Alterations in ATP-sensitive potassium channel sensitivity to ATP in failing human hearts

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ATP-sensitive K⁺ channels [Iₖ(ATP)] make an important contribution to the increase of outward K⁺ conductance and shortening of the action potential duration during conditions of metabolic inhibition such as ischemia and hypoxia in mammalian hearts1,2). Although Iₖ(ATP) in a variety of mammalian species are closed by ATP with the half-maximal inhibition at 20–100 μM3), alterations of channel characteristics have been reported in experimentally induced hypertension and myocardial hypertrophy in feline hearts4). In humans, similar to these other mammalian preparations, metabolic inhibition causes action potential shortening and contractile failure in isolated human myocytes5). And previous studies suggest that Iₖ(ATP) also plays a role in these effects in human myocytes5). However, little is known about the involvement of preexisting heart disease on characteristics of Iₖ(ATP) in human heart7,8). In the present study, to address the involvement of preexisting heart disease on channel characteristics, we have examined the action potential and the whole cell membrane current response to metabolic inhibition and have characterized single-channel Iₖ(ATP) in isolated cardiac myocytes from patients with congestive heart failure and compared these characteristics with those from donor hearts.

Isolation of myocytes: The technique for isolating human myocytes was identical to that previously reported by this laboratory8). Human specimens were minced into small pieces using a razor blade and washed three times, for 7 min each time, in oxygenated Ca²⁺-free Tyrode solution with constant agitation by a magnetic stirring bar to remove Ca²⁺. The tissue fragments were then incubated in oxygenated Ca²⁺-free Tyrode solution containing 300–350 U/ml collagenase (type V. Sigma. St. Louis, MO), 0.5 U/ml protease (type X X I V. Sigma), and 1 mg/ml bovine serum albumin (Sigma).

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which was stirred continuously at 37°C. Single cells were isolated from the minced tissue by gravity filtration through a 200-µm nylon mesh. The digestion process was repeated multiple times until no more cells could be obtained. Myocytes were stored at room temperature in a modified “KB medium”\(^\text{11}\). Only Ca\(^{2+}\)-tolerant, clearly striated, rod-shaped cells without any blebs were studied.

Solutions: The cardioplegic solution for human specimens contained (in mM) 27 NaCl, 20 KCl, 1.5 MgCl\(_2\), 5.0 N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES), and 274 glucose (pH 7.0). Normal Tyrode solution contained (in mM) 140.0 NaCl, 5.4 KCl, 1.8 CaCl\(_2\), 0.5 MgCl\(_2\), 5.0 HEPES, and 5 glucose (pH 7.4 with NaOH). Ca\(^{2+}\)-free Tyrode solution was made by omitting CaCl\(_2\) from the normal Tyrode solution. The modified KB-solution had the following composition (in mM): 25 KCl, 10 KH\(_2\)PO\(_4\), 116 KOH, 80 glutamic acid, 10 taurine, 14 oxalic acid, 10 HEPES, and 11 glucose (pH 7.0 with KOH). The composition of the internal (pipette) solution used for the action potential and whole cell recording was (in mM) 120 potassium aspartate, 20 KCl, 1 KH\(_2\)PO\(_4\), 1 MgCl\(_2\), 5 ethylene glycol-bis (β-aminoethyl ether) - N, N', N'-tetraacetic acid (EGTA), and 5 HEPES (pH 7.2). Normal Tyrode solution was used as external solution during the action potential and whole cell recordings. In excised inside-out patch recordings, the pipette (external) solution contained (in mM) 150 KCl and 5.0 HEPES (pH 7.4). The cytosolic surface of the membrane was perfused with a bath solution containing (in mM) 120 potassium aspartate, 30 KCl, 2 MgCl\(_2\), 5 HEPES, and 5 EGTA (pH 7.2). Various concentrations of Na\(_7\)-ATP were added to the bath solution.

Cyanide (1 mM) was added to the bath solution during action potential and whole cell recordings. In metabolic inhibition experiments, glucose was replaced by 10 mM 2-deoxyglucose at least 20 min before exposure to cyanide. All agents were purchased from Sigma Chemical.

The experimental chamber (0.2 mM) was continuously perfused with bath solution at a rate of 6~7 ml/min, and complete solution exchange was achieved within 5.0 ± 1.2 s (n = 16) by measuring the current change induced by changing the solution from 5.4 mM K\(^+\) solution to 150 mM K\(^+\) solution. To facilitate the rapid exchange of test solutions, the mouth of a fine-bore polyethylene inlet tube was positioned within 1 mm of the patch.

