Interrelation Between Pinacidil and Intracellular ATP Concentrations on Activation of the ATP-Sensitive K⁺ Current in Guinea Pig Ventricular Myocytes

Keiko Nakayama, Zheng Fan, Fumiaki Marumo, and Masayasu Hiraoka

The patch-clamp technique was used to study the relation between pinacidil and intracellular ATP concentration ([ATP]ᵢ) on the activation of the outward K⁺ current in guinea pig ventricular myocytes. Pinacidil shortened the action potential duration, exhibiting stronger effect at 2 mM [ATP], than at 5 mM [ATP]. Pinacidil at 5 μM or higher concentrations activated the time-independent outward current at potentials positive to −80 mV, and the pinacidil-activated current was suppressed by increasing [ATP], from 2 to 5 mM. The dose-response curve of pinacidil at different [ATP], showed a shift to the right and a depression of the maximum response at increased [ATP]. The pinacidil-induced shortening of the action potential duration and outward current were inhibited by application of 0.3–1.0 μM glibenclamide. In single-channel current recordings, pinacidil activated the intracellular ATP-sensitive K⁺ channel current without changing the unitary amplitude, and increased open probability of the channel, an effect dependent on [ATP]. The pinacidil-activated single-channel current was blocked by glibenclamide. These results prove the notion that pinacidil activates the ATP-sensitive K⁺ channel current, which explains the action potential shortening in cardiac cells after application of pinacidil. (Circulation Research 1990;67:1124–1133)

Recently, considerable attention has been paid to a group of agents—which includes cromakalim (BRL 34915), nicorandil, and pinacidil—because of their unique abilities to produce a potent vasodilatory action and to promote action on the potassium channel of vascular smooth muscle cells. Because of the latter action, these agents are called potassium channel openers. All of these agents also affect cardiac membranes to shorten the action potential duration (APD) and, sometimes, to hyperpolarize the resting membrane potential. Therefore, they are assumed to modulate the potassium channel activity of cardiac membranes as well. An actual target of potassium channel openers has not been known until recently, when two of them, cromakalim and nicorandil, were shown with the single-channel recording technique to activate the ATP-sensitive K⁺ channel current (Iₖ,ATP) of ventricular myocytes. As pinacidil has an effect similar to the others on the APD and the background potassium current, its target is assumed to be the same K⁺ channel. Indeed, Arena and Kass showed that pinacidil activated the time- and voltage-independent outward K⁺ current, which was blocked by glibenclamide, a potent blocker of Iₖ,ATP. From these observations, they suggested that the target for pinacidil in the heart is Iₖ,ATP. A preliminary study by Escande et al. with the single-channel recording technique indicated that pinacidil activated this channel, but the detailed mechanism of its action on Iₖ,ATP remains unclear.

The ATP-sensitive K⁺ channel is characterized by a strong inhibition of activity when the intracellular ATP concentration ([ATP]ᵢ) is increased above the millimolar level. Therefore, modulation of this channel by any agent depends on the level of [ATP]. Our previous study demonstrated that nicorandil produced a prominent enhancement of Iₖ,ATP at low [ATP], whereas its effect was antagonized by increasing [ATP]. The present study was done to characterize the pinacidil-activated K⁺ current in intact cells under the condition of different [ATP]. Direct evidence of the pinacidil action on Iₖ,ATP was further explored by means of the single-channel recording technique.
Methods

Preparation

Single ventricular myocytes were isolated from both ventricles of adult guinea pigs that weighed 250–350 g. Our technique to isolate single myocytes has been described in a previous report.16

Solutions

The composition of Tyrode’s solution was (mM) NaCl 144, NaH₂PO₄ 0.33, KCl 4.0, CaCl₂ 1.8, MgCl₂ 0.53, glucose 5.5, and HEPES 5.0, with pH adjusted to 7.3–7.4 by addition of NaOH. The high K⁺-low Cl⁻ solution had the following composition (mM): glutamic acid 70, taurine 15, KCl 30, KH₂PO₄ 10, MgCl₂ 0.5, glucose 11, EGTA 0.5, and HEPES 10, with pH adjusted to 7.4 by addition of KOH. Pinacidil, a gift of Shionogi Pharmaceutical Co. (Osaka, Japan), was dissolved as a stock solution in 1% 0.1N HCl and diluted appropriately before study. Gibenclamide, a gift of Hoechst Japan (Tokyo), was dissolved as 0.2 mM stock solution in 2% dimethyl sulfoxide (DMSO) and diluted into the test solution to obtain the final concentration indicated in the text. The final concentration of DMSO contained in the test solution was less than 0.01%.

