:water partitioning at pH 8.29, 2 pH units above drug pK_a. The neutral compound predominates in a ratio of 99:1 at this pH. Cross-saturated solutions of 2-octanol (Eastman Kodak Co., Rochester, NY) (2 ml) and Tris buffer pH 8.29 (2 ml) were rocked for 1 hr at room temperature after addition of the drug. The aqueous phase was removed after centrifugation at 1500 rpm for 5 min. Initial drug concentration in the aqueous phase (200 μg/ml) and final drug concentration were measured by reverse phase high-performance liquid chromatography (Laboratory Data Control Div., Milton Roy Co., Riviera Beach, FL, with Lichrosorb RP-18, 10 μ, 25 cm × 4.6 mm column, Alltech Associates Inc., Deerfield, IL). The mobile phase consisted of 43% 0.05 M $NaClO_4 \cdot H_2O$ (adjusted to pH 4 with 0.1 $HClO_4$) and 57% acetonitrile. The mobile phase was degassed before use, and flow rate was 1.5 ml/min. Absorbance was measured at 200 nm with detector range of 0.1 a.u.f.s.

Concentrations of 1, 5, 10, 50, 100 and 200 μg/ml were used to calibrate the assay of W6211. Two separate calibration curves were used, one ranging from 1 to 50 μg/ml and the other from 50 to 200 μg/ml. For each of these curves there was a linear relationship between the amount of drug injected and the observed peak height. The average correlation coefficient for the six calibration curves used was 0.998 ± 0.003.

Potential limitations of methods. Because it is not possible to clamp the Na current at physiologic temperature and $[Na]_o$, we instead inferred the available Na conductance from $\dot{V}_{max}$ of phase 0 of action potentials elicited at short, constant latencies (Walton and Fozzard, 1979) at physiologic temperature and $[Na]_o$. Although time-honored (Weidmann, 1955) and widely used, $\dot{V}_{max}$ did not correlate linearly with measurements of I_{Na} in voltage-clamped preparations at very low temperature and in very low $[Na]_o$ (Cohen *et al.*, 1984)—conditions that might affect the biological processes that we wanted to observe. From these observations, Cohen *et al.* (1984) concluded that the monoexponential nature of I_{Na} activation and recovery in the presence of lidocaine would not be reflected in $\dot{V}_{max}$ measurements. However, records from this and other laboratories have demonstrated repeatedly a monoexponential activation and recovery of $\dot{V}_{max}$ in the presence of local anesthetics (Grant *et al.*, 1980, 1982; Kohlhardt and Seifert, 1983; Gintant *et al.*, 1983; Broughton *et al.*, 1984; Strauss *et al.*, 1984; Stambler *et al.*, 1985; fig. 4). Although caution must be exercised in using $\dot{V}_{max}$ as a measure of I_{Na}, we consider it the best measure that can currently be obtained under physiologic conditions.

Statistical analysis. To determine if extracellular pH had an effect on different action potential characteristics shown in tables 1 to 3, the Wilcoxon signed rank test was used. In each experiment, action potential characteristics were recorded at more than one pH value. The slope of each variable as a function of pH was determined and then tested to determine if the slopes across animals differed significantly from 0.

Results

Recovery kinetics in drug-free solutions. Synthetic buffer systems may alter $\dot{V}_{max}$ recovery kinetics during lidocaine exposure (Stambler *et al.*, 1985), so we performed experiments in drug-free solutions over the test pH range. Averaged action potential characteristics are shown in table 1.

Acidosis caused a significant reduction in $\dot{V}_{max}$ ($P < .005$), a trend toward depolarization ($P = .10$) and no detectable difference in action potential duration. Other reports note a decrease in I_{Na} during extracellular acidosis (Woodhull, 1973; Yatani *et al.*, 1984) as well as membrane depolarization and widening of action potential (Spitzer and Hogan, 1979).