1. RESULTS

Action potential shortening during metabolic inhibition in human myocytes isolated from heart failure patients (HF) and donor hearts. Figure 1A shows examples of the action potential and the response to metabolic inhibition in isolated human myocytes from a patient with HF and a donor recorded in the whole cell current-clamp mode. The resting membrane potential in a human myocyte from HF was -66 ± 8.8 mV and that from donor was -79 ± 7.2 mV. Although the action potential duration at 50% was not significantly different between HF and donors, the action potential duration at 90% repolarization was longer, and the late repolarization phase (phase 3) was slower in HF than in donors. Upon exposure to cyanide (1 mM) in the presence of 2-deoxyglucose (10 mM), action potential shortening and slight hyperpolarization of resting membrane potential developed in both groups. However, the action potential duration at 90% repolarization was significantly smaller in HF than in donors at 30 min exposure to cyanide. The action potential duration at 90% repolarization was 24.7 ± 4.1% (n = 15) of control in HF, whereas it
Figure 1 Effect of metabolic inhibition on action potentials recorded in human myocytes from heart failure patients (HF) and donor heart

A: representative action potential and response to metabolic inhibition in an isolated human myocyte from HF and donor recorded under whole cell current-clamp configuration. After exposure to cyanide (1 mM) in presence of 2-deoxyglucose (10 mM), shortening of action potential occurred in both groups (●). After 30-min exposure to cyanide, action potential duration shortened to 26.5% of control measured at 90% repolarization in HF and to 61.9% of control in donor. Action potential recovered partially after additional exposure of glibenclamide (0.5 μM) in continued presence of cyanide and deoxyglucose in both groups (▲). B: changes in action potential durations at 90% repolarization (APD90) were plotted vs time in human myocytes isolated from HF and donors. APD90 decreased during metabolic inhibition, which was reversed after exposure to glibenclamide (0.5 μM) in both groups. Vertical bar through each point represents SD from 15 cells with HF and from 11 cells with donors. Normalized values were significantly different at 2.5 min (P < 0.05), 5 min (P < 0.01), 7.5–30 min (P < 0.001), 32.5–37.5 min (P < 0.01), and 40–42.5 min (P < 0.05) between 2 groups.

Table 1 summarizes the action potential parameters and their response to the metabolic inhibition in both groups. These results indicate that action potential shortening occurs sooner and to a greater extent in HF than in donors. There were no significant interpatient differences in each group in the action potential parameters.

Whole cell membrane current during metabolic inhibition was 58.7 ± 5.9% (n = 10, p < 0.001) of control in donors measured at 30 min exposure to cyanide. The shortening of the action potential was partially reversed by glibenclamide (0.5 μM) in both groups. Figure 1B shows the plot of the action potential duration at 90% repolarization change vs time of cyanide exposure from 15 different cells in HF and 11 different cells in donors. The action potential shortening at 30 min exposure to cyanide was significantly greater in HF than in donors (p < 0.001).
inhibition in human myocytes isolated from HF and donors. Because alterations in K⁺ conductance could be responsible for the different response of the action potential to metabolic inhibition between the two groups, the characteristics of whole cell K⁺ currents were studied. Figure 2 shows the whole cell membrane current characteristics and response to metabolic inhibition in human myocytes from HF and donors. Figure 2 A and B (top panels), shows examples of the response to a voltage step from a holding potential of -40 mV to 0 mV for 300 ms in human myocytes from HF (A) and donor (B). Nifedipine (5 μM) and tetrodotoxin (10 μM) were included in the bath solution to block Ca²⁺ and Na⁺ current. After exposure to cyanide (1 mM) in the presence of 2-deoxyglucose (10 mM), the outward whole cell current at 0 mV increased in both traces, but both the original current magnitude and the response to the metabolic inhibition were different in the two cells. Figure 2 A and B (bottom panels), shows the averaged steady-state current-voltage (I-V) relationships before and during exposure to cyanide (1 mM) in human myocytes from HF (A, n = 16) and donors (B, n = 11). In the control condition, the average slope conductance at the reversal potential was 8.4 ± 0.8 nS (n = 16) in HF and 12.7 ± 1.4 nS (n = 11) in donors. The slope conductance in HF was significantly smaller than that in donors (p < 0.01). Metabolic inhibition increased the slope conductance in both groups. However, the slope conductance of the membrane currents during metabolic inhibition in HF was significantly greater (28.5 ± 2.9 nS, n = 16) than that in donors (20.4 ± 1.8 nS, n = 11, p < 0.01). The current increase was recovered to nearly the control level by subsequent exposure to glibenclamide (0.5 μM) in the continued exposure to cyanide and 2-deoxyglucose in both groups. There were no significant interpatient differences associated with age and gender in either group. However, the control currents without metabolic inhibition may be contaminated by the background

