Testosterone Induces Cytoprotection by Activating ATP-Sensitive K⁺ Channels in the Cardiac Mitochondrial Inner Membrane

Fikret Er, MD*; Guido Michels, MD*; Natig Gassanov, MD; Francisco Rivero, MD; Uta C. Hoppe, MD

Background—Whereas in the past, androgens were mainly believed to exert adverse effects on the cardiovascular system, recent experimental data postulate a benefit of testosterone for recovery of myocardial function after ischemia/reperfusion injury. Thus, we examined whether testosterone might improve myocardial tolerance to ischemia due to activation of mitochondrial (mitoK\text{ATP}) and/or sarcoplasmatic (sarcK\text{ATP}) K\text{ATP} channels.

Methods and Results—In a cellular model of ischemia, testosterone significantly decreased the rate of ischemia-induced death of cardiomyocytes that could be prevented by 5-hydroxydecainoic acid but was unaffected by the sarcK\text{ATP} blocker HMR1098 and the testosterone receptor antagonist flutamide. To index mitoK\text{ATP}, mitochondrial flavoprotein fluorescence was measured. Testosterone induced a highly significant increase in mitochondrial flavoprotein fluorescence in intact myocytes and isolated mitoplasts that could be abolished by 5-hydroxydecainoic acid. Testosterone-mediated flavoprotein oxidation of mitoplasts was K⁺ dependent and ATP sensitive. In mitoplast-attached single-channel recordings, testosterone directly activated an ATP-sensitive K⁺ channel of the inner mitochondrial membrane. Addition of the K\text{ATP} channel opener diazoxide and pinacidil to the cytosolic solution activated the ATP-sensitive K⁺ current comparable to testosterone, whereas 5-hydroxydecainoic acid and glibenclamide inhibited the testosterone-induced current. Patch-clamp experiments of intact myocytes in whole-cell configuration did not demonstrate any effect of testosterone on sarcK\text{ATP} channels.

Conclusions—Our results provide direct evidence for the existence of cardiac mitoK\text{ATP} and a link between testosterone-induced cytoprotection and activation of mitoK\text{ATP}. Endogenous testosterone might play a more important role in recovery after myocardial infarction than is currently assumed. (Circulation. 2004;110:3100-3107.)

Key Words: ion channels ■ hormones ■ myocytes ■ preconditioning ■ electrophysiology

Male gender is a classic risk factor for cardiovascular disease. The mortality and incidence of coronary heart disease in middle-aged men is much higher than in premenopausal women of similar age.1 This significant gender difference has been attributed at least in part to negative effects of testosterone on the cardiovascular system.1,2 Androgens have been associated with an increased cardiovascular risk by adversely affecting the plasma lipid and lipoprotein profile, thrombosis, cardiac hypertrophy, and suspected proatherogenic effects.3,4 However, beside these genetic, mainly testosterone receptor–mediated pathways, recent studies demonstrate another, genomic-independent way of testosterone action. Short-term administration of testosterone acutely induces vasodilation in the systemic, coronary, and pulmonary vascular beds.5 Thus, testosterone might actually increase myocardial tolerance to ischemia. Indeed, testosterone substitution in orchiectomized rats improved recovery of myocardial function after ischemia/reperfusion injury.6

Although this beneficial effect might have been related in part to acute coronary vasodilation by testosterone,7 we hypothesized that in addition testosterone might exhibit direct cytoprotective actions on the myocardium. Mitochondria are increasingly recognized as key players in cell survival.7 Indirect evidence suggests that multiple diverse signaling pathways converge on ATP-sensitive potassium channels of the inner mitochondrial membrane (mitoK\text{ATP}) as the final effectors of cytoprotection against necrotic and apoptotic cardiac or neural ischemic injury.8–11 First published data that provided evidence for the existence of mitoK\text{ATP} came from patch-clamping liver fused giant mitoplasts.12 Subsequently, characterization of putative mitoK\text{ATP} channels has utilized reconstitution of detergent-solubilized mitochondrial membranes into proteoliposomes.13–15 However, both preparations bear the risk of contamination by membrane fragments of other organelles and the sarcolemma.16 Indeed, recently the involvement and even the existence of mitoK\text{ATP} have been...
questioned by several investigators since KATP channel-independent effects of pharmacological KATP channel openers and inhibitors on mitochondrial metabolism and membrane potential have been identified.16–19 The patch-clamp technique of freshly isolated, single mitochondria solves this problem, but its implementation is a severe technical challenge. Here we patch-clamp single mitoplasts (2- to 5-μm vesicles of inner mitochondrial membrane) isolated from rat cardiocytes to measure single-channel KATP currents directly. Our results provide direct evidence for the existence of cardiac mitoKATP and a link between testosterone-induced cytoprotection and activation of mitoKATP.

