Exercise training alters an anoxia-induced, glibenclamide-sensitive current in rat ventricular cardiocytes

KORINNE N. JEW AND RUSSELL L. MOORE
Department of Kinesiology and Applied Physiology, University of Colorado
Cardiovascular Institute, University of Colorado, Boulder, Colorado 80309-0354

Received 25 May 2001; accepted in final form 21 December 2001

Jew, Korinne N., and Russell L. Moore. Exercise training alters an anoxia-induced, glibenclamide-sensitive current in rat ventricular cardiocytes. J Appl Physiol 92: 1473–1479, 2002.—The effect of training on properties of a sarcolemmal ATP-sensitive K+ current (IKATP) was examined in left ventricular cardiocytes isolated from sedentary (Sed) and trained (Tr) female Sprague-Dawley rats. Whole cell patch-clamp techniques were used to characterize IKATP, an anoxia-inducible, glibenclamide-sensitive current. An anoxic condition was induced by superfusing cells with a buffer that was equilibrated with 100% N2, maintained under a layer of argon, and that contained 2-deoxy-D-glucose. Over a 1-h period of anoxia, 59% of Tr cells and 85% Sed cells expressed IKATP. In those cells that did express IKATP, the time to expression of the current during the anoxic period occurred significantly later in cells from the Tr group compared with the Sed. Peak IKATP density was significantly lower in the Tr cells compared with the Sed cells. These results indicate that the onset and magnitude of IKATP were altered by training. These alterations in IKATP may be reflective of processes that contribute to training-induced cardioprotection against ischemia-reperfusion damage.

ATP-sensitive K+ current; ATP-sensitive K+ channel; ischemia-reperfusion

EXERCISE TRAINING HAS BEEN associated with improved recovery of the heart from ischemia-reperfusion (I/R) insult (4, 5, 24, 37, 40). The cellular basis for this training-induced cardioprotective effect has not been identified, although various candidate adaptations have been proposed. The most prominent include a training-induced enhancement of myocardial antioxidant defense systems (37) and an increased expression of ~70- to 72-kDa heat shock protein (HSP72) (7, 26). Both types of adaptations have been strongly correlated to the increased resistance of the trained heart to the negative functional consequences of I/R challenge (7, 26, 37). However, exactly how these types of cellular adaptations contribute to the protection of the heart from I/R injury is not known.

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Address for reprint requests and other correspondence: K. N. Jew, Dept. of Kinesiology and Applied Physiology, Campus Box 354, Univ. of Colorado, Boulder, CO 80309-0354.

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METHODS

Animal Model

Female Sprague-Dawley rats, aged 3–4 mo, were randomly assigned to a sedentary (Sed) group (n = 10) and a run-trained (Tr) group (n = 12). All animals were housed in a 12:12-h light-dark cycle and given standard rat chow and water ad libitum. Animals in the Tr group underwent at least 20 wk of treadmill running. During the first 6 wk, daily running duration was 10 min and was prolonged biweekly in 10-min intervals. Running grade was 5%, and treadmill speed ranged from 20 to 28 m/min. During the next 6 wk, running grade was increased to 10%, and treadmill speed ranged from 20 to 35 m/min. The final training protocol consisted of treadmill running 5 days/wk up to a 10% grade for 1 h/day at 20 m/min for 15 min, 28 m/min for 30 min, and 35 m/min for 15 min. All animals were 9–11 mo of age when killed for cardiocyte isolation, at which time the adrenal glands, thymus, and spleen were dissected and weighed and the plantaris muscle was dissected, homogenized, and assayed for citrate synthase activity (41). This study was conducted under the guidelines accepted by the American Physiological Society and received prior approval from the Institutional Animal Care and Use Committee at the University of Colorado, Boulder campus.

Cardiocyte Isolation

Cardiocytes were obtained from the left ventricle and septal free wall by methods previously described in detail (30). All chemicals and reagents were acquired from Sigma Chemical (St. Louis, MO) unless otherwise noted. Isolated cardiocytes were suspended in growth medium and placed on the stage of an inverted microscope (Olympus). Whole cell patch-clamp techniques. Glass coverslips were removed from growth medium free wall by methods previously described in detail (30).