Table 1: Action potential response to metabolic inhibition in human myocytes isolated from HF and donor hearts

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<thead>
<tr>
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<th>HF</th>
<th>Donors</th>
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<tr>
<td>n</td>
<td>15</td>
<td>11</td>
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<tr>
<td>APD50, ms</td>
<td></td>
<td></td>
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<tr>
<td>Control</td>
<td>195.3 ± 16.4</td>
<td>187.5 ± 15.6</td>
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<tr>
<td>Cyanide(30min)</td>
<td>44.0 ± 4.1 **</td>
<td>96.8 ± 8.2</td>
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<tr>
<td>Glibenclamide</td>
<td>167.4 ± 19.0</td>
<td>157.0 ± 14.5</td>
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<tr>
<td>APD90, ms</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>344.0 ± 26.3 *</td>
<td>301.4 ± 24.7</td>
</tr>
<tr>
<td>Cyanide(30min)</td>
<td>87.4 ± 9.1 **</td>
<td>175.4 ± 16.9</td>
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<tr>
<td>Glibenclamide</td>
<td>314.2 ± 28.0</td>
<td>281.1 ± 20.5</td>
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<tr>
<td>Resting membrane potential</td>
<td>-66 ± 8.8</td>
<td>-79 ± 7.2</td>
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Values are means ± SD; n, no. of hearts; HF, heart failure; APD50 and APD90, action potential duration measured at 50% and 90% repolarization, respectively.
*P < 0.05, **P < 0.001, different from donors using 2-way analysis of variance and analysis of covariance.
Figure 2  Effect of metabolic inhibition on whole cell membrane current in human myocytes from HF and donor heart

A, top panel: representative whole cell membrane current and response to metabolic inhibition in isolated human myocyte from HF recorded in whole cell voltage-clamp configuration. Membrane was voltage clamped to 0 mV for 300 ms from a holding potential of −40 mV (●). Ca\(^{2+}\) current was blocked by nifedipine (5 nM), and Na\(^{+}\) current was blocked by tetrodotoxin (10 nM) in bath solution. Application of cyanide (1 mM) to bath solution (including 2-deoxyglucose, 10 mM) increased outward current (▲). This effect was partially reversed after additional application of glibenclamide (0.5 nM). Bottom panel: averaged steady-state current-voltage (I-V) relationships before (●), during exposure to cyanide (▲), and after additional exposure to glibenclamide (0.5 nM) in human myocytes from HF (n=16). Whole cell current was increased by metabolic inhibition, and this increase was reversed by glibenclamide. TP, test potential. Vertical bar through each point represents SD.

B, top panel: representative whole cell membrane current and response to metabolic inhibition in isolated human myocyte from donor heart under same conditions as A. Current response to cyanide (▲) was less than in HF. Bottom panel: averaged steady-state I-V relationships in control (●), during exposure to cyanide (1 mM), and during additional exposure to glibenclamide (0.5 nM) in human myocytes from donors (n=11). Metabolic blockade increased membrane current, but effects were less than in HF. Vertical bar through each point represents SD.

I\(_{K1}\) and may not be generated by \(I_{KATP}\). To evaluate the \(I_{KATP}\) activity more precisely, whole cell differential current was compared between the two groups. Figure 3 illustrates the whole cell differential current obtained by subtraction of the control current from the test current in each group. The current magnitude of the differential current at 0 mV was greater in HF than in the donor. The differential current at 0 mV was greater in HF than in the donor.
Figure 3  Effect of metabolic inhibition on whole cell membrane current evaluated by differential current measurement

A, top panel: representative whole cell differential current obtained by subtraction of control current from current during metabolic inhibition in an isolated human myocyte from HF. Membrane was voltage clamped to 0 mV for 300 ms from a holding potential of -40 mV. Recording conditions were as in Figure 2. Bottom panel: averaged steady-state I-V relationship of differential current in human myocytes from HF (n=10). Vertical bar through each point represents SD. B, top panel: representative whole cell differential current obtained by subtraction of control current from current during metabolic inhibition in isolated human myocyte from donor. Bottom panel: averaged steady-state I-V relationship of differential current in human myocytes from donors (n=8). Vertical bar through each point represents SD.