TABLE 1

Action potential characteristics in drug-free solutions

Values are mean ± S.E.M. Statistical significance of differences was determined by using the Wilcoxon signed-rank test on slopes of lines connecting data points for each muscle at each pH tested.

	pH			
	6.5 *n* = 4	6.9 *n* = 9	7.4 *n* = 13	7.9 *n* = 6
RMP (mV)[a]	85.5 ± 2.7	85.7 ± 4.2	86.2 ± 3.0	87.0 ± 5.1
APD (msec)	239 ± 44	194 ± 33	205 ± 26	198 ± 37
$\dot{V}_{max}$ (V/sec)[b]	179 ± 35	226 ± 19	248 ± 22	258 ± 28

[a] Abbreviations: *n*, number of papillary muscles; RMP, resting membrane potential; APD, action potential duration; and $\dot{V}_{max}$, maximal upstroke velocity of phase 0.
[b] $P < .005$.

TABLE 2

Action potential characteristics and $\dot{V}_{max}$ blockade in lidocaine 15 μM

	pH			
	6.5 *n* = 4	6.9 *n* = 11	7.4 *n* = 16	7.9 *n* = 9
RMP (mV)[a]	87.9 ± 1.5	82.9 ± 3.8	83.7 ± 3.4	84.3 ± 3.0
APD (msec)[b]	200 ± 20	183 ± 16	180 ± 26	179 ± 34
$\dot{V}_{max}$ (V/Sec)[b]	210 ± 38	220 ± 30	242 ± 29	265 ± 29
b_0[c]	0.20 ± 0.04	0.35 ± 0.09	0.38 ± 0.06	0.30 ± 0.07
τ_r (msec)[c]	230	194 ± 52	125 ± 27	86 ± 26

[a] $P < .0005$.
[b] $P < .005$.
[c] Abbreviations: b_0, proportion of $\dot{V}_{max}$ blockade at end of steady-state action potential and τ_r, time constant of $\dot{V}_{max}$ reactivation. Other abbreviations and values are as reported in table 1. The Wilcoxon signed-rank test was used to determine statistical significance of differences.

TABLE 3

Action potential characteristics and $\dot{V}_{max}$ blockade in W6211 15 μM

Abbreviations and values are as reported in table 2.

	pH			
	6.5 *n* = 5	6.9 *n* = 17	7.4 *n* = 25	7.9 *n* = 19
RMP (mV)[a]	88.0 ± 2.8	85.5 ± 2.6	86.7 ± 2.7	87.7 ± 2.9
APD (msec)[b]	235 ± 23	221 ± 25	219 ± 23	219 ± 24
$\dot{V}_{max}$ (V/sec)[a]	203 ± 23	227 ± 23	257 ± 27	268 ± 29
b_0	0.36 ± 0.11	0.46 ± 0.13	0.33 ± 0.09	0.19 ± 0.11
τ_r (msec)	154 ± 36	110 ± 26	60 ± 15	53 ± 16

[a] $P < .005$.
[b] $P < .05$.

Recovery of $\dot{V}_{max}$ proceeded very quickly in drug-free solutions at all tested pH values. Using a 3% fractional $\dot{V}_{max}$ reduction as the limit of resolution of this technique, we were able to determine τ_r at only one pH in only one muscle—at pH 6.9, τ_r was 94 msec. Prior studies from this laboratory and others using similar methodology have also noted unmeasurable or very short time constants of recovery of $\dot{V}_{max}$ in drug-free solutions (Chen *et al.*, 1974; Grant *et al.*, 1980, 1982; Broughton *et al.*, 1984; Stambler *et al.*, 1985).

Effect of pH and buffer on action potential characteristics and recovery kinetics in lidocaine. Introduction of lidocaine 15 μM at pH 7.4 resulted in significant shortening of the action potential duration (194 ± 26 to 183 ± 24 msec, $P < .005$) but no significant changes in membrane potential (83.8 ± 3.4 to 83.9 ± 3.3 mV) or steady-state $\dot{V}_{max}$ (237 ± 26 to 238 ± 28 V/sec). As pH was changed from 7.9 to 6.5 in the presence of lidocaine, there was a significant depolarization of the membrane, widening of the action potential and decrease in $\dot{V}_{max}$ (table 2).

