Mild Alkalization and Acidification Differentially Modify the Effects of Lidocaine or Mexiletine on Vasorelaxation Mediated by ATP-sensitive K⁺ Channels

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Background: The previous study by the authors showed that the class Ib antiarrhythmic drug lidocaine impairs but mexiletine augments vasorelaxation mediated by adenosine triphosphate-sensitive K⁺ channels. Lidocaine and mexiletine have different values of the negative logarithm of the drug-proton dissociation constant, indicating that the ion channel-blocking effects of these drugs under different pH levels may vary. However, the role of pH in the effects of lidocaine and mexiletine on vasodilation mediated by K⁺ channels has not been studied. Therefore, the current study was designed to examine whether the inhibition and augmentation of vasorelaxation in response to an adenosine triphosphate-sensitive K⁺ channel opener, levcromakalim, by the clinically relevant concentrations of lidocaine or mexiletine are modified by mild alkalization or acidification in the isolated rat aorta.

Methods: Rings of the rat aorta without endothelium were suspended for isometric force recording. Three types of modified Krebs-Ringer solutions (pH 7.2, 7.4, and 7.6) were prepared by changing the composition of NaCl and NaHCO₃. During contractions in response to phenylephrine (3 × 10⁻⁷ M), relaxations in response to levcromakalim (10⁻⁸ to 10⁻⁵ M) were obtained. Lidocaine (10⁻⁸ to 10⁻⁵ M), mexiletine (10⁻⁸ to 10⁻⁵ M), or glibenclamide (10⁻⁵ M) was applied 15 min before the addition of phenylephrine.

Results: Relaxations in response to levcromakalim, which are abolished by the selective adenosine triphosphate-sensitive K⁺ channel antagonist glibenclamide (10⁻⁵ M), were not different among the three pH groups. In the normal Krebs-Ringer solution of pH 7.4, lidocaine significantly reduced these relaxations in a concentration-dependent fashion. Alkalization of pH 7.6 augmented the inhibitory effect of lidocaine on these relaxations, whereas acidification of pH 7.2 substantially abolished this effect. In contrast, mexiletine pH independently augmented relaxations in response to levcromakalim. Glibenclamide (10⁻⁵ M) abolished these relaxations in arteries treated with mexiletine (10⁻⁵ M) in any pH group.

Conclusions: These results suggest that even under conditions of such mild alkalosis or acidosis, vasorelaxation via adenosine triphosphate-sensitive K⁺ channels is dependent on pH in the presence of clinically relevant concentrations of lidocaine but not mexiletine.

THE class Ib antiarrhythmic drugs lidocaine and mexiletine reportedly inhibit cardiac Na⁺ channels, resulting in their antiarrhythmic action.¹ Most antiarrhythmic drugs exist as both charged and uncharged forms of these drugs. The uncharged form seems to dissolve readily into the lipid phase of the cell membrane and reaches the binding sites of Na⁺ channels.² Because the ratio of the uncharged form compared with the charged form of antiarrhythmic drugs is determined by the negative logarithm of the drug-proton dissociation constant (pKa) of the drug and pH of the external solution, it is conceivable that extracellular pH plays an important role in the Na⁺ channel-blocking effects of each antiarrhythmic drug. Lidocaine and mexiletine have different pKa values, indicating that the Na⁺ channel-blocking effects of these antiarrhythmic drugs under different pH levels may vary.¹ However, even in cardiac myocytes, the role of mild changes of pH in the effects of lidocaine and mexiletine on ion channels has not been demonstrated.