Methods

Preparation of Myocytes and Mitoplasts
Single ventricular cells were isolated from Sprague-Dawley rats (weight, 200 to 250 g) by enzymatic digestion, as previously described.20 Freshly isolated myocytes were used within 12 hours. For mitoplast imaging and patch-clamp studies, myocytes were labeled with tetramethylrhodamine ethyl ester 6 μmol/L (TMRE) (Sigma) and Mitotracker Green 1 μmol/L (Molecular Probes, Inc), respectively, to facilitate identification of intact mitoplasts after further subcellular purification. Isolated intact mitochondria and mitoplasts were prepared as previously described.21,22 Mitoplasts appeared as transparent 2- to 5-μm vesicles with bright red and green fluorescence, indicating retention of TMRE and Mitotracker Green, respectively.

Flavoprotein Fluorescence and Mitochondrial Imaging
Images were obtained as previously described with a Leica TCS SP2 confocal laser-scanning microscope equipped with a DM IRE2 inverted fluorescence microscope and argon/helium-neon lasers at room temperature 21°C to 22°C.8 To localize mitochondria in isolated myocytes and intact mitoplasts, TMRE fluorescence was excited with the 543-nm line of a helium-neon laser, and emission was recorded at >600 nm. For flavoprotein fluorescence, measurement fluorescence was excited at 488 nm with an argon laser, and the emission was recorded at 505 to 535 nm. In each experiment the endogenous flavoprotein fluorescence of the myocytes or mitoplasts was recorded. To determine the redox potential, the maximum fluorescence was determined with dinitrophenol (DNP) (Sigma), and minimum fluorescence was determined with sodium cyanide (CN) (Sigma), respectively. The difference between DNP and CN fluorescence intensity was assumed as 100% fluorescence. The fluorescence intensity with the test substances was calculated accordingly.
adjusted to 7.4 with KOH. To eliminate K⁺ in some experiments, KCl was replaced by osmotically equivalent mannitol 280 mmol/L.

**Electrophysiology**

Mitoplasts were approached with glass pipettes having tip resistance values of ~30 to 50 MΩ and drawn toward the tip by applying gentle suction. Seal resistance values of 10 to 15 GΩ were obtained, and experiments were performed in mitoplast-attached mode with symmetrical bath and pipette solution composed of KCl 150 mmol/L and HEPES 10 mmol/L; pH was adjusted to 7.2 with KOH. The sarcolemmal I_{KATP} of isolated myocytes was recorded with the use of standard microelectrode whole-cell patch-clamp technique. The bath solution contained the following (in mmol/L): NaCl 135, KCl 5, glucose 10, MgCl2 1, HEPES 10; pH was adjusted to 7.4 with NaOH. The pipette solution was composed of the following (in mmol/L): KCl 120, NaCl 15, EGTA 10, MgCl2 1, HEPES 10, and Mg-ATP 1; pH was adjusted to 7.2 with KOH. Experiments were performed at room temperature (21°C to 22°C). Currents were recorded and digitized with an Axopatch 200B amplifier and Digidata 1200 interface (Axon Instruments) with the use of custom software.

