Myocyte Volume and Function in Response to Osmotic Stress
Observations in the Presence of an Adenosine Triphosphate-Sensitive Potassium Channel Opener

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Background—Hypothermic hyperkalemic cardioplegia results in significant myocyte swelling and impaired contractility. These detrimental effects may be eliminated by the addition of an adenosine triphosphate-sensitive potassium (K\text{ATP}) channel opener. This study evaluated the hypothesis that a K\text{ATP} channel opener (diazoxide) would benefit volume homeostasis by limiting volume and subsequent contractility changes during osmotic stress.

Methods and Results—Isolated rabbit ventricular myocyte volume and contractility were evaluated using video microscopy and field stimulation after exposure to osmotic stress at 37°C. Myocytes were exposed to Tyrode’s physiological solution for 20 minutes and test solution for 20 minutes, and then reexposed to Tyrode’s for 20 minutes. Test solutions included control Tyrode’s (1T) and osmotically altered Tyrode’s (2.6T, 0.9T, and 0.6T) solutions with or without the K\text{ATP} channel opener diazoxide. Severe osmotic stress (2.6T and 0.6T) resulted in significant cell shrinkage and swelling, respectively. This was unchanged by the addition of diazoxide. Mild hyposmotic stress (0.9T) resulted in significant cell swelling that was eliminated by the addition of diazoxide. Cell swelling was associated with reduced contractility.

Conclusions—Cell swelling, but not shrinkage, was detrimental to myocyte contractility. Diazoxide eliminated volume change due to mild hyposmotic stress, similar to that previously noted with hyperkalemic cardioplegia, but did not alter volume change secondary to severe osmotic stress. (Circulation. 2005;112[suppl I]:I-219–I-223.)

Key Words: contractility ■ myocytes ■ stress

Isolated cardiac myocytes demonstrate a significant reduction in contractility after osmotic stress and ischemia. Paradoxically, our laboratory and others have demonstrated a similar finding after hyperkalemic cardioplegia, which is used clinically to protect myocytes from ischemia during cardiac surgery.1-3 Interestingly, hyperkalemic cardioplegia, osmotic stress, and ischemia also result in myocyte swelling.4-7 It has been our hypothesis that this myocyte structural change may be one of the mechanisms of myocardial stunning after cardiac surgery. Many investigators have linked myocardial edema and cell swelling to a decline in myocardial function; however, the mechanisms underlying myocardial stunning are varied and largely unknown.8

Our laboratory previously demonstrated the elimination of myocyte swelling secondary to hyperkalemic cardioplegia by the addition of an adenosine triphosphate-sensitive potassium (K\text{ATP}) channel opener.3 Moreover, we have also noted the prevention of myocyte contractile dysfunction after hyperkalemic cardioplegia with the amelioration of cellular swelling after the addition of a K\text{ATP} channel opener. This work has been supported by other investigators who have demonstrated a reduction in myocardial edema in isolated heart models and better preservation of isolated myocyte contractile function resulting from the addition of a K\text{ATP} channel opener during hyperkalemic cardioplegia.2,9 The K\text{ATP} channel also has been shown to play an important role in ischemic preconditioning, which has been demonstrated to prevent osmotic cell swelling and to ameliorate ischemic cell swelling at the cellular and mitochondrial levels.10,11 K\text{ATP} channel openers have not been previously shown to play a direct role in cell volume regulation, however, and the results of previous studies may have been due to their other cardio-protective properties in preventing ischemic injury. This study examined whether a K\text{ATP} channel opener would ameliorate the volume and contractility changes observed after osmotic stress in the absence of ischemia.

Methods
Experiments were approved by the Animal Studies Committee at Washington University. Animals received humane care in compliance with the Guide for the Care and Use of Laboratory Animals.