$\dot{V}_{max}$ was reduced in test pulses with short coupling intervals, and b_0, the amount of block present at the beginning of diastole, averaged 38% at pH 7.4. There was a monoexponential relationship between $\dot{V}_{max}$ reduction and coupling interval in each experiment, with correlation coefficients averaging over 0.95. The averaged values show a 45% slowing of dissociation as pH was lowered from 7.9 to 7.4, 55% further slowing from 7.4 to 6.9 and 19% more from 6.9 to 6.5. The values of τ_r at pH 7.4 and 6.9 are in good agreement with the experiments of Broughton *et al.* (1984) and imply that using 15 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, a nonbicarbonate buffer system with a buffering capacity similar to that of physiologic HCO_3^-/CO_2, did not significantly alter the kinetics of drug dissociation in this system (Stambler *et al.*, 1985).

Predicted results when neutral drug molecules predominate. Extracellular protons are thought to enter Na channels (Woodhull, 1973), and Broughton *et al.* (1984) assumed that a drug molecule within a channel was still ionizable in accordance with the difference between the drug pK_a and the extracellular pH. The ratio of neutral to charged drug molecules was termed R and was defined as 10^{pH-pK_a}. Their proposed scheme of drug-membrane interaction is shown in figure 1. Protonation and deprotonation of channel-bound drug molecules were assumed to be rapid. They designated ℓ_c as the aggregate rate constant for the process of unbinding of charged drug molecules and their subsequent diffusion away through the aqueous inner pore of the channel protein. They likewise designated ℓ_n as the rate constant for dissociation of neutral drug molecules. In addition to egress via the channel pore, these lipid-soluble molecules might exit the channel protein through its hydrophobic amino acid segments into the neutral core of the lipid bilayer. From these assumptions, the following relationship of τ_r to R, ℓ_n and ℓ_c was derived.

$$\tau_r = \frac{1 + R}{\ell_n R + \ell_c} \qquad (2)$$

This led to the predicted relationship of τ_r and R shown in figure 2. τ_r nears a minimum value as the number of neutral drug molecules exceeds that of charged drug molecules, suggesting that no further pH potentiation will occur when pH is significantly higher than pK_a. From their experimental values of τ_r and R at pH values of 6.9 and 7.4, they calculated values for ℓ_n of 2.3×10^{-2} ms^{-1} and ℓ_c of 2.1×10^{-3} ms^{-1}. Our calculated values for ℓ_n (1.9×10^{-2} ms^{-1}) and ℓ_c (3.9×10^{-3} ms^{-1}) are in good agreement despite the difference in buffers in the two studies.

Action potential characteristics and recovery kinetics in W6211. Table 3 and figure 3 show the changes in action potential characteristics during superfusion of cardiac muscle with W6211 15 μM. The reduction in action potential duration seen with lidocaine was not evident with W6211 (232 ± 21 to 228 ± 23 msec, $P = .28$), and there was no significant change in resting membrane potential (87.0 ± 3.0 in both) or resting $\dot{V}_{max}$ (247 ± 30 to 250 ± 34 V/sec, $P = .46$). As with lidocaine, a decrease in pH from 7.9 to 6.5 brought a significant depolarization of the membrane, lengthening of action potential duration and decrease in $\dot{V}_{max}$ (table 3).

