Na\textsuperscript{+}/H\textsuperscript{+} Exchange Inhibition With HOE642 Improves Postischemic Recovery due to Attenuation of Ca\textsuperscript{2+} Overload and Prolonged Acidosis on Reperfusion

Hinrik Strömer, MD; Mark C.H. de Groot, PhD; Michael Horn, PhD; Christian Faul; Andrea Leupold; James P. Morgan, MD, PhD; Wolfgang Scholz, MD; Stefan Neubauer, MD

Background—Na\textsuperscript{+}/H\textsuperscript{+} exchange inhibition with HOE642 (cariporide) improves postischemic recovery of cardiac function, but the mechanisms of action remain speculative. Because Na\textsuperscript{+}/H\textsuperscript{+} exchange is activated on reperfusion, it was hypothesized that its inhibition delays realkalinization and decreases intracellular Na\textsuperscript{+} and, via Na\textsuperscript{+}/Ca\textsuperscript{2+} exchange, Ca\textsuperscript{2+} overload. Attenuated Ca\textsuperscript{2+} overload and prolonged acidosis are known to be cardioprotective.

Methods and Results—Left ventricular developed and end-diastolic pressures were measured in isolated buffer-perfused rat hearts subjected to 30 minutes of no-flow ischemia and 30 minutes of reperfusion (37°C) with or without 1 μmol/L HOE642 added to the perfusate 15 minutes before ischemia. Intracellular Ca\textsuperscript{2+} concentration ([Ca\textsuperscript{2+}]) and pH\textsubscript{r} were measured with aequorin (n=10 per group) and \textsuperscript{31}P NMR spectroscopy (n=6 per group), respectively. HOE642 did not affect preischemic mechanical function, [Ca\textsuperscript{2+}], or pH\textsubscript{r}. Mechanical recovery after 30 minutes of reperfusion was substantially improved with HOE642: left ventricular developed pressure (in percent of preischemic values) was 92±3 versus 49±7 and left ventricular end-diastolic pressure was 16±3 versus 46±5 mm Hg (P<0.05 for HOE642-treated versus untreated hearts). End-ischemic [Ca\textsuperscript{2+}], was significantly lower in HOE642-treated than in untreated hearts (1.04±0.06 versus 1.84±0.02 μmol/L, P<0.05). Maximal intracellular Ca\textsuperscript{2+} overload during the first 60 seconds of reperfusion was attenuated with HOE642 compared with untreated hearts: 2.0±0.3 versus 3.2±0.3 μmol/L (P<0.05). pH\textsubscript{r} was not different at end ischemia (≈5.9±0.05). Realkalinization was similar in the first 90 seconds of reperfusion and significantly delayed in the next 3 minutes (eg, 6.8±0.07 in HOE642-treated hearts compared with 7.2±0.07 in untreated hearts; P<0.05).

Conclusions—HOE642 improves postischemic recovery by reducing Ca\textsuperscript{2+} overload during ischemia and early reperfusion and by prolonging postischemic acidosis. (Circulation. 2000;101:2749-2755.)

Key Words: stunning, myocardial — myocardium — calcium — reperfusion

Intracellular Ca\textsuperscript{2+} overload on reperfusion after prolonged ischemia is an important pathophysiological factor that contributes to reduced postischemic recovery of mechanical function.\textsuperscript{1,2} It has been demonstrated that the Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger is largely responsible for the excessive Ca\textsuperscript{2+} influx during ischemia and reperfusion, which occurs secondary to intracellular Na\textsuperscript{+} overload.\textsuperscript{3–5} It has been hypothesized that during early reperfusion, Ca\textsuperscript{2+} overload is aggravated by the activation of the Na\textsuperscript{+}/H\textsuperscript{+} exchanger, with protons extruded in exchange for Na\textsuperscript{+} on realkalinization.