Increasing evidence suggests that adenosine triphosphate (ATP)-sensitive K⁺ channels play an important role in physiologic and pathophysiologic vasodilation.³ Previous studies, including ours, showed the inhibitory or augmenting effects of lidocaine and mexiletine on the activity of ATP-sensitive K⁺ channels.⁴⁻⁹ pH changes may be capable of modifying these effects of class Ib antiarrhythmic drugs on vasodilation mediated by ATP-sensitive K⁺ channels because extracellular pH seems to contribute to the effects of lidocaine and mexiletine on Na⁺ channels by changing the ratio of the uncharged to the charged form of the drugs.¹⁻³ However, the role of pH changes in the vasodilation mediated by K⁺ channels has not been well-studied. Therefore, the current study was designed to examine whether the inhibition and augmentation of vasorelaxation in response to an ATP-sensitive K⁺ channel opener, levcromakalim, by the clinically relevant concentrations of lidocaine or mexiletine are modified by the mild alkalization or acidification in the isolated rat aorta.

Methods

The study was approved by the institutional animal care and use committee (Wakayama Medical College, Wakayama, Japan). The experiments were performed on
Drugs

The following pharmacologic agents were used: dimethyl sulfoxide, glibenclamide, lidocaine hydrochloride, and phenylephrine (Sigma, St. Louis, MO). Mexiletine hydrochloride and levromakalim were gifts from Boehringer Ingelheim Pharm. KG. (Ingelheim, Germany) and SmithKline Beecham Pharmaceutical Company (Betchworth, Surrey, Great Britain), respectively. Drugs were dissolved in distilled water such that volumes of less than 60 μl were added to the organ chambers. Stock solutions of levromakalim (10⁻⁵ M) and glibenclamide (10⁻⁵ M) were prepared in dimethyl sulfoxide (3 × 10⁻⁴ M). The concentrations of drugs are expressed as final molar concentration.

Statistical Analysis

The data are expressed as mean ± SD; n refers to the number of rats from which the aorta was taken. Statistical analysis was performed using repeated measures analysis of variance, followed by the Scheffé F test for multiple comparison. Differences were considered to be statistically significant when P was less than 0.05.

Results

During submaximal contractions in response to phenylephrine (3 × 10⁻⁷ M), a selective ATP-sensitive K⁺ channel opener, levromakalim (10⁻⁸ to 10⁻⁵ M) induced concentration-dependent relaxations in the rat thoracic aorta without endothelium (fig. 1). These relaxations, which are abolished by a selective ATP-sensitive K⁺ channel antagonist, glibenclamide (10⁻⁵ M), were not different among the three pH groups (fig. 1). At normal pH (pH 7.4), lidocaine (3 × 10⁻⁵, 10⁻⁴ M) significantly reduced relaxations in response to levromakalim in a concentration-dependent fashion (fig. 2). Alkalinization (pH 7.6) augmented the inhibitory effect of these concentrations of lidocaine (fig. 2 and table 1). However, acidification (pH 7.2) substantially abolished this effect of lidocaine on vasodilator responses to levromakalim, although it seemed to be a tendency of the shift in the concentration–response curve (fig. 2 and table 1). In contrast to lidocaine, mexiletine (3 × 10⁻⁵, 10⁻⁴ M) augmented relaxations in response to levromakalim in the pH-independent fashion (fig. 3 and table 2). Glibenclamide (10⁻⁵ M) abolished these relaxations in arteries treated with mexiletine (10⁻⁴ M) in any pH group (fig. 4). Neither lidocaine nor mexiletine produced any effects on contractions to phenylephrine in any pH group (data not shown).

Discussion

This is the first study showing the differential role of pH changes in the effects of the class Ib antiarrhythmic drugs lidocaine and mexiletine on vasorelaxation mediated by K⁺ channels. Our results suggest that even under conditions of such mild alkalosis or acidosis, vasorelaxation via ATP-sensitive K⁺ channels is dependent on pH.
in the presence of clinically relevant concentrations of lidocaine but not mexiletine.

In the current study, glibenclamide, which has been shown to be a selective antagonist of ATP-sensitive K⁺ channels, abolished relaxations in response to levcromakalim. These results are consistent with our recent study of the isolated rat aorta showing that vasorelaxation to levcromakalim is completely inhibited by glibenclamide. Our previous finding in the rat aorta that glibenclamide did not affect relaxations in response to levcromakalim was confirmed in this study.