Figure 4 shows the effect of pH on W6211 blockade and recovery kinetics. Because W6211 is an order of magnitude less lipid-soluble than lidocaine (table 4), one might expect less

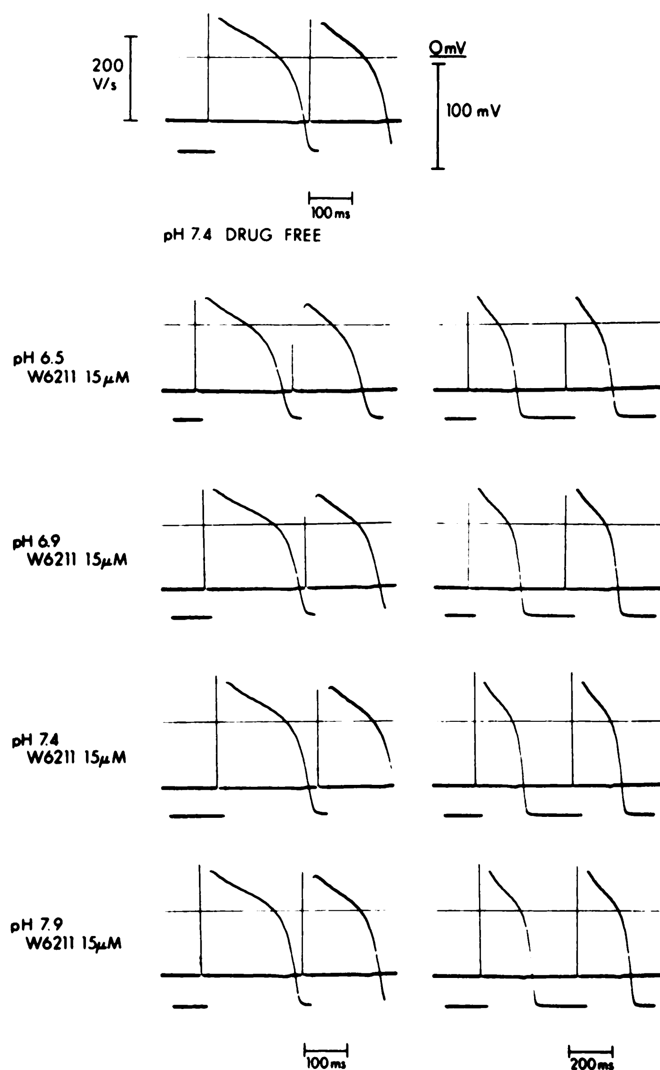


Fig. 3. Action potentials and $\dot{V}_{max}$ in W6211. Top panel shows drug-free steady-state action potential (left) followed by extrastimulus at very short coupling interval. $\dot{V}_{max}$ is only minimally reduced in the second impulse. Panels below show the pH-dependent effects of W6211 on steady-state action potential characteristics, and on $\dot{V}_{max}$ of test impulses at short (left column) and longer (right column) coupling intervals. At all coupling intervals, $\dot{V}_{max}$ of the test impulse is more reduced in solutions of lower pH. Note the difference in time scale in left and right columns.

TABLE 4
Comparison of lidocaine and W6211

	Lidocaine	W6211
pK_a	7.86	6.29
Molecular weight	234	250
Lipid solubility[a]	2.2	1.2
pH	R (neutral/charged)	
6.5	0.04	1.58
6.9	0.12	4.0
7.4	0.35	12.6
7.9	1.0	40

[a] log P octanol:water.