Pretreatment with Na\textsuperscript{+}/H\textsuperscript{+} exchange has been shown to provide substantial improvement of postischemic recovery in vivo and in isolated muscle preparations: amiloride, the classic Na\textsuperscript{+}/H\textsuperscript{+} exchange inhibitor, improved postischemic recovery and attenuated intracellular Ca\textsuperscript{2+} overload.\textsuperscript{5–7} However, its effect on Na\textsuperscript{+}/H\textsuperscript{+} exchange is rather nonspecific, because it also inhibits Na\textsuperscript{+}/Ca\textsuperscript{2+} exchange,\textsuperscript{6} the T-type Ca\textsuperscript{2+}- and the tetrodotoxin-sensitive Na\textsuperscript{+} channels.\textsuperscript{8} The first specific Na\textsuperscript{+}/H\textsuperscript{+} exchange inhibitor, HOE694, demonstrated cardioprotective effects in ischemia-reperfusion injury.\textsuperscript{8,9} Efforts to further improve potency and specificity led to the development of HOE642 (cariporide), a selective Na\textsuperscript{+}/H\textsuperscript{+} exchanger subtype I (the prevailing cardiovascular subtype) inhibitor. In a variety of ischemia models, HOE642 revealed a similar cardioprotective effect.\textsuperscript{10} The preliminary results of a large multicenter trial (Guardian Trial) revealed beneficial effects of the drug in patients with acute coronary syndromes.\textsuperscript{11} However, results of studies on the precise mechanisms of action of HOE642 in ischemia-reperfusion have not been

Received September 9, 1999; revision received December 30, 1999; accepted January 4, 2000.
From the Medizinische Universitätsklinik (H.S., M.C.H.d.G., M.H., C.F., A.L., S.N.), Würzburg, Germany; Harvard-Thorndike Laboratory (J.P.M.), Cardiovascular Division, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Mass; and the HMR Deutschland GmbH (W.S.), Frankfurt/Main, Germany.
Correspondence to Hinrik Strömer, MD, Medizinische Universitätsklinik, Josef-Schneider-Straße 2, 97080 Würzburg, Germany. E-mail stromer@mail.uni-wuerzburg.de
© 2000 American Heart Association, Inc.
Circulation is available at http://www.circulationaha.org
reported. It has been hypothesized that the cardioprotective effect of Na\(^+\)H\(^+\) exchange inhibition is mediated by delayed realkalinization on reperfusion with consequent attenuation of Na\(^+\) overload and, thus, intracellular Ca\(^{2+}\) overload. A Both preservation of acidosis and reduced Ca\(^{2+}\) overload were shown to be cardioprotective. To test this hypothesis for HOE642 during ischemia-reperfusion, measurements of intracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_i\)) and pH\(_i\) with high temporal resolution are required. In the present study, we used aequorin bioluminescence and \(^{31}\)P NMR spectroscopy techniques to assess [Ca\(^{2+}\)]\(_i\) and pH\(_i\), respectively, in an isolated heart model.

## Methods

### Isolated Heart

Studies were conducted according to the guidelines of the American Physiological Society Principles for Research Involving Animals and Human Beings. Isolated hearts were dissected and perfused as described previously. Briefly, male Wistar rats aged 8 to 10 weeks with a body weight of 300 to 330 g and a heart weight of 1.2 to 1.3 g were anesthetized with intraperitoneal pentobarbital, and the hearts were retrogradely perfused within 30 seconds after thoracotomy with an oxygenated (95% O\(_2\)/5% CO\(_2\), pH 7.4, at 37 \(^\circ\)C) Krebs-Henseleit solution containing (in mmol/L) NaCl 118, KCl 4.7, NaHCO\(_3\) 23, MgCl\(_2\) 1.2, CaCl\(_2\) 1.5, and dextrose 5.2. Left ventricular (LV) isovolumic pressure was recorded with an intraventricular latex balloon attached to a Statham P 23 XL transducer. The hearts were perfused at a coronary perfusion pressure of 60 to 70 mm Hg. This flow rate was kept constant except during no-flow ischemia.

### LV Pressure Recordings

LV pressure tracings were digitized with a 12-bit AD converter (sampling rate 1 kHz) and stored on magnetic disk. LV developed and end-diastolic pressures (LVPD and LVEDP, respectively), time to peak contraction (TP), time constant of exponential pressure decay (tau), and oscillatory Ca\(^{2+}\) (oscill max) were measured. Maximum and minimum values of the first derivative of pressure normalized by LVPD (+dP/dt/LVPD and −dP/dt/LVPD, respectively) were derived from the pressure tracing.