Measurements of I_{KATP}

Measurements of I_KATP were recorded from cardiocytes of 10 Sed (1–5 cells sampled per animal) and 12 Tr rats (3–5 cells sampled per animal) by using whole cell patch-clamp techniques. Glass coverslips were removed from growth media and used to form the bottom of a flow-through chamber and placed on the stage of an inverted microscope (Olympus). The external bathing solution contained (in mM) 150 NaCl, 5.4 KCl, 3.6 CaCl2, 1.2 MgCl2, and 10 HEPES at pH 7.4. Nifedipine (5 μM) was added to the external solution to suppress the slow, inward Ca^{2+} current. Current recordings were made at ambient temperature (25 ± 1°C) by using fire-polished, low-resistance (1.3–2.5 MΩ) glass pipettes containing an internal buffer composed of (in mM) 150 KCl, 5 HEPES, and 20 EGTA at pH 7.3. Whole cell currents were elicited and amplified by using the Axopatch 1D amplifier (Axon Instruments, Foster City, CA) in voltage-clamp mode and recorded onto a personal computer using pClamp 5.0 software (Axon Instruments).

Cardiocytes were voltage clamped to a holding potential of −45 mV, and an outward current was recorded during voltage steps of 1-s duration to −15 and 0 mV. Each protocol was preceded by a transient rectangular 10-mV hyperpolarizing pulse, and the resulting current data were used to estimate cell capacitance as previously described (2).

I_{KATP} expression during anoxia. Recordings of the baseline outward current were taken 1 min after electrical access to each cardiocyte was gained. At this time, cells were superfused with an external solution that was bubbled with room air while a constant flow of room air was passed over the superfusion on the flow-through chamber. After baseline current recording, anoxic conditions were induced by superfusing the cells with an external solution equilibrated with 100% N2 and contained 2-deoxy-D-glucose (5 mM). In addition, a layer of argon was passed over the cells to exclude ambient oxygen. Current recordings were made every other minute after induction of anoxia. The anoxia-inducible I_{KATP} was defined as the mean current occurring during the last 200 ms of each 1-s voltage step (8) that exceeded the current recorded under nonanoxic control conditions. Our criterion for determining when an anoxia-inducible I_{KATP} was expressed was designated as the time at which the recorded current was greater than or equal to the “mean + 3 SDs” of the five previous current recordings (e.g., an I_{KATP} expression at 45 min was 3 SDs greater than the combined mean of the currents recorded at 35, 37, 39, 41, and 43 min). A representative example of the experimental induction of this current is provided in Fig. 1. Current was measured every minute after initial I_{KATP} expression. Cardiocytes not expressing I_{KATP} within 1 h were labeled as “nonexpressers.” Key characteristics assessed included the time at which I_{KATP} expression occurred after the induction of the anoxic condition and the peak I_{KATP} density (I_{KATP} corrected for baseline cell capacitance) occurring after current expression. The I_{KATP} was found to be sensitive to glibenclamide; 4 μM glibenclamide were sufficient to completely abolish the current defined as I_{KATP} (Fig. 1). Finally, we adopted the method of Benndorf et al. (2) to examine the anoxia-induced current during a protocol consisting of a 3-s voltage ramp from +80 mV to −100 mV (see Fig. 1C). As previously described (2), the anoxia-induced current was virtually ohmic (Fig. 1C). Furthermore, in our experiment, the reversal potential for the anoxia-induced current was −86.5 mV; this was very close to the theoretical Nernst K+ reversal potential of −85.4 mV as would be expected under the conditions of our experiment. This suggests that the anoxia-induced current that we studied and identified as I_{KATP} was, in fact, carried by potassium.

Data Analysis

Electrophysiological data analysis was performed by using custom-made IDL 4.0 software (Research Systems, Boulder, CO). Statistical analyses were performed by using SPSS 10.0 software (SPSS, Chicago, IL). Simple between-group (Sed vs. Tr) analyses were conducted by using a Student’s t-test. Between-group comparisons across time were made by using a repeated-measures analysis of variance. All data are presented as means ± SE. To reduce the possibility of committing a type II interpretive error, i.e., a false negative, significance was reported at both the P < 0.05 and P < 0.10 levels (50).