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I-219
Isolation of Myocytes

New Zealand white rabbits of either sex (3 to 4 kg; Myrtle, Rapid Tree, TN; Harlen, Indianapolis, IN) were anesthetized intramuscularly (xylazine 14.0 mg/kg, acepromazine 1.3 mg/kg, and ketamine 83.0 mg/kg) and given heparin intravenously (3000 U). Cardectomy was performed and the hearts were perfused in a retrograde fashion with 37°C Tyrode’s physiological solution containing (in mmol/L) 130 NaCl, 5 KCl, 0.4 KH₂PO₄, 3 MgCl₂, 5 HEPES, 15 taurine, 10 glucose, and 5.7 creatine, at a height of 80 cm. The pH was adjusted to 7.25 by titrating with 20% NaOH. A solution containing 1.8 mmol/L of CaCl₂ in Tyrode’s physiological solution was perfused for 5 minutes. Extracellular Ca²⁺ was washed out by perfusion of 0.1 mmol/L of Na₂EGTA in Tyrode’s physiological solution for 5 minutes. Solutions were equilibrated with 95% O₂–5% CO₂. Chamber temperature was controlled by a water bath and extrapolated to result in cellular volume change similar to that observed after hyperkalemic cardioplegia in previous experiments (6% to 7% volume change).³ Solution osmolarity was measured with a freezing point depression osmometer (Osmette S, Precision Systems). A stock solution of diazoxide was made by dissolving the reagent in dimethyl sulfoxide (DMSO, 0.1%), which has been shown to have no effect on cell volume.¹² Baseline volume measurements were recorded at 10, 15, and 20 minutes and averaged. Volume measurements were recorded every 5 minutes during the test solution and reperfusion periods. Baseline contractility was recorded after 15 minutes of control perfusion. Contractility measurements were recorded at 10 and 20 minutes after reexposure to 1T. Volumes are presented relative to baseline. Filled symbols represent significant differences as indicated.

Statistical Analysis

Results are expressed as mean ± standard error of the mean with N equal to the number of cells in each group. A repeated-measures analysis of variance was used for sequential time-based measurements for each test solution against its own baseline (volume) value. Using Fisher’s least significant difference test, post hoc multiple comparisons between different solutions were made separately during test solution and reperfusion periods. Contractility studies were analyzed by analysis of variance using Fisher’s post hoc test. Statistical significance was defined as P<0.05. Statistical analysis was performed using Stat View 5.0 (Abacus Concepts, Inc).

Results

The osmolarity (mOsm/L) of the test solutions were 196.6 ± 1.1, 293.5 ± 1.5, 324.1 ± 1.9, and 838.7 ± 4.0 for the 0.6T, 0.9T, 1T, and 2.6T groups, respectively. Osmolarity was not significantly increased by the addition of diazoxide.

Baseline Myocyte Volume

In the control 1T group, myocyte volume remained stable throughout the entire perfusion period (Figure 1).
Myocyte Volume and Severe Hyperosmotic Stress
Perfusion with 2.6T solution resulted in significant myocyte shrinkage (40.7% ± 1.1%, \( P < 0.0001 \) versus 1T) that was not altered by the addition of diazoxide (40.6% ± 0.9%, \( P < 0.0001 \) versus 1T and baseline, Figure 1). On reexposure to 1T solution, the cell volume in both groups returned to baseline within 10 minutes.

Myocyte Volume and Severe Hyposmotic Stress
Perfusion with 0.6T solution resulted in significant myocyte swelling (33.0% ± 2.9%, \( P < 0.0001 \) versus 1T and baseline) that was not altered by the addition of diazoxide (35.3% ± 3.1%, \( P < 0.0001 \) versus 1T and baseline, Figure 2). On reexposure to 1T solution, the cell volume in both groups significantly decreased (\( P < 0.05 \) versus baseline and 1T).

Myocyte Volume and Mild Hyposmotic Stress
Perfusion with 0.9T solution resulted in significant myocyte swelling (7.0% ± 1.4%, \( P < 0.05 \) versus 1T and baseline) that was significantly reduced by the addition of diazoxide (Figure 2).

Myocyte Contractility
Baseline myocyte contractility is represented in the Table. The only significant difference in contractility parameters between groups at baseline was in maximum velocity of shortening between the 0.6T group and the 2.6T group. Each parameter was subsequently normalized to its own baseline value.

Myocyte Contractility and Severe Hyperosmotic Stress
Myocyte contractility was significantly better preserved after exposure to the hyperosmotic 2.6T with diazoxide solution when compared with hyposmotic solutions (0.6T and 0.6T + DZX, Figure 3, Figure 4, and Figure 5). The myocyte contractility after exposure to hyperosmotic solution, however, was not significantly different from control 1T solution (Figures 3, 4, and 5).