Fig. 1. Concentration–response curves for levcromakalim (10⁻⁸ to 10⁻⁵ M) in the absence and in the presence of glibenclamide (10⁻⁵ M), obtained in rat thoracic aortas without endothelium in Krebs-Ringer solutions at different pH values. Data are shown as mean ± SD and expressed as percent of maximal relaxation induced by papaverine (3 × 10⁻⁴ M; 100% = 1,180 ± 334 mg [n = 4] and 990 ± 354 mg [n = 4] for control rings and rings treated with glibenclamide in pH 7.2; 1,080 ± 407 mg [n = 4] and 1,030 ± 332 mg [n = 4] for control rings and rings treated with glibenclamide in pH 7.4; 960 ± 86 mg [n = 4] and 1,006 ± 104 mg [n = 4] for control rings and rings treated with glibenclamide in pH 7.6, respectively). *Difference between control rings and rings treated with glibenclamide is statistically significant (P < 0.05).

Fig. 2. Concentration–response curves for levcromakalim in the absence or in the presence of lidocaine (10⁻⁵, 3 × 10⁻⁵, 10⁻⁴ M), obtained in rat thoracic aortas without endothelium in Krebs-Ringer solutions at different pH values. Data are shown as mean ± SD and expressed as percent of maximal relaxation induced by papaverine (3 × 10⁻⁴ M; 100% = 1,300 ± 306 mg [n = 4], 1,197 ± 473 mg [n = 4], 1,233 ± 222 mg [n = 6], and 1,193 ± 231 mg [n = 6] for control rings and rings treated with 10⁻⁵, 3 × 10⁻⁵, or 10⁻⁴ M lidocaine in pH 7.2; 100% = 1,357 ± 260 mg [n = 4], 1,310 ± 274 mg [n = 6] for control rings and rings treated with 10⁻⁵ or 10⁻⁴ M lidocaine in pH 7.6, respectively). *Difference between control rings treated with lidocaine is statistically significant (P < 0.05).
to nitric oxide donors also reinforces the selectivity of glibenclamide on ATP-sensitive $K^+$ channels in this preparation.\textsuperscript{6}

In the rat aorta, relaxations induced by levcromakalim were not different in any pH group. These results are consistent with the evidence that modulation of vasorelaxation in response to ATP-sensitive $K^+$ channel openers induced by alkalinization or acidification within such physiologically ranged changes of pH has not been demonstrated. Although in isolated coronary and cerebral arteries decreased extracellular pH, beyond the values in the current study, reportedly produces vasorelaxation mediated by ATP-sensitive $K^+$ channels, it seems that the mild degree of changes in the extracellular pH in our study is at least partly because of these differential results.\textsuperscript{13,14}

In the current study, mild pH changes affected the inhibitory effect of lidocaine on vasodilation in response to levcromakalim, whereas they did not alter the augmenting effect of mexiletine. It may be possible that the mechanism of observed differences in mild changes of pH effects between these two antiarrhythmic drugs is the result of alterations in the ionization of ATP-sensitive $K^+$ channel protein, which affects its ability to respond more to lidocaine than to mexiletine. Lidocaine and mexiletine have different pKa values of 7.85 or 9.3.

### Table 1. Effect of Acidification and Alkalization on Relaxations to Levcromakalim in the Rat Aorta without Endothelium Treated with Lidocaine

<table>
<thead>
<tr>
<th>pH</th>
<th>Control</th>
<th>$10^{-5}$ M</th>
<th>$3 \times 10^{-5}$ M</th>
<th>$10^{-4}$ M</th>
</tr>
</thead>
<tbody>
<tr>
<td>−log EC\textsubscript{50}</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.2</td>
<td>6.65 ± 0.09</td>
<td>6.66 ± 0.05</td>
<td>6.55 ± 0.18</td>
<td>6.44 ± 0.12</td>
</tr>
<tr>
<td>7.4</td>
<td>6.62 ± 0.09</td>
<td>6.50 ± 0.08*</td>
<td>6.45 ± 0.10</td>
<td>6.29 ± 0.11</td>
</tr>
<tr>
<td>7.6</td>
<td>6.65 ± 0.05</td>
<td>6.61 ± 0.11</td>
<td>6.30 ± 0.08*</td>
<td>6.18 ± 0.08*</td>
</tr>
</tbody>
</table>