Whole-Cell Current Recordings

The patch-clamp technique of the whole-cell configuration17 was used to record membrane potentials and currents by using a patch-clamp amplifier (model 8900, Dagan Corp., Minneapolis). Details of the recording technique and the data-acquisition systems are described in our previous reports.7,16 When the ramp voltage-clamp method was used in some experiments, an intelligent arbitrary function synthesizer (model 1731, NF Instruments, Yokohama, Japan) was used to supply the command pulse. The composition of the pipette solution was (mM) KCl 120, K₃ATP (Sigma Chemical Co., St. Louis) 5.0, HEPES 5.0, and K₂BAPTA (Dojin Co., Tokyo) 5.0, with pH adjusted to 7.2 with KOH. In pipette solutions having different ATP contents, the concentration of K₃ATP was changed, and at the same time, the concentration of KCl was varied so as to maintain the final K⁺ concentration constant at 150 mM. The internal perfusion technique as originally described by Soejima and Noma18 was used for changing [ATP]. Series resistance compensation was adjusted to minimize the duration of the capacitive surge on the current trace. At the end of each experiment, liquid junction potential was checked, and if more than ±2 mV, the value of the membrane potential was corrected accordingly. The temperature of the perfusion chamber was maintained at 33–35°C. The solution in the chamber flowed continuously by gravity with a speed of 1.2–1.5 ml/min. The chamber volume of 0.8 ml was completely exchanged 1 minute after a solution change. Control data were taken 5 minutes after changing to the appropriate solution. Drug effects were tested 5–10 minutes after the drug-containing solution was changed.

Single-Channel Current Recordings

Single-channel current recordings were made at room temperature in the inside-out patch configuration17 with the same patch-clamp amplifier used in the whole-cell experiments. Currents and voltage signals were recorded with a videocassette recorder (HR-S 5500, Victor Co., Tokyo) through a pulse code modulation converting system (RP-882, NF Instruments). Recorded signals were filtered off-line through an eight-pole Bessel low-pass filter (48 dB/octave, FV-665, NF Instruments) at 500 Hz and digitized at 5 kHz using an analog-to-digital converter (CED 1401, Cambridge Electronic Design Ltd., Cambridge, UK) to store into the disk of an IBM-AT personal computer for later analysis. When the single-channel recording with the inside-out patch configuration was done, the bath solution (intracellular solution) contained (mM) KCl 140, glucose 5.5, EGTA 2, and HEPES 5, with pH adjusted to 7.3 with KOH. The drug was dissolved in the bath solution at the concentration indicated in the text. The composition of the pipette solution (extracellular medium) was (mM) KCl 140, CaCl₂ 1.8, MgCl₂ 0.53, glucose 5.5, and HEPES 5, with pH adjusted to 7.3 by addition of KOH. The drug effects usually were examined 2–3 minutes after the test solutions were changed, except for calculation of open probability at different [ATP], as described below.

Calculation of Open Probability (ATP-Sensitive K⁺ Channel Current)

Open probability (Pₒ) was calculated as

\[ P_o = \frac{\sum n_i t_i}{N T} \]

where \( t_i \) is the duration of an \( n_i \)th number of open channels, \( N \) is the available channel in the patch, and \( T \) is the total recording time (30 seconds in this study). The numbers of available channels were estimated as the maximum open channels at free [ATP]. To avoid possible influence from the rundown of the channel, total exposure times to different [ATP], with or without the presence of pinacidil were limited to be less than 4 minutes in any patch. The availability of the channels was reevaluated after washout of pinacidil and ATP. Only those patches showing \( P_o \geq 0.98 \) at the second exposure to ATP-free solution were selected for the calculation (assuming \( P_o = 1.0 \) at the first exposure to 0 mM [ATP]).