**Single-Channel Analysis**

Open probability (defined as the relative occupancy of the open state during active sweeps), availability (fraction of sweeps containing at least 1 channel opening), and I_{peak} (the peak ensemble average current, obtained visually) were analyzed from single-channel and multichannel patches at ~70 mV (if not otherwise indicated). In the latter case, they were corrected for the number of the channels in the patch (n). n was defined as the maximum current amplitude observed, divided by the unitary current. Peak current was corrected by division through n. The availability was corrected by the square root method: \( (1 - \text{availability}_{\text{uncorrected}})^{1/n} \). The corrected open probability was calculated on the basis of the corrected number of active sweeps, ie, total open time divided by \( (n \times \text{availability}_{\text{corrected}}) \times \text{number of test pulses} \times \text{pulse length} \). Closed time and first-latency analyses were performed in patches where n=1. Time constants of open-time (\( \tau_{\text{open}} \)) and closed-time histograms (\( \tau_{\text{closed, fast}}; \tau_{\text{closed, slow}} \)) were obtained by a maximum likelihood estimate for monoexponential (open-time histograms) and biexponential function (closed-time histograms). Single-channel amplitudes were
Effects of Testosterone, Diazoxide, 5-HD, and ATP on Single-Channel Behavior of MitoKATP

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control n</th>
<th>Testosterone n</th>
<th>Diazoxide n</th>
<th>Testosterone + 5-HD n</th>
<th>Testosterone + ATP n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Open probability, %</td>
<td>0.92 ± 0.25</td>
<td>21</td>
<td>4.98 ± 0.60*</td>
<td>19</td>
<td>6.29 ± 1.80*</td>
</tr>
<tr>
<td>Availability, %</td>
<td>17.23 ± 3.50</td>
<td>21</td>
<td>28.20 ± 4.30*</td>
<td>21</td>
<td>33.40 ± 4.70*</td>
</tr>
<tr>
<td>Mean open time, ms</td>
<td>1.07 ± 0.36</td>
<td>21</td>
<td>0.67 ± 0.15</td>
<td>21</td>
<td>0.70 ± 0.10</td>
</tr>
<tr>
<td>r_popen, ms</td>
<td>0.36 ± 0.04</td>
<td>16</td>
<td>0.65 ± 0.08*</td>
<td>19</td>
<td>0.56 ± 0.10*</td>
</tr>
<tr>
<td>Mean closed time, ms</td>
<td>3.52 ± 1.73</td>
<td>7</td>
<td>1.47 ± 0.47</td>
<td>7</td>
<td>1.77 ± 0.47</td>
</tr>
<tr>
<td>r_closs, transition, ms</td>
<td>0.42 ± 0.05</td>
<td>4</td>
<td>0.40 ± 0.09</td>
<td>7</td>
<td>0.42 ± 0.06</td>
</tr>
<tr>
<td>r_closs, state, ms</td>
<td>49.30 ± 14.80</td>
<td>4</td>
<td>10.03 ± 3.60*</td>
<td>7</td>
<td>17.60 ± 4.00*</td>
</tr>
<tr>
<td>Mean first latency, ms</td>
<td>66.40 ± 14.90</td>
<td>7</td>
<td>33.60 ± 8.90</td>
<td>7</td>
<td>37.60 ± 7.20</td>
</tr>
<tr>
<td>Amplitude, pA</td>
<td>−0.83 ± 0.07</td>
<td>16</td>
<td>−0.78 ± 0.07</td>
<td>19</td>
<td>−0.89 ± 0.12</td>
</tr>
<tr>
<td>I_{peak} TA</td>
<td>38 ± 16</td>
<td>21</td>
<td>116 ± 69</td>
<td>21</td>
<td>96 ± 27</td>
</tr>
<tr>
<td>Inactivation, %</td>
<td>46 ± 5</td>
<td>21</td>
<td>42 ± 4</td>
<td>21</td>
<td>47 ± 3</td>
</tr>
</tbody>
</table>

*P<0.05 compared with control; †P<0.05 compared with testosterone.

determined by direct measurements for conductance calculation or as the maximum of gaussian fits to all-point amplitude histograms. Time-dependent inactivation was determined after 150 ms as percentage of the respective I_{peak}. The voltage dependence of activation was analyzed with the use of the Boltzmann function.

