Brief Communications

Effects of Proton Buffering and of Amiloride Derivatives on Reperfusion Arrhythmias in Isolated Rat Hearts
Possible Evidence for an Arrhythmogenic Role of Na\(^+\)-H\(^+\) Exchange

Steven C. Dennis, William A. Coetzee, Edward J. Cragoe Jr., and Lionel H. Opie

We investigated the hypothesis that an accelerated Na\(^+\)-H\(^+\) exchange on reperfusion may lead to a displacement of the 3[Na\(^+\)][Ca\(^{2+}\)]\(_{\text{io}}\) equilibrium in favor of an arrhythmogenic rise in cytosolic [Ca\(^{2+}\)]. Supporting evidence was obtained by subjecting isolated rat hearts to 15 minutes of low-flow (5\% of control) ischemia and 2 minutes of reperfusion in the presence of a Krebs-Henseleit HEPES buffer (pH 7.4) containing lactate (10 mM). At first, the [HEPES] was fixed at 5 mM; then, 2 minutes before reflow, either the [HEPES] was varied from 50 to 1 mM to slow H\(^+\) washout, or increasing concentrations of 5-(N,N-dimethyl)-amiloride (K\(_{\text{a}}\), 7 \(\mu\)M) or 5-(N,N-hexamethylene)-amiloride (K\(_{\text{a}}\), 0.2 \(\mu\)M) were added for inhibition of Na\(^+\)-H\(^+\) exchange. In each case, reperfusion ventricular arrhythmias were reduced by 69–73\% (\(p<0.001\)). (Circulation Research 1990;66:1156–1159)

Although an increase in cellular Ca\(^{2+}\) on myocardial reperfusion has been implicated in the development of reflow injury\(^1\) and arrhythmias,\(^2\) the mechanism of Ca\(^{2+}\) elevation remains unclear. One explanation may be that the accumulated Na\(^+\) in the previously underperfused myocardium\(^3\) exchanges for Ca\(^{2+}\).\(^4\) Calcium influx on cardiac reperfusion is increased by reductions in the transsarcolemmal [Na\(^+\)] gradient.\(^5\)

Alternatively, an exchange of Na\(^+\) for Ca\(^{2+}\) could also result from the recovery from ischemia-induced acidosis (Figure 1). An accelerated efflux of accumulated H\(^+\) in exchange for Na\(^+\) would be expected to raise Na\(^+\)\(_{\text{f}}\) and Ca\(^{2+}\)\(_{\text{f}}\) activity,\(^9,10\) even in the absence of Na\(^+\),K\(^+\)-ATPase inhibition.\(^8\)

In this report, we present preliminary evidence to suggest that Na\(^+\)-H\(^+\) exchange may be an important factor in the putative Ca\(^{2+}\) overload thought to underlie cardiac reperfusion arrhythmias.

Materials and Methods

Male Long-Evans rat hearts were perfused at 37\°C with a 100\% oxygenated Krebs-Henseleit (HEPES) buffer (pH 7.4) containing 3 mM K\(^+\), 1.25 mM Ca\(^{2+}\), and 10 mM sodium \(l\)-lactate. The replacement of HCO\(_3\)- with HEPES was intended to suppress H\(^+\) release via HCO\(_3\)-/Cl\(^-\) exchange,\(^13\) and the addition of lactate was designed to reduce H\(^+\)-lactate\(^-\) coefflux.\(^14\)

Perfusion Procedure

First, hearts (0.9–1.1 g wet wt) were perfused with a 5-mM HEPES medium at a constant aortic pressure of 73 mm Hg (10 kPa) for 15 minutes. During this time flexible platinum wire electrodes were attached to the right atrium and left ventricle and filtered (low-pass, –3 dB at 70 Hz) recordings of the electrocardiogram were made by use of a Grass Model 79D physiograph (Grass Instrument, Quincy, Massachusetts).

Provided the heart rate (280±3 beats/min, \(n=108\)) was regular in rhythm, the constant pressure coronary flow (11.5±0.1 ml/min/g wet wt, \(n=108\)) was replaced with 15 minutes of low flow (0.63±0.002 ml/min/g wet wt, \(n=108\)) from an Atto SJ 1211 peristaltic pump (Tokyo, Japan). For the initial 13 minutes of ischemia, the hearts continued to be supplied with a 5-mM HEPES buffer; then, 2 minutes before reperfusion, different media were given via a sidearm on the aortic cannula.