Measurements of Cell Surface Area

Cell surface areas were estimated from the cell capacitance assuming a specific membrane capacitance of 1 μF/cm². Cell capacitance was calculated from current responses induced by 1-mV depolarizing pulses from a holding potential of 90 mV.19
Figure 1. Effects of pinacidil on action potential duration (APD) of guinea pig ventricular myocytes in two different intracellular ATP concentrations ([ATP]). Panel A: Effect of 5 and 10 μM pinacidil (P) on action potentials recorded from a cell dialyzed with an internal solution containing 5 mM ATP (5-ATP). Panel B: Effects of 5 and 10 μM pinacidil on action potentials recorded from a cell dialyzed with an internal solution containing 2 mM ATP (2-ATP). Panel C: Summarized data of the effects of pinacidil on APD. Values of APD are expressed by relative APD at 90% repolarization (APD90) compared with APD90 in the control as 100%. Multiple comparisons among data in the control, 5, and 10 μM pinacidil were done using the Student-Newman-Keuls method. Unpaired t test was used to judge statistical significance between the data of 2 and 5 mM [ATP], at the same pinacidil concentration. Action potentials were elicited at a stimulation frequency of 0.067 Hz.

Statistical Analysis
Data are expressed as mean±SD. Comparisons between two groups of data were evaluated by paired or unpaired t test. Comparisons between more than two groups were evaluated by Student-Newman-Keuls method for critical difference. A value of p<0.05 was considered significant.

Results

Effect of Pinacidil on Membrane Potentials
Effects of pinacidil on membrane potentials of ventricular myocytes were examined at two different [ATP]i, a normal (5 mM) and a low (2 mM) level, while the preparations were stimulated at a constant frequency of 0.067 Hz. At 5 mM [ATP], 10 μM pinacidil decreased action potential amplitude and shortened APD both at 20% (APD20) and 90% repolarization (APD90), whereas 5 μM pinacidil did not produce any changes in these parameters. At 2 mM [ATP], however, 5 μM pinacidil decreased action potential amplitude and shortened both APD20 and APD90, as did 10 μM pinacidil. Furthermore, the degree of the APD shortening by the same concentration of pinacidil was significantly larger at the low than at the normal [ATP]i (Figure 1, Table 1). The resting potential was not changed by pinacidil at either [ATP].

Effect of Pinacidil on Steady-State Current
Figure 2A illustrates membrane current responses to depolarizing or hyperpolarizing voltage steps from the holding potential of -30 mV before and during the application of 10 μM pinacidil using a pipette solution containing 2 mM ATP. Pinacidil increased outward current at a potential positive to around -80 mV, and this increase was time-independent. At potentials negative to -90 mM, pinacidil decreased inward current during pulses (see “Discussion”). The steady-state current-voltage relation, in which current was measured at the end of the 2-second test pulses, showed increased outward current with a loss of negative slope region at voltages between -60 and -20 mV (Figure 2B). There was no change in the reversal potential. Similar results as shown in Figure 2 were confirmed in four cells, although the degree of increased current by pinacidil differed among different preparations.

Effect of Intracellular ATP Concentration on the Pinacidil-Activated Outward Current
The effects of pinacidil on membrane potentials indicated that pinacidil had a stronger effect on APD at the low [ATP], than at the normal [ATP]. Therefore, we examined the pinacidil-activated outward...
Table 1. Effects of Pinacidil on Membrane Potentials of Guinea Pig Ventricular Myocytes at Two Different Intracellular ATP Concentrations