Averaged steady-state I-V relationships of the differential currents demonstrated that the slope conductance at 0 mV was significantly greater in HF (20.9 ± 2.6 nS, n = 14) than in donors (9.2 ± 1.1 nS, n = 9, p < 0.001). These results suggest that macroscopic I_{KATP} induced by metabolic inhibition has greater conductance in HF than in donors.

I_{KATP} activity in human myocytes. To gain further insight into the different response to metabolic inhibition in HF, single-channel I_{KATP} characteristics were compared between HF and donors.

Figure 4A shows I_{KATP} activity in isolated human myocytes from HF and donor hearts recorded in the exercised inside-out patch configuration at -40mV. Upon formation of the excised inside-out patch configuration in the bath solution containing 5 mM ATP, I_{KATP} activity did not appear in either group. After exposure to the bath solution without ATP, channel activity appeared immediately, and when bath solution was again switched to the 5 mM
Figure 4 Characteristics of ATP-sensitive K⁺ channels \([I\text{\textsubscript{K\textsubscript{ATP}}}]\) in isolated human myocytes from HF and donors in excised inside-out patch recordings

A: examples of \(I\text{\textsubscript{K\textsubscript{ATP}}}\) activity in human myocytes isolated from HF and donor in inside-out patch configurations at a holding potential (HP) of \(-40\) mV. Pipette and bath solutions included 150 mM K⁺, and bath solution also included 2 mM Mg²⁺. No channel activity was detected initially in presence of ATP (5 mM) in bath solution (cytosolic side of membrane) in both groups. After exposure of patches to ATP-free bath solution, channel activity appeared in both groups. More than 5 channels were activated during exposure to ATP-free bath solution in HF, whereas 2 channels were active in donor. Arrowheads indicate zero-current level (baseline level).

B: unitary current traces of \(I\text{\textsubscript{K\textsubscript{ATP}}}\) activity in human myocytes from HF and donor. Unitary amplitudes of inward directed currents increased with hyperpolarizing holding voltages. Currents were zero at HP = 0 mV (K⁺ equilibrium potential (Ek)). Outward conductances were detected at holding voltages more positive than Ek in both groups. C: I - V relationships obtained from B. Slope conductance did not differ between HF and donor. Inward currents demonstrated a linear slope conductance of 86 pS in both groups. Outward currents exhibited a slope conductance of 54 pS, indicating inward rectification.

ATP-containing solution, the channel activity ceased in both groups. When the excised inside-out patch configuration was formed in the bath solution without ATP, \(I\text{\textsubscript{K\textsubscript{ATP}}}\) activity in both groups occurred with continuous bursts. \(I\text{\textsubscript{K\textsubscript{ATP}}}\) behavior was first characterized in both groups. Figure 4B shows the conductance characteristics of \(I\text{\textsubscript{K\textsubscript{ATP}}}\) in isolated human myocytes from HF and donor hearts recorded in inside-out patches with 0 mM ATP in the bath solution. The single-channel opening of \(I\text{\textsubscript{K\textsubscript{ATP}}}\) activity was recorded with holding potentials ranging from +20 to -80 mV in both groups.
Zero-current potential was at ~0 mV in symmetrical internal and external K⁺ concentrations (K⁺ equilibrium potential) in both groups. Channel activity exhibited bursting separated by relatively long interburst intervals. The unitary amplitude increased with hyperpolarization and the mean open lifetime increased at depolarization similar to other species. The I-V relationships demonstrated weak inward rectification, and slope conductance of the inward current averaged 84 ± 3 pS (n = 20) in HF and 85 ± 3 pS (n = 14) in donors (Figure 4C). The slope conductance of the outward current averaged 59 ± 3 pS (n = 20) in HF and 56 ± 3 pS (n = 12) in donors in the presence of Mg²⁺ (2 mM) in the bath. These values were not significantly different from each other between the two groups.