Reperfusion Media

In these media, either the Na HEPES–to–NaCl ratio was varied to alter the H\(^+\)\(_{\text{o}}\) buffering capacity, or
Measurements

Electrophysiological recordings were obtained from intracellular microelectrode (15- to 25-MΩ) recordings in an isolated superfused guinea pig papillary muscle during sequential 10-minute exposures to dimethyl sulfoxide (DMSO) (0.04%) and DMSO plus amiloride · HCl · 2H₂O (50 μM), DMA (50 μM), or HMA (1 μM). Whereas DMSO increased membrane potential (Eₘ) from ~86 to ~90 mV and decreased maximum upstroke velocity (dV/dtₘₚₖ) from 568 to 542 V/sec, amiloride and its analogues had no effect on action potential configuration.

Reperfusion Arrhythmias

Arrhythmias were monitored electrocardiographically. Over the 2-minute reperfusion, either there was a peak of premature ventricular contractions (PVCs) at 30–40 seconds or there was a prompt development of ventricular tachycardia (VT), which frequently degenerated into persistent ventricular fibrillation (VF).

Data Analysis

Results presented are the mean±SEM of the PVC+VT+VF durations expressed as a percent of the reperfusion time. Since the percent incidence of PVCs was relatively minor, the reductions in reperfusion arrhythmias were mainly due to decreases in the VT leading to VF. Statistical significance (p<0.05) was assessed by a Kruskal-Wallis nonparametric one-way analysis of variance.

Results

Buffering Capacity and Reperfusion Arrhythmias

The influence of [HEPES] on the severity of rhythm disturbances in the first 2 minutes of reper-

**FIGURE 1.** Tentative scheme illustrating how recovery from ischemia-induced acidosis may lead to cardiac reperfusion arrhythmias. Hypothesis is that an accelerated Na⁺-H⁺ exchange on reperfusion might be arrhythmogenic. The assumption that arrhythmias are due to secondary efflux of Na⁺, discharge for Ca²⁺, is based on a) observed increases in Ca²⁺ during Na⁺-H⁺ exchange⁹,¹⁰ and b) reported effects of Ca²⁺ on automaticity¹¹ and cellular uncoupling.¹² That Ca²⁺ influx may lead to cytoplasmic acidification and further Na⁺-H⁺ exchange is suggested by the finding that a decrease in intracellular pH is an important accompaniment of the inotropic effect of ouabain.¹⁰
fusion is shown in Table 1. As would be expected if a rapid Na\(^{+}\)-H\(^{+}\) exchange were arrhythmogenic, lowering of the perfusate H\(^{+}\) buffering capacity significantly reduced the incidence of VT and VF.

**Amiloride and Reperfusion Arrhythmias**

An antiarrhythmic effect was also seen when the [HEPES] was fixed at 50 mM and 0, 10, or 50 \(\mu\)M amiloride \(\cdot\) HCl \(\cdot\) 2H\(_2\)O was added in 0.04% DMSO. Here, however, the decrease in reperfusion arrhythmias could not be attributed to a slowing of Na\(^{+}\)-H\(^{+}\) exchange (Table 2). At 10 \(\mu\)M, amiloride would not be expected to inhibit Na\(^{+}\)-H\(^{+}\) or 3Na\(^{-}\)-Ca\(^{2+}\) exchange (Table 3).

**Dimethyl Amiloride and Reperfusion Arrhythmias.** In contrast, the effect of DMA on the incidence of reflow PVCs, VT, and VF was consistent with an arrhythmogenic role for Na\(^{+}\)-H\(^{+}\) exchange (Table 2). At close to the half-maximal inhibitory concentration (\(K_{i}\)) of 7-8 \(\mu\)M, DMA (10 \(\mu\)M) reduced the incidence of arrhythmias from 52% to 27%, and DMA (50 \(\mu\)M) decreased the incidence to 14%.

**Hexamethylen Amiloride and Reperfusion Arrhythmias**

A similar pattern was also seen when HMA, another selective Na\(^{+}\)-H\(^{+}\) exchange inhibitor, was added in 0.01% DMSO (Table 2). Again, the reduction in reflow rhythm disturbances corresponded well with the reported \(K_{i}\), of 0.2 \(\mu\)M.\(^{15}\) As the [HMA] was increased from 0 to 0.2 and 1.0 \(\mu\)M, the incidence of arrhythmias decreased from 76% to 42% and 21%, respectively.