<table>
<thead>
<tr>
<th>[ATP]</th>
<th>n</th>
<th>RMP (mV)</th>
<th>APA (mV)</th>
<th>APD20 (msec)</th>
<th>APD90 (msec)</th>
</tr>
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<tbody>
<tr>
<td>2 mM</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Control</td>
<td>10</td>
<td>-87.6±4.1</td>
<td>134.7±4.4</td>
<td>507.2±218.1</td>
<td>713.2±299.9</td>
</tr>
<tr>
<td>Pinacidil 5 μM</td>
<td>9</td>
<td>-85.6±5.5</td>
<td>128.7±7.0*</td>
<td>300.6±168.6*</td>
<td>434.5±212.5*</td>
</tr>
<tr>
<td>Pinacidil 10 μM</td>
<td>4</td>
<td>-86.5±3.6</td>
<td>123.4±11.2*</td>
<td>163.1±138.0*</td>
<td>245.8±187.1*</td>
</tr>
<tr>
<td>5 mM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>5</td>
<td>-89.8±0.7</td>
<td>136.3±2.9</td>
<td>491.8±185.8</td>
<td>689.1±249.9</td>
</tr>
<tr>
<td>Pinacidil 5 μM</td>
<td>5</td>
<td>-88.0±0.9</td>
<td>135.4±2.0</td>
<td>521.4±209.4</td>
<td>719.6±207.9</td>
</tr>
<tr>
<td>Pinacidil 10 μM</td>
<td>5</td>
<td>-87.6±1.0</td>
<td>133.5±1.7*</td>
<td>405.2±202.5*</td>
<td>571.8±253.2*</td>
</tr>
</tbody>
</table>

Values are mean±SD. [ATP], intracellular ATP concentration; RMP, resting membrane potential; APA, action potential amplitude; APD20, action potential duration at 20% repolarization; APD90, action potential duration at 90% repolarization.

*p<0.05 vs. control.

current at different [ATP]. First, antagonistic action of increasing [ATP] on pinacidil-activated outward current was tested using the internal perfusion technique18 (Figure 3). Under the condition of 2 mM [ATP], 10 μM pinacidil markedly increased the time-independent outward current at potentials positive to −50 mV, and the current-voltage curve lost its negative slope region. When [ATP], was increased to 5 mM in the presence of pinacidil, the increased outward current was nearly halved, and the current-voltage curve regained a slight negative slope. In six myocytes, the outward current at 0 mV increased significantly from 0.13±0.07 nA in the control to 1.06±0.63 nA after the pinacidil application at 2 mM ATP in the pipette solution (p<0.05). An increase in [ATP], to 5 mM in the presence of pinacidil decreased the current to 0.61±0.40 nA.

The dose-response curve of pinacidil-activated current was examined at different [ATP]. A ramp voltage-clamp method was used to produce a slow depolarization (200 mV/30 sec) from −120 to 80 mV. Figure 4 presents typical results obtained with different [ATP]. The pinacidil dose-dependently activated the outward current at a voltage positive to −80 mV in each [ATP], and the degree of the increase also was dependent on [ATP]. The pinacidil effect was the most prominent at 2 mM [ATP], among the three conditions. A quantitative estimation of the type of experiments shown in Figure 4 was achieved by expressing the dose-response curve as the current density. The current density induced by pinacidil was measured as the current level at 0 μM, because it was nearly zero during the control (Figure 5). At 2 mM [ATP], the current density showed a sigmoidal dependence on the pinacidil concentration, with a nearly maximal response at 100 μM (159.4±14.8 μA/cm²; n=5). We could not judge whether the response at 100 μM pinacidil was really saturated or not, because all the cells died after application of the drug at higher concentrations. At 5 mM [ATP], a sigmoidal dependence of the current density on the pinacidil concentration shifted to the right, and at the same time, the maximal response at 100 μM pinacidil was markedly suppressed to 16.8±4.5 μA/cm² (n=4). The response was saturated when the concentration of pinacidil was higher than 100 μM. At 10 mM [ATP], the maximal current induced by pinacidil was reduced further (15.8±4.0 μA/cm²; n=6) and shifted to develop at the higher concentration (300 μM). The current density decreased to 10.3±2.5 μA/cm² at 500 μM. The decrease of the current density response to 500 μM pinacidil in comparison to that at