Simulated Ischemia and Cell Injury

The cell injury model has been described previously. Briefly, cardiomyocytes were incubated in a 1.5-mL tube in buffer containing the following (in mM/L): NaCl 119, NaHCO3 25, KH2PO4 1.2, KCl 4.8, MgSO4 1.2, HEPES 10, CaCl2 1, glucose 11. In some experiments test substances were added to the buffer, as indicated. After 20 minutes of incubation, cells were centrifuged to a pellet, and gaseous diffusion was prevented with a layer of mineral oil. After 60 minutes of pelleting, probes of each sample were mixed with hypertonic trypan blue solution, as previously described. Cells permeable to trypan blue were counted as dead by 2 blinded investigators (G.M., N.G.) and expressed as a percentage of total cells counted (>200 for each sample).

Materials and Statistical Analysis

In some experiments, ATP, androstenedione, diazoxide, dihydrotestosterone (testosterone), flutamide, glibenclamide, 5-hydroxydecanoic acid (5-HD), pinacidil, paxilline, the adenine nucleotide translocator (ANT) blocker carboxyatractyloside (Sigma), or HMR1098 (Aventis Pharm) was added to the bath solutions, as indicated. Pooled data are presented as mean±SEM. Comparisons between groups were performed with 1-way ANOVA. Probability values of P<0.05 were deemed significant.

Results

Testosterone Induces Mitochondrial Flavoprotein Oxidation in Intact Myocytes

To obtain initial evidence regarding whether testosterone exhibited effects on myocardial mitochondria, mitochondrial flavoprotein fluorescence was measured in isolated rat ventricular myocytes. Confocal images of single cardiomyocytes were obtained at baseline, testosterone loaded, fully oxidized with 100 μmol/L DNP, and fully reduced with 5 μmol/L CN (Figure 1A to 1E). There were virtually no differences in fluorescence intensity between baseline and CN. Testosterone (10 μmol/L) significantly increased the mitochondrial flavoprotein fluorescence relative to DNP oxidation to 48.8±2.6% (n=6; P<0.001 versus baseline; Figure 1B to 1D, 1F), whereas androstenedione (100 μmol/L), a precursor of testosterone, did not induce flavoprotein oxidation (n=5). The testosterone-induced flavoprotein oxidation was dose dependent (10⁻⁹ mol/L: 4.7±0.5%, n=3; 10⁻⁸ mol/L: 9.7±1.2%, n=3; 10⁻⁷ mol/L: 10.1±1.6%, n=5; 10⁻⁶ mol/L: 37.8±3.1%, n=4; 10⁻⁵ mol/L: 87.0±3.1%, n=6; 10⁻⁴ mol/L: 95.2±2.9%, n=5) (Figure 1H). Thus, the first increase of flavoprotein oxidation could be observed at physiological testosterone concentrations in men. The KATP channel blocker 5-HD virtually abolished the testosterone-induced mitochondrial oxidation (Figure 1G). 5-HD (100 μmol/L) blocked 79% to 90% of the testosterone-induced flavoprotein oxidation at testosterone concentrations of 10⁻⁹ to 10⁻⁴ mol/L (P<0.001). Addition of the testosterone receptor antagonist flutamide (10 μmol/L) did not prevent the testosterone (10 μmol/L)–induced flavoprotein oxidation (46.8±5.9%; n=4), indicating that the effect was not mediated by the testosterone receptor. Recently, mitochondrial Ca²⁺-activated K⁺ channels (mitoKCa) have been implicated to play a role in cytoprotection. However, the mitoKCa antagonist paxilline (10 μmol/L) exhibited no effect on testosterone-induced flavoprotein oxidation (n=4), excluding a contribution of mitoKCa₅. These results suggested that testosterone oxidized cardiac mitochondria in a testosterone receptor–independent way, possibly by activating mitoKATP.

Testosterone Directly Oxidizes Mitoplasts in a K⁺-Dependent and ATP-Sensitive Manner

To examine whether testosterone exerted a direct effect on mitochondria, measurements of mitochondrial flavoprotein fluorescence were repeated with the use of isolated mitoplasts (Figure 2A to 2E). Confocal images of cardiac mitoplasts revealed that testosterone (10 μmol/L) directly and significantly increased mitoplast flavoprotein fluorescence to 60.5±1.9% relative to the fully oxidized state with 100 μmol/L DNP (n=5; P<0.001 versus baseline; Figure 2B to 2D, 2F). Elimination of K⁺ in the bath entirely abolished the testosterone-induced oxidation of mitoplasts (n=4), indicating that the testosterone effect was mediated via mitochondrial K⁺ influx (Figure 2G). Similar to intact myocytes, 5-HD (100 μmol/L) suppressed the testosterone-induced mitoplast oxidation (Figure 2F), whereas flutamide (10 μmol/L) did not affect the testosterone-induced mitoplast oxidation (n=3).
Testosterone Activates K<sub>ATP</sub> Channels of the Inner Mitochondrial Membrane