The open-time histogram was formed at −40 mV, which could be best described by a single exponential function with the mean open lifetime of 1.4 ± 0.2 ms (n = 17) in donors. The close-time histogram during a burst was best fitted by a single exponential function with an averaged mean closed time during a burst at −40 mV of 1.2 ± 0.2 ms (n = 20) in HF and 1.1 ± 0.2 ms (n = 17) in both HF and donors. Closed times between bursts distributed variably and required several orders of magnitude for complete fitting, indicating the existence of several sequential transitions before channel opening. These values were not different from each other between the two groups, nor from those in other mammalian species previously reported. These results suggest that channel conductance and open bursting behavior of I_{KATP} are unchanged in HF.

Sensitivity of human I_{KATP} to ATP. Figure 5 illustrates examples of I_{KATP} activity during exposure to 10 μM, 100 μM, and 5 mM ATP bath solution in HF and in the donor recorded at −40 mV.
Figure 6 Concentration-dependent inhibition of $I_{\text{KATP}}$ by ATP in human myocytes isolated from HF and donor heart in inside-out patch recordings

Relationship between concentration of ATP in bath solution and open-state probability ($P_o$) in HF and donors was evaluated by single-channel open events. ATP concentration at half-maximal channel inhibition was 131.0 $\mu$M in HF and 26.1 $\mu$M in donor heart. Hill coefficient of fitted curve was 1.9 in HF and 2.0 in donors. Vertical bar through each point represents SD from 14-18 HF cells and from 9-11 donor cells.

During exposure to 100 $\mu$M ATP, one channel was observed and at least three channels became active after switching the bath solution to 10 $\mu$M ATP in both patches. $I_{\text{KATP}}$ activity in HF was greater than in the donor during exposure to 10–100 $\mu$M ATP solutions. The channel open-state probability ($P_o$) evaluated during exposure to 100 $\mu$M ATP solution at $-40$ mV was 0.25 ± 0.55 ($n = 14$) in HF and 0.04 ± 0.31 ($n = 10$, $p < 0.001$) in donors. $P_o$ during exposure to ATP-free solution at $-40$ mV was 0.34 ± 0.59 ($n = 13$) in HF and 0.31 ± 0.53 ($n = 9$) in donors. Although these values did not achieve statistical significance, it appeared that $P_o$ during exposure to ATP-free solution was greater in HF compared with donors. The higher channel activation rate in HF may cause greater macroscopic $I_{\text{KATP}}$ conductance.

To evaluate the difference of channel activity between the two groups in more detail, channel activity was evaluated in different ATP concentrations. Channel activity was evaluated by $P_o$. $P_o$ was changed in a concentration-dependent manner in both groups. Figure 6 illustrates the concentration-dependent effect of ATP on $I_{\text{KATP}}$ in both groups. The concentration-dependent activation of each $I_{\text{KATP}}$ was fit by the least-squares method using the Hill equation

$$P_o = \frac{V_{\text{max}}}{1 + ([\text{ATP}]/IC_{50})^H}$$

Where $V_{\text{max}}$ is the maximal $P_o$, $IC_{50}$ is the concentration of ATP at half-maximal channel inhibition, and $H$ is the Hill coefficient. The relationship between the concentration of ATP and channel activity was fitted by the Hill equation with a similar Hill coefficient value of the fitted curve of 1.9 in HF and of 2.0 in donors. However, the relationship between concentration of ATP and channel activity was significantly different. The $IC_{50}$ in HF was 131.0 $\mu$M, whereas the value in donors was 26.1 $\mu$M, indicating that the threshold of opening of $I_{\text{KATP}}$ in HF is significantly lower than in donors.

II. DISCUSSION

The major findings in this study are as follows.
1) During metabolic inhibition by treatment with cyanide (1 mM) and 2-deoxyglucose (10 mM), human action potential shortening occurred sooner and to a greater extent in HF than in donors. The shortening of the action potential was partially reversed by glibenclamide (0.5 $\mu$M) in both groups.
2) The whole cell membrane current response to metabolic inhibition, evaluated by the differential current measurement, was also greater and sooner in HF than in donors.
3) Single human and ventricular $I_{\text{KATP}}$ characteristics recorded in inside-out patches
in HF were substantially similar to those in donors and to other mammalian hearts. 4) In contrast, the half-maximal inhibition of ventricular $I_{\text{KATP}}$ by ATP was significant greater in HF than in donors. These results suggest that although channel behavior of $I_{\text{KATP}}$ is not altered in HF, the channel is less sensitive to ATP inhibition in HF than in donor cells and will therefore be activated at higher ATP concentrations. The altered sensitivity of $I_{\text{KATP}}$ to ATP may cause the altered response of the action potential to metabolic inhibition in HF heart.