![Figure 2](http://circres.ahajournals.org/)

**Figure 2.** Effect of pinacidil (P) on membrane current. Panel A: Superimposed current traces before (○) and during (●) the application of 10 μM pinacidil at four different test voltages (Vt). Voltage protocol is shown at the bottom. Panel B: Effect of 10 μM pinacidil on current-voltage relation. Current values were measured as the level at the end of 2-second depolarizing or hyperpolarizing test pulses. HP, holding potential.
300 μM was observed in four of six cells. By these sigmoidal curves, the following K_d values were calculated: 77.0 and 69.0 μM at 2 and 5 mM [ATP], respectively, and 158 μM at 10 mM [ATP].

**Block of the Pinacidil-Induced Changes in Membrane Potential and Current by Glibenclamide**

We examined the effects of glibenclamide on the changes in action potentials and outward current induced by pinacidil because Arena and Kass described the suppression of the pinacidil-activated current by glibenclamide. Figure 6A illustrates the effects of glibenclamide on the pinacidil-induced action potential change at 2 mM [ATP]. Application of 0.3 and 1.0 μM glibenclamide prolonged APD, which was shortened by the pretreatment of 100 μM pinacidil. APD_90 was shortened by 100 μM pinacidil from 481 ± 77 (n = 5) to 177 ± 70 msec. The shortened APD_90 by pinacidil was prolonged by 0.3 μM glibenclamide to 292 ± 167 msec (p < 0.01 vs APD_90 in 100 μM pinacidil) and further by 1.0 μM to 416 ± 135 msec (p < 0.01) (Figure 6B). Therefore, 1.0 μM glibenclamide completely antagonized the shortening of APD induced by 100 μM pinacidil. The pinacidil-activated outward current also was almost completely suppressed by 0.3 μM glibenclamide (Figure 7). This result was confirmed in three myocytes.

**Effect of Pinacidil on the Single-Channel Current**

The effect of pinacidil on the single-channel current was studied using the inside-out patch configurations. Figure 8 shows a typical example of the records at a membrane potential of +40 mV (Figure 8A), as well as amplitude histograms (Figure 8B). When the intracellular solution did not contain ATP, prominent openings of the single channels were observed (Figure 8A, recording a); the openings were largely inhibited by 2 mM [ATP], (recording b). Application of 30 μM pinacidil in the presence of 2 mM [ATP] activated the current again (recording c). The amplitude of the pinacidil-activated channels at 2 mM [ATP], (2.53 pA) was not different from that in the ATP-free solution (2.48 pA, calculated by the difference between the third and fourth open level in recording a), confirming our recent report. Addition of 0.01% DMSO did not influence the pinacidil-activated channel activity (recording d), whereas 0.3 μM glibenclamide dissolved in 0.01% DMSO completely inhibited channel activity (recording e). Values of Po before and after the addition of DMSO in

![Figure 3](http://circres.ahajournals.org/)

**Figure 3. Effect of different intracellular ATP concentrations ([ATP]) on the pinacidil-activated outward current.** Panel A: Current traces recorded in response to depolarization to 0 mV for 2 seconds from −30 mV at different stages of treatment. ○, Control with 2 mM [ATP], (2-ATP); ●, application of 10 μM pinacidil (P) in 2 mM [ATP]; ▲, application of 10 μM pinacidil in 5 mM [ATP], (5-ATP). Panel B: Effect of 10 μM pinacidil on the current-voltage relation at different ATP concentrations in the pipette solution. Data were taken from the same experiment shown in panel A. Current values were measured as the level at the end of 2-second test voltages from −30 mV.