RESULTS

Animal Model

Training did not significantly affect tibial lengths, adrenal weights, or thymus and spleen weight in these animals (Table 1). Body weight, however, was significantly greater in the Tr group. These results are consistent with previous studies using female Sprague-Dawley rats in our laboratory (29, 30, 34, 35). Citrate synthase activities of plantaris muscle homogenates were significantly increased by run training. These data provide verification that our treadmill training
protocol was effective in producing a trained state without eliciting overt signs of stress in this animal model, as has been described previously (29, 30, 34, 35).

Characteristics of I\textsubscript{K\textsubscript{ATP}} Expression

In our experiments, 26 of 44 (59%) cells in the Tr group expressed I\textsubscript{K\textsubscript{ATP}} compared with 28 of 33 cells (85%) in the Sed group. In those cells that did exhibit an anoxia-induced current, time to expression of I\textsubscript{K\textsubscript{ATP}} occurred ∼30% later in cells isolated from Tr rats compared with those from Sed rats (Fig. 2; training effect, P < 0.05). The mean capacitance of those cells that did express I\textsubscript{K\textsubscript{ATP}} was 95 ± 8 pF in Sed cells and 114 ± 10 pF in Tr cells (P = 0.122).

Once I\textsubscript{K\textsubscript{ATP}} was expressed, the current increased rapidly to a plateau value over 10 min of current expression and the magnitude of current expression across time was greatest in Sed myocytes (Fig. 3; training × time interaction, P = 0.05). Peak I\textsubscript{K\textsubscript{ATP}} density (time independent) assessed during a 45-mV command potential step (−45 to 0 mV) was significantly lower in the Tr group compared with the Sed (Fig. 4; training effect, P < 0.05). When a smaller command potential step (−45 to −15 mV) was used, the between-group

Table 1. Characteristics of rats used in isolated cardiocyte experiments

<table>
<thead>
<tr>
<th></th>
<th>Tr</th>
<th>Sed</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>292 ± 6</td>
<td>274 ± 4</td>
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<td>Tibial length, mm</td>
<td>40.3 ± 0.3</td>
<td>39.9 ± 0.7</td>
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<td>Thymus wt/body wt, (× 10\textsuperscript{-6})</td>
<td>35 ± 4</td>
<td>50 ± 7</td>
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<td>Spleen wt/body wt, (× 10\textsuperscript{-4})</td>
<td>22 ± 1</td>
<td>24 ± 1</td>
<td>0.120</td>
</tr>
<tr>
<td>Left adrenal wt/body wt, (× 10\textsuperscript{-6})</td>
<td>116 ± 5</td>
<td>108 ± 4</td>
<td>0.166</td>
</tr>
<tr>
<td>Right adrenal wt/body wt, (× 10\textsuperscript{-6})</td>
<td>108 ± 5</td>
<td>100 ± 5</td>
<td>0.257</td>
</tr>
<tr>
<td>Citrate synthase activity, (\mu\text{mol}\cdot\text{min}^{-1}\cdot\text{g wet tissue}^{-1})</td>
<td>24.9 ± 2.7</td>
<td>15.5 ± 1.2</td>
<td>0.005</td>
</tr>
</tbody>
</table>

Values are means ± SE. Tr, trained; Sed, sedentary.
suggesting that our anoxia protocol was sufficient to elicit the opening of KATP (ANOVA simple effects: $P < 0.05$ at 2, 8, and 10 min and $P < 0.1$ at 3, 4, and 9 min after the onset of current expression). $I_{K_{\text{ATP}}}$ density data in this figure represent data collected by using a step potential of 45 mV (−45 to 0 mV). Sample sizes are as indicated in Fig. 2. Similar results were obtained by using the smaller (−45 to −15 mV) command potential step (i.e., Sed vs. Tr $I_{K_{\text{ATP}}}$ density difference, ANOVA simple effects: $P < 0.05$ at 8 and 10 min and $P < 0.1$ at 2, 3, and 9 min).