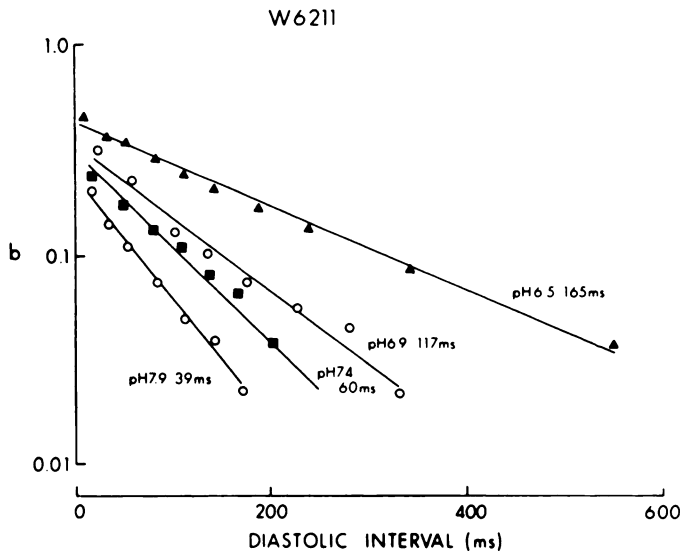


Fig. 4. Kinetics of $\dot{V}_{max}$ recovery after W6211 blockade in a single guinea pig papillary muscle exposed to the drug at varying extracellular pH. Natural logarithm of block (b) is plotted on the abscissa, and the diastolic coupling interval is plotted on the ordinate. Data points are the average of five measurements, and the lines through them were derived by a fit of equation 1 to the data. The slope of each line represents the inverse time constant, τ_r, the overall time constant of $\dot{V}_{max}$ recovery at each pH. The linearity of each group of points illustrates the monoexponential nature of $\dot{V}_{max}$ recovery as a function of diastolic interval. As pH falls from 7.9 to 6.5, τ_r rises from 39 to 165 msec.

$\dot{V}_{max}$ reduction and an attenuation of pH-induced changes in dissociation kinetics (Broughton *et al.*, 1984). Neither of these effects was seen. The amount of block at the beginning of diastole, b_0, was comparable for both drugs at pH 7.4, averaging 0.33 for W6211 and 0.38 for lidocaine. In addition, $\dot{V}_{max}$ recovery in W6211 slowed by 13% as pH fell from 7.9 to 7.4, 83% from 7.4 to 6.9 and 40% more from 6.9 to 6.5. We observed, therefore, a pH-dependent change in $\dot{V}_{max}$ recovery kinetics despite an up to 10-fold excess of neutral drug molecules. Our calculated value for ℓ_n for W6211 is 2.0×10^{-2} ms^{-1} and for ℓ_c is -2.6×10^{-2} ms^{-1}. The value for ℓ_n for W6211 is very similar to that calculated previously for lidocaine. The implications of a negative value for ℓ_c will be discussed.

Lipid solubility determinations. Calculation of P, the ratio of octanol:water partitioning at 2 pH units above drug pK_a, was based on measured drug concentrations in the aqueous phase before and after partitioning, using the equation:

$$P = \frac{(Cb - Ca)}{Ca}$$

Cb is the concentration in water before partitioning, and Ca is the concentration in water after partitioning. Each concentration is determined by the equation:

$$C = \frac{(H - I)}{S}$$

where H is the peak height, I is the intercept and S is the slope of the calibration curve.

In three replicate assays, log P was 1.25 ± 0.04 (range 1.21–1.29) (table 4).

Discussion

Several investigations from this and other laboratories have examined the slowing of recovery from local anesthetic blockade of Na channels in myocardium exposed to extracellular acidosis. This pH potentiation of drug effect, which should be of clinical importance in the treatment of ischemic cardiac arrhythmias, was first reported in acutely ischemic canine myocardium (Kupersmith *et al.*, 1975; Hondeghem, 1976). Grant *et al.* (1980; 1982) demonstrated slowing of recovery of $\dot{V}_{max}$ from block in acidotic guinea pig papillary muscle during exposure to lidocaine and quinidine. A kinetic scheme was proposed in the latter papers for protonation and deprotonation of channel-bound drug molecules, with extracellular acidosis generating ever larger amounts of the more slowly dissociating charged form. Broughton *et al.* (1984), in a study of the contribution of lipid solubility, went on to formulate expressions predicting the effect of drug pK_a. By assuming that charged and neutral forms of blocked channels are in continuous equilibrium with external hydrogen ions, they concluded that the recovery time constant τ_r would reach a minimum value once pH exceeded pK_a. In other words, the scheme predicts that a drug which is predominantly neutral over the test pH range should dissociate at an essentially constant rate despite changes in extracellular pH.

The major finding of this study is that cardiac Na channels blocked by predominantly neutral molecules (W6211) as well as those blocked by physicochemically similar but predominantly charged molecules (lidocaine) show continuous variation of τ_r with extracellular pH. In addition, the mathematical expressions generated by this scheme, when solved using experimental data for W6211, yield a negative estimate for ℓ_c, the aggregate time constant of dissociation of charged drug molecules from blocked channels. This study suggests that this view of channel blockade is incomplete in its portrayal of the interactions of the drug, the channel, the membrane and extracellular hydrogen ions.