**Discussion**

To our knowledge, this study is the first to investigate the possible involvement of Na\(^{+}\)-H\(^{+}\) exchange in the genesis of cardiac reperfusion arrhythmias. Previous studies of myocardial Na\(^{+}\)-H\(^{+}\) exchange have all been conducted in cultured cells or sarcolemmal vesicles, where increases in Na\(^{+}\), and Ca\(^{2+}\), could be readily measured.\(^{6-10}\)

In our reperfused hearts, the evidence for a rise in cytosolic Ca\(^{2+}\) is indirect and, therefore, has to be interpreted with caution. For instance, interstitial acidification at lower buffer concentrations\(^{16}\) may not only reduce arrhythmias by slowing Na\(^{+}\)-H\(^{+}\) exchange; protons also compete with Na\(^{+}\) and Ca\(^{2+}\) for access to their voltage-gated channels.\(^{17-18}\)

Similarly, the antiarrhythmic actions of DMA and HMA need not exclusively be due to inhibition of sarcolemmal Na\(^{+}\)-H\(^{+}\) exchange. With prolonged (>5 minutes) exposure, these compounds also have progressive negative chronotropic and vasoconstricting effects (data not shown), which are presumably the result of H\(^{+}\) retention in nodal tissue\(^{18}\) and/or blood vessel walls.\(^{19}\)

Further, although the Na\(^{+}\)-H\(^{+}\) exchange inhibitors appear not to influence myocardial Na\(^{+}\) or Ca\(^{2+}\) channels (Table 3), their specificity has not been fully characterized. Amiloride, for instance, decreases adenylate cyclase activity (\(K_{i}\), 6 \(\mu\)M), slows protein phosphorylation (\(K_{i}\), 1 mM), and blocks \(\alpha_{1}\)-(K, 24–33 \(\mu\)M), \(\alpha_{2}\)-(K, 14 \(\mu\)M), \(\beta\)-(K, 64 \(\mu\)M), and acetylcholine (K, 100 \(\mu\)M) receptors.\(^{15}\)

Nevertheless, the H\(^{+}\) buffering data plus the close association between the incidence of reflow arrhythmias and change in intracellular pH suggests that Na\(^{+}\)-H\(^{+}\) exchange is involved in the genesis of reperfusion arrhythmias. Further studies are needed to explore the role of Na\(^{+}\)-H\(^{+}\) exchange in myocardial reperfusion arrhythmias.

---

**Table 1. Antiarrhythmic Effects of Reduced Perfusion Buffering**

<table>
<thead>
<tr>
<th>[HEPES], pH 7.4 (mM)</th>
<th>Incidence of reflow arrhythmias (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>68±5</td>
</tr>
<tr>
<td>35</td>
<td>69±4*</td>
</tr>
<tr>
<td>25</td>
<td>55±12*</td>
</tr>
<tr>
<td>15</td>
<td>41±4*</td>
</tr>
<tr>
<td>5</td>
<td>34±4*</td>
</tr>
<tr>
<td>1</td>
<td>21±3*</td>
</tr>
</tbody>
</table>

Values are mean±SEM of six experiments. *p<0.001, describes statistical significance of overall trend.

---

**Table 2. Antiarrhythmic Effects of Amiloride Analogs**

<table>
<thead>
<tr>
<th>Apparent (K_{i}) for Na(^{+})-H(^{+}) exchange inhibition ((\mu)M)</th>
<th>Concentration used ((\mu)M)</th>
<th>Incidence of reflow arrhythmias (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amiloride (\cdot) HCl (\cdot) 2H(_2)O in 0.04% DMSO (84)</td>
<td>10</td>
<td>19±5*</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>12±2*</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>52±10</td>
</tr>
<tr>
<td>DMA in 0.04% DMSO (7)</td>
<td>10</td>
<td>27±8*</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>14±3*</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>76±4</td>
</tr>
<tr>
<td>HMA in 0.01% DMSO (0.2)</td>
<td>0.2</td>
<td>42±12*</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>21±3*</td>
</tr>
</tbody>
</table>