In our experiments, the size of the anoxia-inducible $I_{K_{\text{ATP}}}$ that we recorded ($\sim 15$–$40$ pA/pF) was similar to previously reported values (2, 48). We have estimated that the maximal anoxia-induced conductance recorded in a typical Sed myocyte ($39.3$ pA/pF × 95 pF/cell $\div 0.045$ V $= 82,789$ pS/cell) corresponds to the opening of $\sim 1,037$ KATP channels per cell, assuming a single-channel conductance of 80 pS/channel (2, 3, 46, 49). On the basis of calculations by Knopp et al. (20), there are $\sim 100,000$ channels per cardiocyte in the rat, suggesting that our anoxia protocol was sufficient to elicit the opening of $\sim 1\%$ of the KATP channels. This conclusion corresponds nicely to the data of Fairve and Findlay (11) where they estimated that the opening of $\sim 1\%$ of the total pool of sarcolemmal KATP channels would be sufficient to explain the anoxia-induced abbreviations in action potential duration that they observed in their experiments. These data, together with the anoxia-inducibility, current reversal potential, and the glibenclamide-sensitivity strongly support the idea that the current we studied was the classically defined cardiac sarcolemmal ATP-sensitive K⁺ current.

Exercise training significantly altered several temporal characteristics of $I_{K_{\text{ATP}}}$ expression. In response to a 1-h anoxic challenge, in those cells that did express $I_{K_{\text{ATP}}}$, the time to $I_{K_{\text{ATP}}}$ expression was significantly prolonged in the Tr group. In cardiac cells, the open probability of sarcolemmal KATP channels is inversely related to cellular [ATP]. More specifically, it has been suggested that cellular ATP is compartmentalized and that subsarcolemmal [ATP] ([ATP]ss) is the fraction that is centrally important to KATP channel operation (48, 49). Indeed, this concept is useful in explaining why the KATP channels open when intracellular [ATP] is in the millimolar range, whereas the inhibition constant of ATP ($K_i$) is between $\sim 20$ and $200$ μM (31–33). In this context, there are two simple hypothetical explanations for the observed alterations in the frequency and onset of current expression with training: 1) [ATP]ss in the proximity of the KATP channels was better preserved in Tr cells; or 2) the responsiveness of KATP channels to a reduction in [ATP]ss was blunted by training (i.e., the $K_i$ for the KATP channel was effectively reduced by training).

With regard to the first possibility, there is strong evidence that ATP derived from glycolysis preferential inhibits the KATP channels (48, 49). It appears that pyruvate kinase and/or phosphoglycerate kinase, enzymes that mediate ATP-generating steps of glycolysis, are physically associated in close proximity with KATP channels and, therefore, may be important in maintaining elevated [ATP] in a KATP channel microenvironment (48, 49). However, in the context of our experimental design, finding suppresses the responsiveness of left ventricular cardiocytes to anoxic challenge with respect to the quantitative and temporal characteristics of $I_{K_{\text{ATP}}}$ expression.

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![Graph showing anoxia-induced $I_{K_{\text{ATP}}}$ density over 10 min of current expression.](image)

**DISCUSSION**

To our knowledge, this is the first study to investigate the effects of exercise training on the characteristics of the cardiac sarcolemmal $I_{K_{\text{ATP}}}$. The key findings of this study were that, in response to an anoxic challenge, the onset of a glibenclamide-sensitive outward current was markedly delayed and peak current density reduced in cardiocytes isolated from endurance-trained rats. The findings provide evidence that training suppresses the responsiveness of left ventricular cardiocytes to anoxic challenge with respect to the quantitative and temporal characteristics of $I_{K_{\text{ATP}}}$ expression.

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a differential (i.e., Sed vs. Tr) inhibition of $I_{K_{ATP}}$ channels via the effects of subsarcolemmal glycolysis seems highly unlikely in view of the fact that our anoxia condition included the use of 2-deoxy-d-glucose to inhibit glycolysis and to accelerate cellular ATP depletion. We hasten to point out that, in a more physiological context, this hypothetical mechanism might be important because there are reports that training can increase myocardial glucose transport rate and/or an upregulation in the activities of several glycolytic enzymes, including pyruvate kinase (12, 17, 18, 40, 43). It is possible that a training-induced increase in glycolytic ATP production in close proximity to sarcolemmal $K_{ATP}$ channels would serve to suppress anoxia-induced $I_{K_{ATP}}$ expression via an enhanced glycolysis-mediated maintenance of [ATP]$_{ss}$.