![Figure 4](http://circres.ahajournals.org/)

**Figure 4. Effects of pinacidil on the background current induced by 30-second depolarizing ramp from −120 to +80 mV at different intracellular ATP concentrations ([ATP]). [ATP], is indicated at the top of each graph as 2-ATP, 2 mM [ATP]; 5-ATP, 5 mM [ATP]; and 10-ATP, 10 mM [ATP]. ○, Control current before the application of pinacidil (P); ●, current in the presence of 50 μM pinacidil; ▲, current in the presence of 100 μM pinacidil.
the presence of 2 mM [ATP], and 30 μM pinacidil were 12.6±5.1% and 9.7±3.2%, respectively (n=3). Further application of 0.3 μM glibenclamide reduced P₀ to less than 0.1% in five preparations. At 2 mM [ATP], activation of the single-channel current sensitive to the ATP-free solution also was observed in eight of 10 patches in 30 μM pinacidil, and two of five patches in 10 μM pinacidil. With either pinacidil concentration, the amplitude of the single-channel current was not changed from that with the ATP-free solution. P₀ of I_{KATP} in different [ATP], was estimated with and without the presence of 30 μM pinacidil. Figure 9A shows typical recordings of the single-channel current; Figure 9B summarizes data from four to six patches. At each [ATP], (0.1–2.0 mM), pinacidil significantly increased P₀. Furthermore, P₀ of the channel in the presence of pinacidil was dependent on [ATP], because an increase in the latter decreased the former.

**Discussion**

The present study demonstrated that a vasodilator, pinacidil, shortened APD and increased the time-independent outward current in ventricular myocytes. The effects of pinacidil were dependent on [ATP], being accentuated by lowering [ATP], and attenuated by increasing it or by application of glibenclamide. Single-channel current recordings confirmed an activation of the [ATP]-sensitive current by pinacidil without changing the unitary amplitude of the current and the dependence of the activation on [ATP]. These results indicate that pinacidil increases P₀ of the ATP-sensitive K⁺ channel, which explains the shortening of APD of heart cells after drug treatment.

Pinacidil has been shown to shorten the APD of heart muscles and to modify the shape of the T wave on surface electrocardiograms. The effects are attributed to an increase in K⁺ permeability of cardiac membranes.\(^5\)\(^,\)\(^2\) Iijima and Taira\(^4\) actually showed an increase in the outward current using isolated ventricular myocytes, and they interpreted this action as an activation of the background K⁺ current. Arena and Kass\(^5\) recently explored the target of the membrane current system activated by pinacidil and confirmed that pinacidil actually activated the K⁺ current in guinea pig ventricular myocytes, but they interpreted the pinacidil-activated current as I_{KATP} on the basis of its time- and voltage-independent properties, as well as its pharmacological characteristics.
Our study confirmed that pinacidil shortened APD of ventricular myocytes and activated the time-independent outward current. These effects were antagonized by raising [ATP]. All of the above actions by pinacidil were easily blocked by a low concentration of glibenclamide, which has been claimed as a specific inhibitor of $I_{\text{KATP}}^{10-13}$. Furthermore, the single-channel recordings in this and the previous study$^{20}$ proved the actual activation of $I_{\text{KATP}}$ by pinacidil. Therefore, our data presented the confirming evidence that the target of the pinacidil action in heart is indeed $I_{\text{KATP}}$.

The mode of channel activation by pinacidil appears to increase the $P_o$ of $I_{\text{KATP}}$, because marked openings were seen without changing the unitary amplitude. An increase in [ATP], decreased the channel opening without affecting the unitary current amplitude, and the decreased $P_o$ of the channel activity was restored by application of pinacidil (Figures 8 and 9). The results may indicate that ATP and pinacidil share the same binding site or process for channel modulation. Kinetic analysis of channel activity showed an antagonistic action by ATP and

**Figure 7.** Effect of glibenclamide (G) on the pinacidil (P)-induced background current. Current was induced by 30-second depolarizing ramp from -120 to +80 mV. Note that 0.3 μM glibenclamide (A) completely suppressed the pinacidil-activated background current (●).