The scheme of channel blockade utilized by Broughton *et al.* was based on the following assumptions: 1) a large fraction of charged drug molecules in the channel might exit through the aqueous channel pore during diastole (fig. 1), 2) protonation and deprotonation of drug molecules in the channel were extremely rapid processes, and charged and neutral moieties of drug-complexed channels are in continuous equilibrium (fig. 1), 3) the pK_a of the drug was the same whether it was bound to the receptor in the channel or free in solution, 4) the receptor affinity was unaffected by extracellular pH, 5) extracellular protons had unlimited access to the drug within the channel, 6) the receptor site was the same for all three tertiary amine local anesthetic compounds studied and 7) over the test pH range, extracellular protons did not exert significant effects on

transmembrane potential, screening charge, kinetics of channel gating, lipid phase or protein configuration.

The results of other investigations suggest that the first two assumptions bear revision. The first is that charged drug molecules in the channel may exit through the aqueous channel pore during diastole, a time during which the h gate is open but the m gate is closed. The ability of charged drug to escape *via* the channel pore during diastole has been challenged by Yeh (1978) and Yeh and Tanguy (1985). The latter study showed that permanently charged quaternary derivatives of lidocaine escaped from the channel at a very slow rate when the membrane was held at a normal negative resting potential, but that drug escape was enhanced when the membrane was held at less negative potentials. This suggested a voltage-dependent "trapping" of charged drug by the activation gate. On the other hand these findings have yet to be confirmed in experiments on cardiac tissue. Thus it appears reasonable to assume that charged drug molecules cannot escape at a significant rate from the resting channel during diastole. The second assumption is that charged and neutral moieties of drug-complexed channels are in continuous equilibrium. Proton transfer in unbound enzymes takes place rapidly but not instantaneously (Eigen and Hammes, 1963), and it is likely that the finite rate of this exchange process cannot be neglected. Indeed, Schwarz *et al.* (1977) integrated such an assumption in calculating binding and unbinding constants in a study of lidocaine in acidotic skeletal muscle.

Figure 1 shows our original kinetic scheme incorporating the rate constants k_p and ℓ_p for the protonation and deprotonation reactions, respectively. The rates of change of charged blocked channels (B_c) and neutral blocked channels (B_n) are described by:

$$\frac{dB_c}{dt} = k_cD_c[U] + k_pH^+B_n - (\ell_c + \ell_p)B_c \qquad (3)$$

and

$$\frac{dB_n}{dt} = k_nD_n[U] + \ell_pB_c - (k_pH^+ + \ell_n)B_n \qquad (4)$$

where k_c and k_n are aggregate terms describing drug association for charged and neutral molecules during diastole that combines rate constants for binding, diffusion and field effect, and ℓ_c and ℓ_n are aggregate terms describing drug dissociation during diastole that combines rate constants for binding, diffusion and field effect.

Because drug uptake is negligible during diastole, the association rates k_nD_n and k_cD_c are discounted. Inasmuch as the charged drug molecules are now assumed to be trapped once bound, ℓ_c is assumed to be negligible at the resting potentials observed in this study as shown in our amended kinetic scheme (fig. 5). With these assumptions, the rates of change of B_c and B_n can be described by:

$$\frac{dB_c}{dt} = k_pH^+B_n - \ell_pB_c \qquad (5)$$

and

$$\frac{dB_n}{dt} = \ell_pB_c - (\ell_n + k_pH^+)B_n \qquad (6)$$

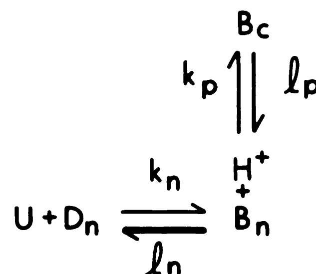
The solution is the sum of two exponentials where the slower exponential time constant is approximated by:

$$\tau_r = \frac{1}{\ell_p} + \frac{1}{\ell_n}\left(1 + \frac{k_pH^+}{\ell_p}\right) \qquad (7)$$