### Aequorin Loading and Normalization of Light Signals

Aequorin was microinjected, and the heart was positioned in an organ bath as described previously. Aequorin light signals were measured with a photomultiplier and digitized and stored as described for LV pressure. The light signals were analyzed for peak systolic (L\(_{\text{sys}}\)) and end-diastolic (L\(_{\text{dias}}\)) light. To signal the magnitude of the aequorin light signal depends on the amount of aequorin that had entered the cell during the loading procedure, the light signal must be normalized according to the following technique: HOE642 does not alter intracellular Ca\(^{2+}\) transients under normoxic conditions (see Results). Therefore, the preischemic amplitude of the Ca\(^{2+}\) light signals (ΔL\(_i\)) was chosen as a normalization reference. Because aequorin is consumed throughout the experiment, light values cannot simply be related to preischemic values as 100%, and aequorin consumption must be taken into account. For the conversion of light to calcium concentration, the method of fractional luminescence is used as described previously:

\[
L(t) = \frac{1 + K_I [Ca^{2+}]_i}{1 + K_E + K_R [Ca^{2+}]_i} \times L_{\text{max}}(t)
\]

where the \(K_I\) value is 4.65 \(\times\) 10\(^6\) mol/L and the \(K_R\) value is 135. \(L_{\text{max}}(t)\) is the maximum light value when all aequorin would be simultaneously activated by Ca\(^{2+}\) at time \(t\).

Time dependency of \(L_{\text{max}}\) due to aequorin consumption can be calculated as the following:

\[
L_{\text{max}}(t) = \int_0^t L(t) dt + L_{\text{max}(t=\text{end})}
\]

which is the time integral of aequorin light signal from time of interest (t) to the end of the experiment (t=\text{end}).

With the use of preischemic control values of 0.68 \(\mu\)mol/L for [Ca\(^{2+}\)]\(_{\text{sys}}\) and of 0.31 \(\mu\)mol/L for [Ca\(^{2+}\)]\(_{\text{dias}}\) as previously reported, and with replacement of these values in equation 1, respectively, the following can be calculated:

\[
L_{\text{sys}} = 1.3 \Delta L
\]

Thus, ΔL\(_{\text{sys}}\) was used in combination with the aequorin light integral from time \(t\) to the end of the experiment to calculate \(L_{\text{max}(t=\text{end})}\). \(L_{\text{max}(t=\text{end})}\) aequorin light values can be converted into [Ca\(^{2+}\)]\(_i\) at any other time point of the respective experiment with the use of equations 1, 2, and 3.

### Analysis of Calcium Overload in the First Minute of Reperfusion

To analyze Ca\(^{2+}\) overload occurring in the first minute of reperfusion, the following parameters were used (Figure 2, bottom). [Ca\(^{2+}\)]\(_{\text{max}}\) was end-ischemic [Ca\(^{2+}\)]\(_i\); peak was the initial peak of Ca\(^{2+}\) signal in the first minute of reperfusion, reflecting the first Ca\(^{2+}\) influx; Oscill\(_{\text{max}}\) was the maximum of the first 10 transients of Ca\(^{2+}\) oscillations in the first minute of reperfusion, reflecting the amount of Ca\(^{2+}\) released from the overloaded sarcoplasmic reticulum during each oscillation on top of the cytosolic Ca\(^{2+}\) overload; L\(_{\text{dias}}\) and L\(_{\text{dias}-60}\) were the time integrals of the first or second 30 seconds of reperfusion, respectively, normalized by L\(_{\text{max}(t=\text{end})}\), a global Ca\(^{2+}\) overload index; and L\(_{\text{dias}}\) and L\(_{\text{dias}-60}\) was the index of Ca\(^{2+}\) overload distribution with respect to time. The time intervals for L\(_{\text{dias}}\) and L\(_{\text{dias}-60}\) were chosen according to preliminary experiments, demonstrating that the major part of the peak of the reperfusion-induced Ca\(^{2+}\) overload is over within 1 minute and that no significant differences could be detected for light integral measurements of ≈1 minute.