Maximal responses to levcromakalim ($10^{-5}$ M)

<table>
<thead>
<tr>
<th>pH</th>
<th>Control</th>
<th>$10^{-5}$ M</th>
<th>$3 \times 10^{-5}$ M</th>
<th>$10^{-4}$ M</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.2</td>
<td>−72.0 ± 8.3</td>
<td>−73.1 ± 11.6</td>
<td>−70.4 ± 8.8</td>
<td>−68.5 ± 6.2</td>
</tr>
<tr>
<td>7.4</td>
<td>−68.1 ± 8.7</td>
<td>−66.0 ± 8.2</td>
<td>−58.0 ± 8.2</td>
<td>−51.9 ± 8.6*</td>
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<tr>
<td>7.6</td>
<td>−78.6 ± 9.8</td>
<td>−78.2 ± 12.5</td>
<td>−67.5 ± 10.5</td>
<td>−61.2 ± 10.0</td>
</tr>
</tbody>
</table>

Data are shown as mean ± SD. Maximal responses to levcromakalim ($10^{-5}$ M) were expressed by percent of maximal relaxation to papaverine ($3 \times 10^{-4}$ M).

* $P < 0.05$ versus pH 7.2.

Fig. 3. Concentration–response curves for levcromakalim in the absence or in the presence of mexiletine ($10^{-5}$, $3 \times 10^{-5}$, $10^{-4}$ M), obtained in rat thoracic aortas without endothelium in the Krebs-Ringer solutions at different pH values. Data are shown as mean ± SD and expressed as percent of maximal relaxation induced by papaverine ($3 \times 10^{-4}$ M; 100% = 1,117 ± 340 mg [n = 6], 1,077 ± 165 mg [n = 6], 1,073 ± 273 mg [n = 6], and 1,053 ± 335 mg [n = 6] for control rings and rings treated with $10^{-5}$, $3 \times 10^{-5}$, or $10^{-4}$ M mexiletine in pH 7.2; 1,113 ± 148 mg [n = 6], 1,100 ± 219 mg [n = 6], 1,185 ± 179 mg [n = 6], and 1,030 ± 129 mg [n = 6] for control rings and rings treated with $10^{-5}$, $3 \times 10^{-5}$, or $10^{-4}$ M mexiletine in pH 7.4; 1,020 ± 165 mg [n = 6], 1,003 ± 231 mg [n = 6], 963 ± 211 mg [n = 6], and 970 ± 195 mg [n = 6] for control rings and rings treated with $10^{-5}$, $3 \times 10^{-5}$, or $10^{-4}$ M mexiletine in pH 7.6, respectively). Differences between control rings and rings treated with mexiletine is statistically significant ($P < 0.05$).
respectively, suggesting that the differential proportion of uncharged form between these antiarrhythmic drugs in the same pH solution may be at least partly because of the differential pH-dependency of these drugs for vasodilation mediated by ATP-sensitive K<sup>+</sup> channels. However, when one considers the previous studies showing the ratio of the uncharged to the charged form of lidocaine and mexiletine in the different extracellular pH, alteration of only 0.2 or 0.4 pH units would be expected to alter the amount of the uncharged drug a very small degree. Therefore, the differential ratio of the uncharged to the charged form of the compound in such mild alkalosis and acidosis may not be responsible for our results regarding the differential modulator effects of lidocaine and mexiletine on vasodilation mediated by ATP-sensitive K<sup>+</sup> channels. pH-dependent effects of lidocaine and mexiletine on Na<sup>+</sup> channels have been demonstrated only in a relatively large extent of pH changes in cardiac myocytes. We cannot rule out the possibility that our results from lidocaine and mexiletine may be mediated by some components other than ATP-sensitive K<sup>+</sup> channels, including G-protein coupled receptors, because it has been demonstrated that the activity of G-protein is related to the inward rectifier K<sup>+</sup> channel modulation in cardiac myocytes.