To directly characterize the effect of testosterone on mitochondrial K<sup>+</sup> flux, single-channel patch-clamp recordings of mitoplasts were performed. Testosterone (10 μmol/L) activated single-channel currents with a unitary conductance of 13.0±1.3 pS (Figure 3C; n=10) when the pipette solution contained 150 mmol/L K<sup>+</sup> (Figure 3A; Table). The channel activity increased with more negative test potentials (Figure 3B, 3D, 3E). These K<sup>+</sup> currents were blocked on application of ATP (5 mmol/L) to the cytosolic solution (Figure 3A). ATP block was prevented by the addition of the ANI blocker carboxyatractyloside (10 μmol/L) to the bath solution. This suggests that ATP was transported via the ANT into the mitoplasts and that the regulatory channel site for ATP faces the mitochondrial matrix. The I-V relation for the testosterone-activated current showed that the reversal potential estimated by linear regression was 0 mV, which is nearly equal to the equilibrium potential for K<sup>+</sup> (E<sub>K</sub>) when an intramitochondrial K<sup>+</sup> concentration of 180 mmol/L is assumed (Figure 3B, 3C). Flutamide (10 μmol/L) did not inhibit testosterone-induced K<sup>+</sup> channel activation (n=4). Androstenedione (100 μmol/L) had no effect on mitoplast-attached single K<sup>+</sup> channel activity compared with control (n=3) (Figure 4B). Addition of the K<sub>ATP</sub> channel openers diazoxide (100 μmol/L) or pinacidil (100 μmol/L) to the cytosolic solution activated the ATP-sensitive K<sup>+</sup> current comparable to testosterone, whereas 5-HD (100 μmol/L) and 5-HD (100 μmol/L) did not influence the single-channel amplitude (Figure 4C). Notably, ATP (5 mmol/L) also abolished the increase of mitoplast flavoprotein fluorescence on exposure to testosterone (n=4) (Figure 2H), providing further evidence for the contribution of a putative ATP-sensitive K<sup>+</sup> channel.

Figure 3. Single-channel mitoplast-attached mitoK<sub>ATP</sub> current recordings. A, Testosterone-activated I<sub>mitoKATP</sub> at −70 mV (Testo) (10 μmol/L) (left), which was blocked by ATP 5 mmol/L (right). Bottom tracings, Ensemble averages (Testo, 240 tracings; Testo+ATP, 360 tracings). B, I<sub>mitoKATP</sub> at various potentials (V<sub>m</sub>). C, Single-channel amplitude (I) as a function of test potentials. Mean values were derived from Boltzmann fits of individual experiments (n=3): V<sub>0.5</sub> = −51.8±10.5 mV, slope = −9.9±4.9 mV.

Figure 4. Drug effects on single mitoK<sub>ATP</sub> channels. A, Testosterone-induced activation of I<sub>mitoKATP</sub> at −70 mV (Testo) (10 μmol/L) (middle) compared with control (left) was blocked by 5-HD 100 μmol/L (Testo+5-HD) (right). Bottom tracings, Ensemble averages (control, 120 sweeps; Testo, 180 sweeps; Testo+5-HD, 240 sweeps). B, Agonistic effect of pinacidil (100 μmol/L) on mitoK<sub>ATP</sub> at various test potentials (V<sub>m</sub>). Pinacidil increased the mean open time (4.21±0.59 vs 0.46±0.20 ms; n=3), open probability (66.61±9.9 vs 39.45±7.8%; n=3), and single-channel amplitude (−0.91±0.02 vs −0.48±0.04 pA; n=3) compared with control (n=3; V<sub>m</sub> = −60 mV; P<0.01). C, Drug effects on mitoK<sub>ATP</sub> open probability at −70 mV: androstenedione (Andro) (n=3), testosterone (Testo) (n=19), diazoxide (Diaz) (n=14), testosterone+5-HD (Testo+5-HD) (n=4), testosterone+ATP (Testo+ATP) (n=4), and testosterone+glibenclamide (Testo+Gly) (n=5) compared with control (n=21). *P<0.001 compared with control.