### Measurement of pH\(_i\) With \(^{31}\)P NMR Spectroscopy

For NMR measurements, hearts were perfused in an NMR sample tube and inserted into the bore of a Bruker (AM 300) 7.05-T magnet (150 mm; Oxford Instruments) as previously described. \(^{31}\)P NMR spectra were collected at 121.5 MHz during 30-second intervals by signal-averaging 16 free induction decays with a pulse angle of 45° (recycle time 1.93 seconds). The free induction decays were analyzed in time domain (AMARES fit routine), and the inorganic phosphate (Pi) and phosphocreatine (PCr) resonance areas were

\[
\frac{\text{Pi}}{\text{PCR}} = \text{area of Pi peak} / \text{area of PCR peak}
\]
analyzed (MRUI package). The chemical shift difference between Pi and PCr was used to obtain pH values from a standard curve.\textsuperscript{18}

**Experimental Protocol (Aequorin Experiments)**

After the aequorin loading procedure, the temperature was increased to 37°C within 10 minutes, and the hearts were paced at 5 Hz. After steady state conditions were reached, a pressure-volume relationship was obtained to determine the volume at peak developed pressure (Vol\textsubscript{max}) as previously described.\textsuperscript{13} LV volume was set to 50% of Vol\textsubscript{max} and kept constant for the remainder of the experiment. After stabilization of mechanical function and aequorin light signals, HOE642 was added into the perfusate in the treated group, resulting in a concentration of 1 μmol/L. Vehicle (0.9% NaCl solution) was added to the perfusate in the untreated group (n=10 per group). Fifteen minutes later, no-flow ischemia was initiated. Pacing was discontinued 5 minutes after the initiation of ischemia. Hearts were reperfused after 30 minutes of ischemia. In all hearts, transient ventricular fibrillation occurred. Pacing was reinitiated after stabilization of the cardiac rhythm ~5 minutes after spontaneous defibrillation. The hearts were reperfused for 30 minutes after spontaneous defibrillation.

**Experimental Protocol (NMR Experiments)**

Additional groups of hearts were examined (6 untreated, 6 HOE642 treated) with the NMR spectrometer as described earlier to measure pH\textsubscript{i}. No aequorin loading was performed in these hearts, but otherwise, hearts were subjected to the same protocol.

**Statistical Analysis**

Data are reported as mean±SEM. A paired t test or a repeated measures ANOVA for within-group comparisons was performed where appropriate. A value of P<0.05 indicates significant differences between treated and untreated groups.

**Results**

**Effects of HOE642 on Baseline Cardiac Performance**

No significant changes in mechanical function, [Ca\textsuperscript{2+}], or pH\textsubscript{i} were induced with HOE642 under preischemic (values not shown) conditions, and all preischemic parameters for untreated and HOE642 hearts were similar (Figures 1A to 1F, Table).

**Cardiac Function During Ischemia and Reperfusion**

After the beginning of no-flow ischemia, LVDPP declined to zero within ~5 minutes (Figures 1A and 2). Ischemic contracture developed after ~5 minutes up to 32 mm Hg (Figures 1B and 2). In the second half of the ischemic period, resting pressure declined slightly to 24 mm Hg at the end of the ischemic period (Figure 1B). No differences in mechanical function or resting pressure, respectively, could be detected up to this time point between HOE642-treated and untreated hearts. On reperfusion, LVEDP increased to 60±4.3 mm Hg in untreated hearts compared with 47±2.8 mm Hg in the treated hearts (P<0.05). All hearts showed low-amplitude contractions before going into ventricular fibrillation within 2 minutes (Figure 2). Time of ventricular fibrillation was not abbreviated with HOE642 treatment. After an average of 9±2 minutes, hearts spontaneously defibrillated. In the next 20 minutes of reperfusion, LVEDP slowly declined and LVDPP increased, reaching a stable plateau 20 to 30 minutes after spontaneous defibrillation, resulting in an LVDPP of 49.6±7.7 in untreated and 92.5±3.2 (values are percent of preischemic values) in treated hearts, respectively (P<0.05; Figure 1A). Similarly, at the end of reperfusion, LVEDP was 43±6.8 mm Hg in untreated hearts compared with 15±3.1 mm Hg in the HOE642 group (P<0.05; Figure 1B). Coronary perfusion pressure was not influenced by HOE642 treatment throughout the protocol (Figure 1C).