Myocyte Contractility and Severe Hyposmotic Stress
Myocyte contractility was significantly reduced following exposure to hyposmotic stress (0.6T and 0.6T with diazoxide groups) when compared with the hyperosmotic 2.6T with diazoxide group. When examining velocity of shortening and velocity of relengthening, contractility in the 0.6T group was significantly reduced when compared with the 2.6T group (Figures 4 and 5).

### Myocyte Contractility at Baseline

<table>
<thead>
<tr>
<th>Parameters</th>
<th>0.6T (n=8)</th>
<th>0.6T + Dzx (n=8)</th>
<th>0.9T (n=8)</th>
<th>0.9T + Dzx (n=8)</th>
<th>1T (n=8)</th>
<th>2.6T (n=8)</th>
<th>2.6T + Dzx (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell shortening, %</td>
<td>10.9 ± 0.7</td>
<td>10.8 ± 0.8</td>
<td>10.2 ± 0.8</td>
<td>10.5 ± 0.7</td>
<td>10.4 ± 0.9</td>
<td>10.1 ± 0.7</td>
<td>9.6 ± 0.6</td>
</tr>
<tr>
<td>Maximum velocity of shortening, ( \mu \text{m/s} )</td>
<td>216.3 ± 33.2*</td>
<td>186.1 ± 20.0</td>
<td>178.7 ± 14.2</td>
<td>176.1 ± 14.7</td>
<td>174.1 ± 23.7</td>
<td>147.1 ± 12.8</td>
<td>164.2 ± 14.6</td>
</tr>
<tr>
<td>Maximum velocity of relengthening, ( \mu \text{m/s} )</td>
<td>232.0 ± 27.9</td>
<td>205.6 ± 20.9</td>
<td>210.0 ± 20.8</td>
<td>211.8 ± 20.2</td>
<td>205.6 ± 21.5</td>
<td>186.3 ± 17.4</td>
<td>210.5 ± 12.4</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM. 0.6T indicates severe hyposmotic Tyrode’s; 0.9T, mild hyposmotic Tyrode’s; 1T, normosmotic Tyrode’s; 2.6T, severe hyperosmotic Tyrode’s. Dzx indicates diazoxide.

*\( P < 0.05 \) vs 2.6T.
Myocyte Contractility and Mild Hyposmotic Stress

Exposure to mild hyposmotic stress (0.9T) resulted in a significant reduction in contractility when compared with the severe hyperosmotic (2.6T with diazoxide) solution. Velocity of relengthening was also significantly reduced when compared with the 2.6T group alone (Figures 3, 4, and 5). This reduced contractility was not significantly different from the control 1T group.

Myocyte Contractility and the K<sub>ATP</sub> Channel Opener Diazoxide

Diazoxide had no effect on contractility between groups at each level of osmotic stress (0.6T versus 0.6T with diazoxide, 0.9T versus 0.9T with diazoxide, or 2.6T versus 2.6T with diazoxide). The addition of diazoxide to the mild hyposmotic group (0.9T) eliminated the observed differences in contractility when compared with the 2.6T with diazoxide group (Figures 3, 4, and 5).

Discussion

Myocyte volume change occurs after hypothermic hyperkalemic cardioplegia, ischemia, and osmotic stress.4,7 Our laboratory previously demonstrated that the addition of either a non-specific (pinacidil) or claimed mitochondrial-specific (diazoxide) K<sub>ATP</sub> channel opener to hyperkalemic cardioplegia ameliorated the associated cell swelling and reduced contractility.3 In addition, we have demonstrated that opening the K<sub>ATP</sub> channel resulted in a decrease in myocyte size at normothermic temperature.3 Ischemic preconditioning has been demonstrated to provide resistance to osmotic cell swelling and to ameliorate ischemic cell swelling at the cellular and mitochondrial levels,10,11 and the K<sub>ATP</sub> channel is known to play an important role in ischemic preconditioning.13 This led to the hypothesis that a K<sub>ATP</sub> channel opener would participate in cellular volume homeostasis, and specifically, would ameliorate the cellular volume change secondary to osmotic stress in the absence of cardioplegia.