The ATP-sensitive K<sup>+</sup> channel is a complex of two proteins: the sulfonylurea receptor, which is a member of the ATP-binding cassette transporter family, and a smaller protein, Kir6.1 or 6.2, which belongs to the

<table>
<thead>
<tr>
<th>pH</th>
<th>Control</th>
<th>Mexiletine</th>
<th>Mexiletine + Glibenclamide</th>
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</thead>
<tbody>
<tr>
<td>7.2</td>
<td>6.61 ± 0.12</td>
<td>6.72 ± 0.07</td>
<td>6.62 ± 0.15</td>
</tr>
<tr>
<td>7.4</td>
<td>6.64 ± 0.09</td>
<td>6.70 ± 0.17</td>
<td>6.69 ± 0.08</td>
</tr>
<tr>
<td>7.6</td>
<td>6.71 ± 0.13</td>
<td>6.78 ± 0.25</td>
<td>6.73 ± 0.15</td>
</tr>
</tbody>
</table>

Maximal responses to levocromakalim (10<sup>-5</sup> M)

<table>
<thead>
<tr>
<th>pH</th>
<th>Control</th>
<th>Mexiletine</th>
<th>Mexiletine + Glibenclamide</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.2</td>
<td>−67.8 ± 7.8</td>
<td>−76.0 ± 16.0</td>
<td>−80.7 ± 6.3</td>
</tr>
<tr>
<td>7.4</td>
<td>−58.9 ± 5.2</td>
<td>−69.3 ± 11.6</td>
<td>−72.7 ± 7.8</td>
</tr>
<tr>
<td>7.6</td>
<td>−66.9 ± 11.3</td>
<td>−74.3 ± 8.2</td>
<td>−74.5 ± 4.5</td>
</tr>
</tbody>
</table>

Data are shown as mean ± SD. Maximal responses to levocromakalim (10<sup>-5</sup> M) were expressed by percent of maximal relaxation to papaverine (3 × 10<sup>-4</sup> M).

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Fig. 4. Concentration–response curves for levocromakalim in the presence of mexiletine (10<sup>-4</sup> M), glibenclamide (5 × 10<sup>-6</sup> M), or both, obtained in rat thoracic aorta without endothelium in the Krebs-Ringer solutions at different pH values. Data are shown as mean ± SD and expressed as percent of maximal relaxation induced by papaverine (3 × 10<sup>-4</sup> M; 100% = 1,328 ± 299 mg [n = 5] and 1,360 ± 217 mg [n = 5]) for rings treated with 10<sup>-4</sup> M mexiletine or 10<sup>-6</sup> M mexiletine plus 10<sup>-3</sup> M glibenclamide in pH 7.2; 1,015 ± 130 mg [n = 5] and 1,065 ± 30 mg [n = 5] for rings treated with 10<sup>-4</sup> M mexiletine or 10<sup>-6</sup> M mexiletine plus 10<sup>-3</sup> M glibenclamide in pH 7.4; 1,032 ± 183 mg [n = 5] and 1,088 ± 132 mg [n = 5] for rings treated with 10<sup>-4</sup> M mexiletine or 10<sup>-6</sup> M mexiletine plus 10<sup>-3</sup> M glibenclamide in pH 7.6, respectively. *Differences between rings treated with 10<sup>-6</sup> M mexiletine and 10<sup>-4</sup> M mexiletine plus 10<sup>-3</sup> M glibenclamide are statistically significant (P < 0.05).
inward rectifier K\(^+\) channel family.\(^{16}\) Because recent direct functional and biochemical studies have shown that the sulfonylurea receptor of the ATP-sensitive K\(^+\) channel is a primary target of the openers of this channel, it is likely that the differential pH-dependent effects of lidocaine and mexiletine on vasorelaxation to ATP-sensitive K\(^+\) channel openers are caused by the effects of these class Ib antiarrhythmic drugs on the sulfonylurea receptor of ATP-sensitive K\(^+\) channels in vascular smooth muscle cells.\(^{17}\) However, further biochemical studies are necessary to clarify the mechanisms responsible for the effects of lidocaine and mexiletine on ATP-sensitive K\(^+\) channels.