After reperfusion, TP was prolonged and +dP/dt/LVDP was decreased compared with preischemic values. Relaxation was impaired after reperfusion as indicated by an increase of tau and decrease in −dP/dt/LVDP. These abnormalities could be completely prevented with HOE642 treatment (Table).

The reported LV pressure results were taken from aequorin experiments. Measurements in NMR experiments showed similar results.

**Intracellular Ca\textsuperscript{2+} During Ischemia and Reperfusion**

In the first few minutes of ischemia, the amplitude of the Ca\textsuperscript{2+} transient increased by ~30% in both groups and fell to zero after pacing was terminated (Figures 1E and 2). During ischemia, intracellular resting Ca\textsuperscript{2+} increased, as indicated by an increase in the aequorin light signal normalized by L\textsubscript{max}(t) (Figures 1E and 2). HOE642 treatment significantly blunted the ischemic Ca\textsuperscript{2+} overload. On reperfusion, intracellular Ca\textsuperscript{2+} markedly increased, reaching its peak after 15 to 20 seconds, in parallel to an increase in LV resting pressure (Figure 2, bottom). Subsequently, pressure and [Ca\textsuperscript{2+}], then slowly decreased until the first Ca\textsuperscript{2+} oscillations occurred (Figure 2). LV pressure showed only minor responses to Ca\textsuperscript{2+}, indicating that the myofilaments were still desensitized at this early stage of reperfusion.

Ca\textsuperscript{2+} overload at end ischemia and on reperfusion was significantly attenuated by HOE642 pretreatment, as indicated by a reduced end-ischemic [Ca\textsuperscript{2+}], reduced peak, and reduced I\textsubscript{0–30}/I\textsubscript{30–60} values (Figure 3). Ca\textsuperscript{2+} overload not only was attenuated by HOE642 but also was delayed, as indicated by a reduced I\textsubscript{0–30}/I\textsubscript{30–60} ratio in treated (0.78±0.09) versus untreated (1.84±0.3, P<0.05) hearts. Within 5 minutes on reperfusion, [Ca\textsuperscript{2+}], declined to preischemic values (Figure 2) with no differences between the groups after the first minute.

Peak systolic and LV end-diastolic [Ca\textsuperscript{2+}], in untreated and HOE642-treated hearts were similar in both groups 30 minutes after reperfusion. There were no differences from preischemic values (Figure 1E), suggesting that the availability of Ca\textsuperscript{2+} to activate the myofilaments was not responsible for postischemic dysfunction.

**pH on Reperfusion**

Preischemic pH\textsubscript{i} was similar for both groups. During ischemia, pH\textsubscript{i} declined at a similar rate and extent, by 1.2 pH units, in both groups (Figure 1D). On reperfusion, pH\textsubscript{i} was rapidly restored in the untreated group within 2 minutes (Figures 1D and 4). In the HOE642-treated group, recovery of pH\textsubscript{i} was similar for the first 90 seconds of reperfusion. However, for the next 3 minutes of reperfusion, realkalinization was significantly delayed in HOE642-treated compared with untreated hearts. Later, pH\textsubscript{i} reached preischemic values in both groups.
**Discussion**

In the present study, we investigated the cardioprotective effects of HOE642, a cardioselective Na⁺/H⁺ exchange inhibitor, during ischemia-reperfusion injury. To our knowledge, this is the first report on [Ca²⁺] and pH measurements of the effects of a Na⁺/H⁺ exchange inhibitor with a temporal resolution of ≤30 seconds, the resolution required to evaluate fast changes of proton and Ca²⁺ homeostasis on reperfusion.
During ischemia, acidosis develops due to ATP breakdown and lactate production. The low pH stimulates pH regulating transport systems such as the \( \text{Na}^+ / \text{H}^+ \) exchanger. After no-flow ischemia, extracellular pH drops secondary to intracellular acidification, thus likely reducing the activity of the \( \text{Na}^+ / \text{H}^+ \) exchanger. On reperfusion, extracellular pH is immediately restored with a rapid increase in extracellular pH, and the \( \text{Na}^+ / \text{H}^+ \) exchanger is reactivated. The \( \text{Na}^+ / \text{H}^+ \) exchanger then contributes to transient \( \text{Na}^+ \) overload linked to \( \text{Ca}^{2+} \) overload via the \( \text{Na}^+ / \text{Ca}^{2+} \) exchanger working in reverse mode (\( \text{Na}^+ \) out, \( \text{Ca}^{2+} \) in). Events during the first few minutes of reperfusion are considered to be the main determinants of reperfusion injury. One of the major factors for reperfusion injury leading to stunning, necrosis, and arrhythmias is the \( \text{Ca}^{2+} \) overload phenomenon. Intracellular acidosis during the early phase of reperfusion can protect the myofilaments against reperfusion injury.