We define the pK_a of the drug-complexed channel as log (k_p/ℓ_p) and term it pK_a'. By assuming that it is the same as the pK_a of the drug in bulk solution (that is, $pK_a = pK_a'$), we estimated ℓ_p and ℓ_n for both drugs using equation 7 and a nonlinear least squares procedure. We found that ℓ_p for lidocaine was negative. Again, this suggests inadequacy of the kinetic model or its underlying assumptions.

The next assumption to be revised concerned the pK_a' of the drug-receptor complex. Although assumed above to be the same as the pK_a of the drug itself, involvement of ionizable groups in the drug-receptor bond may make it different. Alternatively, the pH near or within the channel may differ from the extracellular pH, distorting the predicted amounts of neutral and charged drug molecules. Fitting equation 7 to the experimental data where ℓ_p, ℓ_c and pK_a' were unknowns yielded positive rate constants for all reactions of both drugs: for lidocaine, $\ell_p = 1.1 \times 10^{-2}$ ms^{-1} and $\ell_n = 6.9 \times 10^{-2}$ ms^{-1}; for W6211, $\ell_p = 2.7 \times 10^{-1}$ ms^{-1} and $\ell_n = 6.7 \times 10^{-2}$ ms^{-1}. The pK_a' for lidocaine was 7.48 and for W6211 was 7.35. The fit of the predicted and measured data points is shown in figure 6.

A consistent trend of drug-receptor pK_a' away from the pK_a of the drug might be ascribed to a difference in the pH of the channel and the extracellular space—indeed, analysis of the Na channel blocking effect of phenytoin in nerve led to this conclusion (Morello *et al.*, 1984). We, on the other hand, found the pK_a' to be lower than the pK_a of lidocaine and higher than that of W6211. These inconsistent trends might be due to different and drug-specific conformational changes in the bound drug molecule, resulting in different affinity of the terminal ionizable N group for protons within the channel (Scheiner and Hillenbrand, 1985). Alternatively, this result of our calculations may be due to the relatively large S.D.s of the measurements of recovery time constants, or to the choice of test drugs—although a large range of neutral:charged drug ratios was tested, the range tested with either drug alone was



$$\tau = \frac{1}{\ell_p} + \frac{1}{\ell_n}\left(1 + \frac{1}{R^*}\right)$$

Fig. 5. Revised scheme of interaction of local anesthetic molecules with cardiac Na channel. It is no longer assumed that channel-bound charged drug molecules are able to exit the channel during diastole, and rate constants are given to the processes of protonation (k_p) and deprotonation (ℓ_p) of drug molecules within the channel. Abbreviations: U, unblocked channel; D_c, D_n, charged, neutral drug molecules; B_c, B_n, channels blocked by charged, neutral drug molecules; $R = 10^{pH-pK_a}$ or ratio of neutral to charged drug molecules; $R^* = 10^{pH-pK_a'}$ or ratio of channels blocked by neutral molecules to channels blocked by charged molecules; and $pK_a' = pK_a$ of the drug-receptor complex.