The therapeutic ranges of plasma concentrations of lidocaine and mexiletine used as antiarrhythmic drugs have been reported as 8 \(\times 10^{-6}\) to 5 \(\times 10^{-5}\) and 8 \(\times 10^{-7}\) to 5 \(\times 10^{-5}\) for lidocaine and mexiletine, respectively.\(^{18,19}\) Because approximately 50% of lidocaine and mexiletine is bound to plasma proteins, concentrations of lidocaine or mexiletine used in the current study are within and beyond the free plasma concentrations in the clinical situations, respectively.\(^{20}\) Therefore, our results suggest that in the clinical situations, lidocaine pH-dependently impairs vasodilation mediated by ATP-sensitive K\(^+\) channels, whereas clinically relevant concentrations of mexiletine may not affect these vasodilator effects.

Class Ib antiarrhythmic drugs are usually administered to treat ventricular arrhythmias, including ventricular premature contractions, ventricular tachycardia, and ventricular fibrillation, which can be often seen in patients with ischemic heart disease or during cardiopulmonary resuscitation.\(^{21,22}\) In these situations, vital organs may be subject to hypoxia, leading to acidosis in systemic circulation and local circulation of the organs. During hypoxia, acidosis, and ischemia, ATP-sensitive K\(^+\) channels are activated, resulting in arterial dilation and increased tolerance of tissues to ischemia.\(^{15,23-24}\) Therefore, when one uses lidocaine and mexiletine in these patients with acidosis, it may be speculated that lidocaine does not affect but mexiletine may augment beneficial vasodilator effects induced by activation of ATP-sensitive K\(^+\) channels, which play an important role in regulation of circulation during hypoxia, acidemia, and ischemia. In contrast, several types of ATP-sensitive K\(^+\) channel openers are now available to treat cardiovascular disorders, including hypertension and ischemic heart disease.\(^{25}\) Because the patients with these disease states often have ventricular arrhythmias, lidocaine and mexiletine can be coadministered with ATP-sensitive K\(^+\) channel openers. If these patients are slightly hyperventilated or hypoventilated in combination with metabolic acidosis or alkalosis during anesthesia and intensive care, the effects of these openers will be easily pH-dependently modified by lidocaine but not mexiletine. However, it may be difficult to extrapolate the current in vitro study to the clinical setting because of the following reasons. First, in the current study, many of the reported force changes produced by lidocaine and mexiletine were relatively small. Second, the effects of these antiarrhythmic drugs seem to be limited to vasodilation mediated by ATP-sensitive K\(^+\) channels and to be unrelated to other mechanisms affecting vasoconstriction because lidocaine and mexiletine did not alter baseline tone as well as contractions to phenylephrine in this study. Therefore, in the clinical setting, when multiple factors interact to regulate vascular smooth muscle tone, the role of mild pH changes in inhibitory as well as augmenting effects of lidocaine and mexiletine on ATP-sensitive K\(^+\) channels may be modest.

Even considering our results from conduit arteries, such as the aorta, it is still unclear whether our results have relevance to the smooth muscle function in resistance blood vessels, such as cerebral arterioles. However, because it is well-known that ATP-sensitive K\(^+\) channels play a major role in vasodilation, especially of smaller arteries, the current study also indicates the possibility that lidocaine and mexiletine may differentially produce pH-dependent and -independent modulation of pathophysiologically and pharmacologically induced vasodilator responses via ATP-sensitive K\(^+\) channels in resistance vascular beds.

References


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