**Time Course Parameter of Mechanical Function**

<table>
<thead>
<tr>
<th></th>
<th>No Treatment (n=10)</th>
<th>HOE642 Treatment (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Preischemia</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TP, ms</td>
<td>60.8±1.07</td>
<td>59.5±0.9</td>
</tr>
<tr>
<td>Tau, ms</td>
<td>22.7±0.5</td>
<td>23.4±0.38</td>
</tr>
<tr>
<td>( +!dp/dt/LVDP, \text{s}^{-1} )</td>
<td>26.4±0.6</td>
<td>27.3±0.7</td>
</tr>
<tr>
<td>( -!dp/dt/LVDP, \text{s}^{-1} )</td>
<td>21.6±0.4</td>
<td>21.3±0.2</td>
</tr>
<tr>
<td><strong>Change 30 min after reperfusion, % of</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TP</td>
<td>115±5.4†</td>
<td>105±2.8*</td>
</tr>
<tr>
<td>Tau</td>
<td>133±8.7†</td>
<td>106±3*</td>
</tr>
<tr>
<td>( +!dp/dt/LVDP )</td>
<td>89.8±4.3†</td>
<td>96.8±1.7</td>
</tr>
<tr>
<td>( -!dp/dt/LVDP )</td>
<td>87.4±3.8†</td>
<td>96.6±1.7*</td>
</tr>
</tbody>
</table>

Values are given as mean±SEM.
*
\( P<0.05 \) vs no treatment.
†\( P<0.05 \) vs preischemia.

**Figure 2.** Original chart-strip recording of representative experiments without (A) or with (B) HOE642 treatment. On onset of ischemia, LVDP rapidly decays to zero. Subsequently, ischemic contracture develops. Hearts fibrillate on reperfusion. At 20 to 30 minutes after spontaneous defibrillation, mechanical function stabilizes. Ischemic \( \text{Ca}^{2+} \) overload was blunted with HOE642 treatment. At end of reperfusion, \( \text{Ca}^{2+} \) transients return to preischemic level. Bottom, First 40 seconds of reperfusion are displayed to characterize reperfusion-induced \( \text{Ca}^{2+} \) overload phenomenon (scale enlarged from top). LV pressure increases in parallel with \( \text{[Ca}^{2+}\text{]} \), followed by \( \text{Ca}^{2+} \) oscillations. \( \text{[Ca}^{2+}\text{]} \), peak, and \( \text{Oscill}_{\text{max}} \) are \( \text{Ca}^{2+} \) overload indices as described in Methods. Note that \( \text{Ca}^{2+} \) overload on reperfusion was blunted by HOE642, correlating with improved postischemic recovery of mechanical function.

**Figure 3.** \( \text{Ca}^{2+} \) overload indices of reperfusion as defined in Method section and legend to Figure 2, bottom. *\( P<0.05 \) vs untreated (n=10 per group).
Ca\textsuperscript{2+} Overload and pH\textsubscript{i} on Reperfusion

In the present study, the Ca\textsuperscript{2+} overload phenomenon was characterized in an isolated heart model with a temporal resolution of a few milliseconds. This resolution was sufficient to detect not only a global increase in [Ca\textsuperscript{2+}], on reperfusion, as found with NMR indicator techniques\textsuperscript{5,7} or radioactive calcium,\textsuperscript{21} but also rapid sequences of Ca\textsuperscript{2+} transients as demonstrated in Figure 2 (bottom). This phenomenon is known as “Ca\textsuperscript{2+} oscillation,” characteristic of Ca\textsuperscript{2+} overload.\textsuperscript{22} In addition, pH\textsubscript{i} was measured with a time resolution of 30 seconds.