Notably, ATP (5 mmol/L) also abolished the increase of mitoplast flavoprotein fluorescence on exposure to testosterone (n=4) (Figure 2H), providing further evidence for the contribution of a putative ATP-sensitive K<sup>+</sup> channel.
glibenclamide (10 μmol/L) inhibited the testosterone-induced current (Figure 4A to 4C; Figure 5A to 5H; Table). These results indicate that the testosterone-, diazoxide-, and pinacidil-activated current was mitoK<sub>ATP</sub>.

**Testosterone Does Not Affect Sarcoplasmatic K<sub>ATP</sub> Channels**

To test whether testosterone exhibits any effect on sarcoplasmatic K<sub>ATP</sub> (sarcK<sub>ATP</sub>) channels, which also have been proposed to play a potential role in cytoprotection,26 patch-clamp current recordings in the whole-cell configuration of isolated cardiomyocytes were performed. Currents of freshly isolated cardiomyocytes were consecutively recorded in bath solution (control) and bath solution supplemented with testosterone and pinacidil. As expected, 100 μmol/L pinacidil significantly increased sarcK<sub>ATP</sub> current density measured at 100 mV from 2.23±0.13 pA/pF (control) to 10.85±0.49 pA/pF (n=11; P<0.001) (Figure 6A, 6B). Conversely, testosterone at concentrations of 1 μmol/L (2.24±0.21 pA/pF; n=7), 10 μmol/L (2.26±0.30 pA/pF; n=9), and 100 μmol/L (2.21±0.14 pA/pF; n=11) did not increase sarcK<sub>ATP</sub> current size compared with control cells (P=NS versus control for all concentrations). To probe any blocking effect of testosterone on sarcK<sub>ATP</sub>, myocytes were simultaneously perfused with 100 μmol/L pinacidil and 100 μmol/L testosterone (n=9). The recorded currents were not different compared with pure pinacidil-induced currents (10.98±0.71 versus 10.85±0.49 pA/pF at 100 mV; P=NS), indicating that testosterone does not block sarcK<sub>ATP</sub>.

**Testosterone Prevents Ischemia-Induced Cell Death**

To examine whether testosterone-induced mitoK<sub>ATP</sub> channel opening can protect cardiomyocytes from ischemic cell death, we used an established cell injury model.8,23 After 60 minutes of ischemia, the rate of killed cardiomyocytes in the control group was 47.1±1.8% (n=11). Testosterone (10 μmol/L) significantly protected the cardiomyocytes and decreased the rate of cell deaths to 24.0±1.6% (n=14; P<0.001; Figure 6C), whereas androstenedione (100 μmol/L) did not exhibit cytoprotection (44.0±3.1%; n=8, P=NS versus control). 5-HD abolished the cardioprotective effect of testosterone (testosterone 10 μmol/L plus 5-HD 100 μmol/L: 42.5±1.3%; n=12; P=NS versus control), whereas 5-HD alone had no effect on death rate compared with control (45.8±2.5%; n=6; P=NS). The sarcolemmal K<sub>ATP</sub> channel blocker HMR1098 (100 μmol/L) and the testosterone receptor blocker flutamide (10 μmol/L) did not prevent the testosterone-induced protection of cardiomyocytes (rate of cell death, 23.3±2.6% [n=6] and 25.2±3.2% [n=6], respectively; P=NS versus testosterone, P<0.001 versus control). These results substantiated that the acute protective effect of testosterone is mediated by mitoK<sub>ATP</sub> channel opening in a testosterone receptor–independent manner.

**Discussion**

Our results show that testosterone acutely and directly depolarizes and oxidizes cardiac mitochondria in a K<sup>+</sup>-dependent, ATP-sensitive, and testosterone receptor–independent manner. By patch clamping the cardiac inner mitochondrial membrane, we demonstrate testosterone-induced activation of mitochondrial K<sup>+</sup> channels, which were inhibited by ATP, 5-HD, and glibenclamide, whereas testosterone exhibited no effect on sarcK<sub>ATP</sub> channels. Testosterone protected cardiomyocytes from ischemic cell death.