Values are mean±SEM of six experiments. Apparent \(K_{i}\) data are from Kleyman and Cragoe.\(^{15}\) K values for other ion transport blocking actions are summarized in Table 3. Lower incidence of arrhythmias in amiloride and DMA control hearts is possibly due to presence of relatively high (0.04%) concentration of DMSO. As described in “Materials and Methods,” 0.04% DMSO increases membrane potential and reduces \(dV/dt_{max}\). In the absence of DMSO, control incidence of reperfusion arrhythmias in DMA studies was 66±7% (\(n=6\)). DMSO, dimethyl sulfoxide; DMA, 5-(N,N-dimethyl)-amiloride; HMA, 5-(N,N-hexamethylene)-amiloride. \(K_{sa}\) half-maximal effective concentration. *p<0.01. **p<0.001.
mias and the $K_v$ for Na$^+$.H$^+$ exchange inhibition do support the hypothesis that Ca$^{2+}$ entry on reperfusion may be due to recovery from acidosis (Figure 1). Even though a secondary influx of Ca$^{2+}$ in exchange for Na$^{+}$, has to be extrapolated from studies in cultured cells,$^9,10$ such an influx is a logical explanation for the arrhythmogenic effects of Na$^+$.H$^+$ exchange. Whereas the removal of the incoming Na$^+$ via the Na$^+$.K$^+$-ATPase would only produce a slight hyperpolarization,$^8$ an exchange of Na$^+$ for Ca$^{2+}$ would promote automaticity and cellular uncoupling.$^2,11,12$

A Na$^+$.H$^+$-Ca$^{2+}$-exchange mechanism might also explain why Ca$^{2+}$ influx on reperfusion is a) not blocked by Ca$^{2+}$ channel antagonists and b) not associated with net Na$^+$ efflux.$^1$ The operation of a Na$^+$.H$^+$-Ca$^{2+}$ exchange is also consistent with the observation that arrhythmias are decreased by more gradual reperfusion.$^{20}$ A slower washout of accumulated H$^+$,$^{19}$ would limit the rate of Na$^+$.H$^+$ exchange$^6,7$ and allow more of the incoming Na$^+$ to leave via the Na$^+$.K$^+$-ATPase. Thus, in addition to accounting for our arrhythmia data, the presumed operation of a Na$^+$.H$^+$-Ca$^{2+}$ exchange on reperfusion is also in accord with previous findings.

**Acknowledgment**

The authors thank Lawrence Siko for technical assistance.

**References**

5. Grinwald PM: Calcium uptake during post-ischemic reperfusion in the isolated rat heart. *J Mol Cell Cardiol* 1982;14: 359–365

**Table 3. Ion Transport Blocking Effects of Amiloride, DMA, and HMA**

<table>
<thead>
<tr>
<th></th>
<th>Epithelial Na$^+$ channel</th>
<th>Na$^+$.H$^+$ exchange</th>
<th>Voltage-gated Ca$^{2+}$ channel</th>
<th>Voltage-gated Na$^+$ exchange</th>
<th>3Na$^+$.Ca$^{2+}$ exchange</th>
<th>3Na$^+$.2K$^+$-ATPase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amiloride</td>
<td>0.3</td>
<td>84</td>
<td>600</td>
<td>90</td>
<td>1,100</td>
<td>&gt;3,000</td>
</tr>
<tr>
<td>DMA</td>
<td>&gt;400</td>
<td>7</td>
<td>(&gt;50)</td>
<td>(&gt;50)</td>
<td>550</td>
<td>3,000</td>
</tr>
<tr>
<td>HMA</td>
<td>&gt;300</td>
<td>0.2</td>
<td>(&gt;)</td>
<td>(&gt;1)</td>
<td>100</td>
<td>...</td>
</tr>
</tbody>
</table>

Apparent $K_v$ data are from Kleyman and Craque.$^{18}$ Values in parentheses are suppositions based on action potential measurements described in "Materials and Methods." DMA, 5-(N,N-dimethyl)-amiloride; HMA, 5-(N,N-hexamethylene)-amiloride; $K_v$, half-maximal effective concentration.
Effects of proton buffering and of amiloride derivatives on reperfusion arrhythmias in isolated rat hearts. Possible evidence for an arrhythmogenic role of Na(+)-H+ exchange.
S C Dennis, W A Coetzee, E J Cragoe, Jr and L H Opie

Circ Res. 1990;66:1156-1159
doi: 10.1161/01.RES.66.4.1156

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/66/4/1156