To analyze the effects of HOE642 on reperfusion-induced Ca\textsuperscript{2+} overload, various indices were defined as described in Methods and in Figure 2. In the present report, all Ca\textsuperscript{2+} overload indices were depressed with HOE642 treatment, although differences for Oscillmax were not significant (Figure 3). Furthermore, Ca\textsuperscript{2+} overload on reperfusion was delayed by HOE642, as indicated by a reduced I\textsubscript{0} - I\textsubscript{30} / I\textsubscript{30 - 60} ratio. Although the reduction in Ca\textsuperscript{2+} overload with HOE642 was evident only during the first minute of reperfusion, [Ca\textsuperscript{2+}]\textsubscript{i} was still elevated for the next 4 minutes. The rise in pH\textsubscript{i} makes the myofilaments most sensitive to injury after the first minute of reperfusion. Realkalinization was delayed from the second to the fourth minute of reperfusion by HOE642 (Figures 1D and 4). Therefore, both the effects on [Ca\textsuperscript{2+}]\textsubscript{i} and on pH\textsubscript{i} are cardioprotective and may explain the markedly beneficial effect on postischemic recovery of systolic and diastolic function (Figures 1A, 1B, and 2, Table).\textsuperscript{3,12}

In cardiomyocytes, the Na\textsuperscript{+}/H\textsuperscript{+} exchanger is only 1 of several pH-regulating systems\textsuperscript{1}: protons can leave the cell via the lactate/H\textsuperscript{+} symporter if lactate is present and via the Na\textsuperscript{+}/HCO\textsubscript{3}⁻ symporter depending on the Na\textsuperscript{+} gradient across the cell membrane.\textsuperscript{3} The relative importance of these mechanisms is unknown. In the concentration used for the present study, HOE642 (1 \(\mu\)mol/L) inhibits \textgreater 95% of Na\textsuperscript{+}/H\textsuperscript{+} exchanger subtype I, the predominant subtype in cardiac tissue.\textsuperscript{4,10} Na\textsuperscript{+}/H\textsuperscript{+} exchange is immediately activated on reperfusion by a reestablished pH gradient. Therefore, its inhibition can explain the observed reduction in Ca\textsuperscript{2+} influx (Figure 3). Because pH\textsubscript{i} was found to be similar in both groups during the first 90 seconds of reperfusion (Figure 4), the expected effect of HOE642 on pH\textsubscript{i} during this period was overridden by alternative mechanisms other than Na\textsuperscript{+}/H\textsuperscript{+} exchange, presumably by lactate/H\textsuperscript{+} washout. Subsequently, inhibition of Na\textsuperscript{+}/H\textsuperscript{+} exchange predominates as indicated by the differences in pH\textsubscript{i} 2 to 5 minutes after reperfusion (Figure 4).

The observation that Na\textsuperscript{+}/H\textsuperscript{+} exchange inhibition improves postischemic mechanical function is consistent with results from other groups who used in vivo or in vitro models of cardiac ischemia in various species.\textsuperscript{6,10,23} A decrease in Na\textsuperscript{+} and Ca\textsuperscript{2+} overload was demonstrated during 20 minutes of ischemia with NMR spectroscopy\textsuperscript{7} or \textsuperscript{4}\textsuperscript{Ca\textsuperscript{2+}} using the non-specific Na\textsuperscript{+}/H\textsuperscript{+} exchange inhibitor amiloride in isolated hearts.\textsuperscript{5} Pretreatment with HOE694, a selective, but less potent, Na\textsuperscript{+}/H\textsuperscript{+} exchange inhibitor compared with HOE642, improved cardiac output in the isolated working heart after 20 minutes of global ischemia.\textsuperscript{9} In blood-perfused isolated rabbit hearts subjected to 45 minutes of ischemia and 60 minutes of reperfusion, HOE694 improved postischemic recovery of systolic and diastolic function. High-energy phosphates and pH\textsubscript{i} measured with \textsuperscript{31}P NMR spectroscopy were similar during ischemia. ATP and PCr depletion were attenuated by HOE694 after 60 minutes of reperfusion. pH\textsubscript{i} showed a transient overalkalinization in untreated hearts 5 minutes after the beginning of reperfusion (time resolution 5 minutes), an effect abolished with HOE694.\textsuperscript{8}