Characteristics of isolated human myocytes. A variety of previous studies have demonstrated that human atrial and ventricular specimens with low resting membrane potentials may be associated with preexisting heart disease\textsuperscript{13}--\textsuperscript{15}. Studies using experimental animal models with primary heart disease also revealed low resting membrane potentials\textsuperscript{16}. Consistent with these reports, resting membrane potentials in myocytes isolated from HF patients with elevated pressure and dilatation were depolarized compared with donors. In addition, ventricular myocytes isolated from HF patients were also depolarized to a similar level as human myocytes. Although a variety of studies have been made to address the origin of low resting membrane potential in diseased human heart\textsuperscript{15}, \textsuperscript{14}, \textsuperscript{17}, its underlying mechanism still remains unclear.

It is unlikely that isolated myocytes from HF were damaged or modulated by the isolation procedure or experimental conditions because of the following observations. 1) Isolated ventricular myocytes from both HF and donors appeared morphologically similar without a significant difference in cell size. 2) They could generate action potentials in current-clamp mode. 3) $I_{\text{KATP}}$ activity never appeared during cell-attached patch recordings without metabolic inhibition, but it appeared after metabolic inhibition by cyanide or the formation of excised patches in ATP-free solution in both groups, suggesting that the cellular metabolic function may be maintained in both groups. 4) Extensive study of whole cell Na$^+$ current in our laboratory using myocytes isolated from the same patients as in the present study demonstrated that numerous characteristics of Na$^+$ were unaltered in 137 human atrial specimens\textsuperscript{18}. This result also supports the idea that the isolation procedure is not selective in the present study.

Characteristics of $I_{\text{KATP}}$ in cardiac myocytes isolated from HF heart. In the present study, metabolic inhibition shortened the action potential duration in isolated myocytes from both HF and donors similar to previous studies in other mammalian preparations\textsuperscript{2}, \textsuperscript{3}, \textsuperscript{19} and in human atrial myocytes\textsuperscript{5}. Furukawa et al.\textsuperscript{20} demonstrated greater sensitivity of action potential duration to cyanide in hypertrophied cells. Although our experimental and cellular condition may differ from the one they used, a similar underlying mechanism may cause action potential shortening in the present study (discussed below). The fractional shortening of the action potential duration will be a complex consequence of all the currents following within the cell and could not be used as a quantitative indicator of $I_{\text{KATP}}$ activation. Our results do not rule out a contribution of other currents to enhance action potential shortening: L-type Ca$^{2+}$ currents and delayed-rectifier K$^+$ currents\textsuperscript{20}. Our results of whole cell current measurements showed an increase of outward membrane current following metabolic inhibition. However, the extent of the response to metabolic inhibition was different between HF and donor heart, evaluated by differential current measurements. The altered conductance in the differential current in HF
suggests alterations in $I_{K_{ATP}}$ in these human myocytes. These results are consistent with those obtained from the action potential measurements.

When the intracellular surface was exposed to ATP-free bath solution, $I_{K_{ATP}}$ activity decayed within 5-10 min (rundown), similar to previously reported guinea pig $I_{K_{ATP}}$ results\textsuperscript{12}, which appear to be due to dephosphorylation of the channel protein or regulatory subunits\textsuperscript{17}. This phenomenon was observed in both groups. When inside-out patches were formed in the bath solution containing $\sim 100-200 \mu M$ ATP, the phosphorylated state of $I_{K_{ATP}}$ activity was presumably maintained in both groups. The channel $P_o$ of $I_{K_{ATP}}$ could be evaluated during exposure to $10-100 \mu M$ ATP bath solutions in human myocytes from HF and donors.

Overall characteristics of $I_{K_{ATP}}$ gating in human myocytes from HF and donors were similar to each other: channel slope conductance and kinetics were unchanged in HF. They were also similar to the previous reports in other mammalian heart\textsuperscript{[1, 12, 21]} and in human atrium\textsuperscript{[5, 6]}. $I_{K_{ATP}}$ in ventricle appears to have characteristics similar to those in atrium. One of the major differences between HF and donors was the different sensitivity to ATP for $I_{K_{ATP}}$ channel activation. Recently, Cameron et al.\textsuperscript{4} have reported that the half-maximal inhibition of $I_{K_{ATP}}$ in ventricular myocytes from hypertrophied myocardium was significantly greater than that from normal subjects in feline hearts. This result indicates that during ischemia, $I_{K_{ATP}}$ may be operative earlier in ischemic, hypertrophied myocardium than in normal hearts and that this difference may be caused by a long-term adjustment to conditions of chronically depleted oxygen supplies in hypertrophied myocardium. Although the oxygen supply in atrium is greater than in ventricle, relative depletion of the oxygen supply in HF atria compared with normal atria may result in the similar reduction of sensitivity to ATP.