Osmotic Stress and Myocyte Volume: Effect of a K<sub>ATP</sub> Channel Opener

In this study, exposure to severe hyperosmotic (2.6T) Tyrode’s solution or to severe hyposmotic (0.6T) Tyrode’s solution resulted in significant cell shrinkage (40% below baseline) or swelling (30% above baseline), respectively (Figures 1 and 2). Exposure to mild hyposmotic (0.9T) Tyrode’s solution also resulted in a smaller but significant rate of cell swelling (7.0% above baseline). Although the addition of diazoxide to the severe hyperosmotic (2.6T) or severe hyposmotic (0.6T) Tyrode’s solution showed no effect on cell volume regulation, its addition to mildly hyposmotic Tyrode’s (0.9T) solution significantly ameliorated the associated cell swelling (Figure 2). The addition of diazoxide did not significantly increase the osmolarity of the solution, and therefore change in solution tonicity was not the mechanism by which cell swelling was ameliorated. These results suggest that the K<sub>ATP</sub> channel opener contributed to the myocyte volume regulation only under mild osmotic stress and had no effect on cell volume under severe osmotic stress. The degree of osmotic stress (33% to 40%) in the severe groups may have overwhelmed and exceeded the homeostatic capacity of the K<sub>ATP</sub> channel opener.

The extent of rabbit myocyte swelling after hypothermic hyperkalemic cardioplegia has been reported to be in the range of 6.1% to 10% from baseline.4,5,14,15 The mild hyposmotic solution used in this study was formulated to mimic this degree of cellular volume change. The cell swelling (6% to 7%) due to mild hyposmotic stress (0.9T) was prevented by the addition of a K<sub>ATP</sub> channel opener, similar to that observed
after hyperkalemic cardioplegia. The mechanism may involve a change at the mitochondrial level, as opening the mitochondrial K$_{ATP}$ channel has been demonstrated to result in alteration of both mitochondrial matrix and intermembrane space volumes.\textsuperscript{16} It has been suggested that cellular and mitochondrial volumes are closely related, but their exact relationship has not been clearly defined.\textsuperscript{11} Therefore, further experiments are necessary to evaluate the role of K$_{ATP}$ channel openers in myocyte volume homeostasis.

**Osmotic Stress and Myocyte Contractility: Effect of a K$_{ATP}$ Channel Opener**

Myocyte swelling secondary to hyposmotic stress was associated with a decline in contractility. Results indicate a range of myocyte dysfunction, with the 0.6T solution having the most profound effect on contractility. Interesting, hyposmotic stress (especially in the group with diazoxide) provided superior preservation of contractility compared with control and statistically significantly better contractility versus severe hyposmotic stress (0.6T).

The reduction of contractility after mild hyposmotic stress was similar to that after severe hyposmotic stress despite the difference in cell swelling. This finding may suggest that cell swelling profoundly reduces contractility, regardless of the extent of swelling and regardless of the rapidity with which the cell adapts to large volume change. Although the mechanism behind this finding is still largely unknown, osmotic cell swelling may irreversibly injure the structure of the myocyte as seen in the ischemic model, or cell membrane stretch may change the Ca$^{2+}$ ionic current, which could directly affect contractility.\textsuperscript{17,18}

Activity at the K$_{ATP}$ channel itself cannot be presumed to be responsible for the observations in this study based only on the addition of a channel opener. It has become apparent that the presence of a claimed specific or nonspecific K$_{ATP}$ channel opener or inhibitor does not indicate channel activity (or lack of channel activity). K$_{ATP}$ channel openers may have other nonspecific pharmacological effects.\textsuperscript{19–21} In addition, the molecular composition of the mitochondrial K$_{ATP}$ channel is yet to be elucidated and its existence has been questioned.\textsuperscript{22} Therefore, further experiments are necessary to evaluate the role of K$_{ATP}$ channel openers in myocyte volume homeostasis.

**Study Limitations**

Isolated myocytes were used because they allow for repeated measurements. These findings do not necessarily apply to the whole organ or organism because the complex milieu of the extracellular space, vascular, hormonal, and neural elements is lacking. Caution should therefore be taken before any extrapolation to the clinical setting.

**References**


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