In contrast to our findings, ischemic contracture was reported to be attenuated by a Na\textsuperscript{+}/H\textsuperscript{+} exchange inhibitor, and the incidence of reperfusion-induced arrhythmias was reduced.\textsuperscript{9,10,24} However, either these experiments were performed with regional ischemia in vivo or in vitro, or the duration of global no-flow ischemia was markedly shorter than that in our experiments. It is conceivable that the effects of Na\textsuperscript{+}/H\textsuperscript{+} exchange inhibition on ischemic contracture or reperfusion-induced arrhythmias exist for only a lesser degree of ischemic damage. In accordance with the present report, selective inhibition of Na\textsuperscript{+}/H\textsuperscript{+} exchange did not attenuate ischemic contracture during 40 minutes of no-flow ischemia in isolated buffer-perfused rat hearts\textsuperscript{25} or in blood-perfused rabbit hearts.\textsuperscript{8}

Although there were significant differences for [Ca\textsuperscript{2+}], during ischemia, ischemic contracture was virtually identical in both groups (Figures 1B and 1F). This observation is in accordance with the view that during ischemia, the myofilament metabolism was found to be unaltered by Na\textsuperscript{+}/H\textsuperscript{+} exchange inhibition.\textsuperscript{8}

The reduced accumulation of [Ca\textsuperscript{2+}], during ischemia is likely to render the myocytes less prone to further Ca\textsuperscript{2+} overload on reperfusion. This finding is consistent with previous reports that provide evidence that Na\textsuperscript{+}/H\textsuperscript{+} exchange inhibitors are more effective when given before ischemia rather than at the time of reperfusion.\textsuperscript{27,28}

Study Limitations

The changes in pH\textsubscript{i} and [Ca\textsuperscript{2+}], are independent observations, possibly but not necessarily related. According to current
consensus,3,4,8 improved susceptibility to ischemia is most likely linked to the observed changes in pH_i and [Ca^{2+}]_i, but no direct proof for this interrelation was given by the present data.

The aequorin light signal depends not only on [Ca^{2+}], but also on pH, in the sense that both ions compete for Ca^{2+}-binding sites of the aequorin molecule.29 However, because pH_i was comparable in both groups during ischemia and during the first minute of reperfusion, [Ca^{2+}]_i values quantified with the use of fractional luminescence were underestimated, but the relative differences between the treated and untreated hearts were unaffected.

**Clinical Potential**

In view of the experimental data, it is conceivable that HOE642 may become a clinically useful form of treatment in situations in which ischemia and reperfusion occur, such as cardioplegia during cardiac surgery, acute coronary syndrome, or before acute revascularization with angioplasty, bypass surgery, or thrombolysis. Patients with diagnosed coronary artery disease who are at risk for further ischemic events might be candidates for long-term treatment with HOE642. The preliminary results of a first clinical trial (Guardian Trial), revealing the beneficial effects of HOE642 in patients undergoing high-risk revascularization and preventing Q wave infarcts in patients with acute coronary syndrome, were promising.11

**Acknowledgments**

This work was supported in part by a grant from HMR Deutschland GmbH (Frankfurt/Main, Germany), grant SFB 355 A3 from the Deutsche Forschungsgemeinschaft, Germany (Drz Horn and Neubauer) and a grant from Verum-Foundation for behavior and environment (Dr Strömer).

**References**

Na⁺/H⁺ Exchange Inhibition With HOE642 Improves Postischemic Recovery due to Attenuation of Ca²⁺ Overload and Prolonged Acidosis on Reperfusion
Hinrik Strömer, Mark C. H. de Groot, Michael Horn, Christian Faul, Andrea Leupold, James P. Morgan, Wolfgang Scholz and Stefan Neubauer

Circulation. 2000;101:2749-2755
doi: 10.1161/01.CIR.101.23.2749

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2000 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/101/23/2749

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org//subscriptions/