When activated, the unitary conductance of rat ventricular mitoK<sub>ATP</sub> was in the same range as previously estimated.12,27

---

**Figure 5.** Open (A to D) and closed (E to H) time histograms of single-channel experiments before and after testosterone, diazoxide, and pinacidil. Square root (SQR) of number of events per bin is plotted against their duration. Single \( \tau \) values are shown in the Table. For pinacidil, \( \tau_{\text{open}} = 1.91 \pm 0.25 \text{ ms} \), \( \tau_{\text{closed,fast}} = 0.42 \pm 0.04 \text{ ms} \), and \( \tau_{\text{closed,slow}} = 7.21 \pm 2.16 \text{ ms} \) (n=3).
In contrast to K⁺ flux measurements of reconstituted channels and inside-out recordings of fused giant mitoplasts, mitoK\(_{\text{ATP}}\) in intact mitoplasts was virtually inactive in the absence of any K\(_{\text{ATP}}\) channel openers, which is consistent with the assumption that the mitochondrial inner membrane has a low resting permeability to cations to maintain the mitochondrial transmembrane potential. Because mitoK\(_{\text{ATP}}\) current in mitoplast-attached patches was activated and blocked when testosterone, diazoxide, or pinacidil and ATP, 5-HD, or glibenclamide, respectively, were added outside the pipette, the relevant regulatory sites for these substances on the channel are likely to face the mitochondrial matrix. MitoK\(_{\text{ATP}}\) would then be modulated as matrix concentrations of these substances rise in response to transmembrane diffusion or transport. Because mitoK\(_{\text{ATP}}\) block by ATP was inhibited by the ANT blocker carboxyatractyloside, we hypothesize that ATP enters the mitochondrial matrix via the ANT. Our results now provide a direct link between cytoprotection, mitochondrial oxidation, which was K⁺-dependent and ATP-sensitive, and activation of mitoK\(_{\text{ATP}}\) channels. On the basis of our experiments, we conclude that mitoK\(_{\text{ATP}}\) is recruitable to prevent or mitigate ischemic cardiac and possibly neural injury.

Androgens are known as anabolic sex hormones that act via binding to their nuclear receptor and regulate protein synthesis. In addition, recent experimental data indicated a nongenomic pathway of testosterone action on the cardiovascular system, ie, acute testosterone-mediated vasodilation. Our results now demonstrate, for the first time, an acute direct effect of testosterone on cardiac myocytes as well. Anabolic androgenic steroids have been associated with myocardial ischemia, sudden cardiac death, and hypertension in athletes, leading to the view that androgens are detrimental to the cardiovascular system. However, anabolic androgenic steroids consist of a variety of different steroids with differing pharmacological properties. No clinical study has yet demonstrated a conclusive link between physiological testosterone and fatal cardiovascular events. Epidemiological data and an intervention study rather suggest either a neutral or a beneficial effect of natural circulating androgens on coronary heart disease in men. However, detailed experimental and clinical evaluations of chronic testosterone receptor-mediated actions, which might be different from acute effects, are still lacking. Thus, before we draw any clinical conclusions from our findings that testosterone can directly and acutely protect cardiocytes against ischemic injury by opening mitoK\(_{\text{ATP}}\) channels in isolated cells and mitoplasts, extensive studies of the chronic effects of testosterone in vitro and in vivo are definitely warranted.

Acknowledgments

This study was supported by a grant from the Deutsche Forschungsgemeinschaft (Ho2146/2-1) and by the Marga und Walter Boll Stiftung. We are grateful to N. Henn and M. Weber for technical assistance.

References


Testosterone Induces Cytoprotection by Activating ATP-Sensitive K⁺ Channels in the Cardiac Mitochondrial Inner Membrane
Fikret Er, Guido Michels, Natig Gassanov, Francisco Rivero and Uta C. Hoppe

Circulation. 2004;110:3100-3107; originally published online November 1, 2004; doi: 10.1161/01.CIR.0000146900.84943.E0
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2004 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/110/19/3100

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/