The exact reason for the lower sensitivity of $I_{K_{ATP}}$ to ATP in HF relative to donor heart is unclear at present. Individual channel slope conductance and gating kinetics were unchanged in HF heart as described above. Because the Hill coefficient of the concentration-response relationship of the channels was close to 2 in both HF and donors, two binding sites for ATP may exist on the channel protein in both groups. Intracellular ligands such as ADP, protons, and lactate can influence $I_{K_{ATP}}$ sensitivity to ATP by shifting the concentration-response relationship to the right\textsuperscript{[20-24]}. However, channel modulation by these metabolites can be ruled out in the present study, because the ATP sensitivity of the channel was tested in cell-free excised inside-out patch conditions in both groups. Previous reports suggest that the pertussis toxin sensitive inhibitory G protein $G_i$ couples $I_{K_{ATP}}$ via a membrane delimited pathway in mammalian heart\textsuperscript{[25, 30]}. They have demonstrated that $G_i$ can activate $I_{K_{ATP}}$ by reducing channel sensitivity to ATP. Because an increased level of $G_{\alpha}$ was reported in failing human hearts\textsuperscript{[27]}, $I_{K_{ATP}}$ activity may be enhanced in HF. It has also recently been documented that the sensitivity to ATP of $I_{K_{ATP}}$ is reduced by several-fold in metabolically inhibited guinea pig ventricular myocytes through a Ca\textsuperscript{2+}-dependent phenomenon measured by excised inside-out membrane patches\textsuperscript{30}. Because the activity of many proteases and phospholipases including trypsin is increased by Ca\textsuperscript{2+} concentration, these enzymes may cause the reduced intrinsic sensitivity of the channels to ATP\textsuperscript{30}. ATP binding to the inhibitory site may also be influenced by proteolysis in HF heart, and this process could cause altered sensitivity of $I_{K_{ATP}}$ in HF heart cells. In addition to these modulators, a
lower sensitivity of $I_{\text{KATP}}$ in HF relative to donors may be caused by degeneration of high-affinity $I_{\text{KATP}}$ channels in HF heart. Because heterogeneity of sensitivities among $I_{\text{KATP}}$ has been previously demonstrated, the population of high-affinity channels may be mainly involved in HF. The complex interrelationships among these and other unknown factors would be expected to modulate $I_{\text{KATP}}$ to reduce ATP sensitivity in HF heart.

Limitations and clinical implications. This study is limited by the small number of donor hearts evaluated. The availability of healthy donor heart is usually extremely limited for laboratory investigation, and this is a limitation of cellular electrophysiological studies of human heart. However, our statistical treatment using two-way ANOVA and analysis of covariance can effectively eliminate the statistical errors generated by using a different sample size for each group. The characteristics of $I_{\text{KATP}}$ in myocytes obtained from donor hearts as well as HF hearts were quite homogeneous within each group. These combinations of statistical methods indicated that the only factor influencing the intergroup comparison was HF (HF vs. donor).

The present results suggest that $I_{\text{KATP}}$ can active sooner in vivo ischemic conditions in patients with HF than in ischemic conditions in patients without HF. The alterations in $I_{\text{KATP}}$ in HF may provide a beneficial or adaptive function in this issue. In addition, the response to the therapeutic interventions by the $I_{\text{KATP}}$ modulators in HF may be different from the ischemic condition in normal heart. Further in vivo study is required to confirm the alterations in electrophysiological responses to ischemia in patients with HF.

11) Isenberg G, Klockner U : Calcium tolerant ventricular myocytes prepared by preincubation in KB medium. Pfluegers Arch, 1982 ; 395 : 6～18
13) Gilmour RF, Heger JJ, Prystowsky EN, Zipes DP : Cellular electrophysiologic abnormalities of diseased
human ventricular myocardium. Am J Cardiol, 1983; 51: 137–144