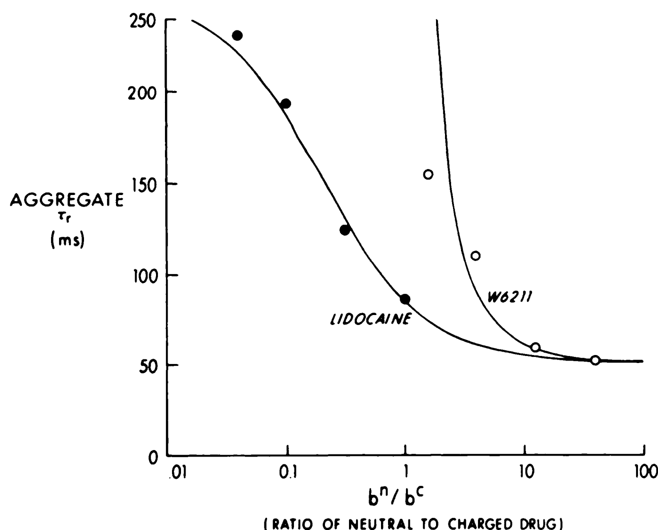


Fig. 6. Actual and predicted values of τ and R for both drugs. Experimental values are represented by closed (lidocaine) and open (W6211) circles, and curves were generated using the new kinetic scheme (fig. 5, equation 7). Drug-receptor pK_a was allowed to vary in these determinations, and its least squares estimate was 7.48 for lidocaine and 7.35 for W6211. Note the difference in the axes compared to figure 2.

either predominantly charged (lidocaine) or predominantly neutral (W6211).

Other simplifying assumptions inherent in the proposed scheme would, if incorrect, mandate more complex analysis. The drug receptor, for instance, is assumed to be accessible to extracellular protons (Woodhull, 1973). Different drugs, though, or even the neutral and charged forms of the same drug may bind at different receptor sites within the channel, bringing nonuniform responses to changes of extracellular pH. The assumption that the receptor has an affinity for the drug that remains constant despite changes in extracellular pH is untested. It is also assumed that the dissociation rate constant for neutral molecules from blocked channels, ℓ_n, is independent of pH. This aggregate term includes the rate of unbinding from the receptor and the rate of diffusion through the membrane (Starmer and Hollett, 1985). These processes may be affected by the physical state of membrane lipids and proteins, and may thereby be affected by pH. Unbinding may depend in part on the conformation of the channel protein, which may vary with pH (Verma and Wallach, 1976). Diffusion may be affected by the physical phase of nearby areas of the bilayer. Because the ionization state of the charged phosphate group may alter phospholipid interactions, extracellular pH may change the temperature at which phase changes occur (Boggs, 1980). Acidosis favors formation of the gel phase of several membrane phospholipids at physiologic temperature (Lee, 1977; Boggs, 1980). This more rigid ordering of side chains in the neutral core of the bilayer may present more of an obstacle to the egress of the neutral drug molecule, and its effect on channel conductance may dissipate more slowly as a result.

Acidosis has other direct and indirect effects on Na channels and on other ions, which were assumed to be negligible for purposes of modelling. In single heart cells, extracellular acidosis depresses peak I_{Na} and slows inactivation (Yatani *et al.*, 1984) $[Ca]_o$ increases with extracellular acidosis but would cause a decrease rather than an increase in τ_r for lidocaine (Betancourt and Dresel, 1979; Oshita *et al.*, 1980). αi_{Ca} decreases with extracellular acidosis (Hess and Weingart, 1980), but this has an unknown effect on Na channel inactivation and on dissociation of local anesthetic molecules. Intracellular acidosis has been noted to hasten Na channel inactivation in nerve (Courtney, 1979; Wanke *et al.*, 1980) [and intracellular alkalosis to reduce inactivation in nerve (Brodwick and Eaton, 1978)] but delay inactivation in muscle (Nonner *et al.*, 1980). In either case, we would not expect large changes in intracellular pH given the buffering ability of synthetic buffers in myocardium exposed to acidosis (Steenbergen *et al.*, 1977; Deitmer and Ellis, 1980) and the length of time required for intracellular pH response to extracellular acidosis (Schwarz *et al.*, 1977).

In summary, two tertiary amine local anesthetics of very different pK_a showed comparable changes in dissociation kinetics over a wide range of physiologic extracellular pH. We conclude that the relative proportion of neutral and charged drug molecules in the extracellular space may not be an important factor in predicting the duration of Na channel block by these compounds. Instead, the pK_a' of the drug-receptor complex may be the more important predicting factor and may differ significantly from the pK_a of the drug itself. In addition, the rate of deprotonation of channel-bound charged drug cannot be neglected and may be an important rate-limiting step.

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