The notion that our observations were the result of a training-induced desensitization of the $K_{ATP}$ channel to reductions in [ATP]$_{ss}$ is appealing for several reasons. This idea has precedence because several pathological conditions and physiological phenomena have been found to affect the $K_{ATP}$ of sarcolemmal $K_{ATP}$ channels, including diabetes (39), channel phosphorylation by protein kinase C (25), changes in extracellular Ca$^{2+}$ concentration (9), and pH (22). Interestingly, oxygen free radicals have been shown to increase the open probability of $K_{ATP}$ channels, presumably by modulation of ATP binding sites on the $K_{ATP}$ channel (45, 46). This is relevant because training is thought to enhance myocardial antioxidant defense mechanisms and suppress the generation of reactive oxygen species in response to I/R stress (7, 37). In a physiological setting, it seems logical that anoxia-induced $I_{K_{ATP}}$ expression would be blunted in Tr cardiocytes due to a lower exposure of $K_{ATP}$ channels to reactive oxygen species. However, this type of mechanism may have been of limited importance in our studies in view of the fact that except for the very earliest part of our anoxia when oxygen was being eliminated from the cell bath, oxygen was carefully excluded from our experimental system. More investigation will be required to identify the mechanisms underlying the observed changes in $I_{K_{ATP}}$ characteristics in the present study.

Training elicited a reduction in the density of the anoxia-induced, glibenclamide-sensitive current recorded. Our calculations indicate that, at peak $I_{K_{ATP}}$, there were $\sim$25% fewer $K_{ATP}$ channels open in the Tr than in the Sed cardiocytes (776 per Tr cell vs. 1,037 per Sed cell). One potential explanation for this finding is that training enhanced the sensitivity of $K_{ATP}$ channels to the inhibitory effects of ATP (i.e., a decrease in $K_{i,ATP}$). Another possible explanation is that, in the absence of training-induced alterations in $K_{i,ATP}$, training elicited a decrease in the sarcolemmal surface density of functional $K_{ATP}$ channels. The present study was not designed to determine which of these mechanisms might underlie the reduction in current density observed in cardiocytes from Tr rats, although future investigations could be developed to address this issue.

The notion that training improves the ability of the heart to withstand I/R insult has long been recognized and is widely accepted (4, 5, 24, 37, 40). At issue is the physiological relevance of our observation that $I_{K_{ATP}}$ expression was blunted in myocytes isolated from trained rats. It has been proposed that the opening of $K_{ATP}$ channels in the face of metabolic stress is a tissue protective mechanism designed to reduce myocardial energy demand via a suppression of myocardial mechanical activity (6, 10, 27, 33). Although a reduction in oxygen demand is clearly desirable, the suppression of myocardial mechanical activity poses the risk of limiting the ability of the heart to perfuse the coronary circulation and, therefore, further exacerbating metabolic stress by limiting oxygen supply. This raises the interesting possibility that elevated HSP72 and reduced reactive oxygen species in Tr cells may represent adaptations to suppress $K_{ATP}$ channel opening and that this is an adaptation designed to preserve tissue mechanical function and myocardial oxygen supply. This idea is conceptually consistent with earlier observations that administration of a $K_{ATP}$ channel blocker can normalize Ca$^{2+}$ transients and myocyte shortening under conditions of metabolic stress (23, 42) and with our recent observations that administration of a $K_{ATP}$ channel blocker during reperfusion is conducive to the functional recovery of the myocardium after ischemic insult, particularly in the hearts from trained rats (15).

In summary, this study provides evidence that training markedly blunts the responsiveness of sarcolemmal $K_{ATP}$ channels to metabolic stress in single, isolated cardiocytes. Future work will be required to determine whether and how the training-induced alterations in $I_{K_{ATP}}$ characteristics observed in this study are involved in the protection of the heart against the negative functional consequences of I/R insult.

This work was supported in part by National Heart, Lung, and Blood Institute Grant HL-40306 (to R. L. Moore); a Graduate Student Foundation Research Grant from the American College of Sports Medicine; a Graduate Student Scholarship for Women from the American College of Sports Medicine; and a Graduate Student Fellowship from the Womens’ Forum of Colorado Foundation, Inc. (to K. N. Jew).

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