**Figure 8.** Effects of pinacidil (P) and glibenclamide (G) on the ATP-sensitive single-channel current. Panel A: Current recordings obtained from a single patch. Membrane potential is held at +40 mV in the inside-out patch configuration. C, Closed level. Recording a: Presence of 0 mM ATP (0-ATP) in the intracellular solution. There are marked openings of the channel activity up to four levels. Recording b: Presence of 2 mM ATP (2-ATP) in the intracellular solution. Most of the channel activity seen in recording a is now inhibited. Recording c: Application of 2 mM ATP and 30 μM pinacidil in the intracellular solution. Marked openings of the channel again are seen. Recording d: Application of 2 mM ATP and 30 μM pinacidil and 0.01% dimethyl sulfoxide (DMSO) in the intracellular solution. There is no apparent change in the channel activity compared with that in recording c. Recording e: Further application of 0.3 μM glibenclamide in the internal solution. Channel activity is completely suppressed. Panel B: Amplitude histograms recorded from the same patch in panel A.
pinacidil, where the channel was partially closed by ATP. However, the dose-response curve of the pinacidil action did not reveal a competitive inhibition by ATP, and the maximal current activated by pinacidil was suppressed by the higher [ATP] in addition to a shift of the curve to the right (Figure 5). Partially, this shift may be due to experimental imperfection because of too few points on the curve near the maximal response, especially at 2 mM [ATP]. Another possibility is that pinacidil may have at least two sites or processes to interact with the openings of $I_{\text{K,ATP}}$. High concentrations of pinacidil may block the channel opening, in addition to its activation, or accelerate the "rundown" phenomenon of this channel activity. Further study is necessary to delineate an exact mechanism, as well as sites of action for pinacidil to activate $I_{\text{K,ATP}}$.

The effects of pinacidil on the current-voltage curve exhibit two characteristic changes worth mentioning in terms of the drug-induced alteration in membrane potential, other than a marked increase of outward current. First, the increased outward current positive to around $-60$ mV caused a loss of the negative slope region in the current-voltage curve. Second, pinacidil produced little change in the current at the resting potential level around $-80$ mV and decreased inward current at potentials negative to $-90$ mV. These two factors could explain a marked shortening of APD without alternation of the resting potential (see Table 1). These effects were similar to those produced by cromakalim and nicorandil, which also were shown to activate $I_{\text{K,ATP}}$. The result that pinacidil enhanced outward but not inward current may be related to the intrinsic properties of $I_{\text{K,ATP}}$, because it has a sigmoidal current-voltage curve, showing outward-going rectification at negative potentials and inward-going rectification at positive potentials. Consequently, activating $I_{\text{K,ATP}}$ will produce very little inward current negative to its reversal. This factor, however, cannot explain a decrease of the inward current negative to $K^+$ equilibrium potential ($E_K$) after pinacidil, and therefore, the decrease of the current may be explained by several other possibilities. First, the activation of the current
by pinacidil may be voltage-dependent. Second, pinacidil may produce inhibition of other currents, such as the inward rectifier K⁺ current (Iₖᵢ). Third, pinacidil may display inhibition on Iₖ_ATP in addition to activating action. A voltage-dependent activation is not likely because single-channel recording revealed that pinacidil activated Iₖ_ATP at potentials both negative and positive to E_K;²⁰ As to inhibitory action on Iₖ_ATP, our preliminary study indicated that pinacidil produced a suppression of channel activity at positive voltages to E_K but no such action at negative voltages. Therefore, inhibitory action seems not to be the right explanation. Although we have not examined the drug effects on outside-out patches, the most likely explanation is that pinacidil inhibits Iₖᵢ from the outer surface of the membrane, because internal application of the drug did not affect the channel activity of Iₖᵢ.²⁰

The results of the present study point out a common mechanism of action on cardiac membranes shared by the three agents cromakalim, nicorandil, and pinacidil. These agents are claimed as K⁺ channel openers,¹ which possess the ability to increase K⁺ permeability in vascular smooth muscle cells and to produce a vasodilatory action. The exact mechanism of the three agents to induce relaxation of vascular smooth muscles is not known, but it is implicated that increased K⁺ permeability has some crucial role for vasodilatation.²⁴ Recently, the existence of Iₖ_ATP as well as its activation were reported.²⁵ Pinacidil also was suggested to activate Iₖ_ATP of vascular smooth muscles because of inhibition of the vasorelaxant effect of Iₖ_ATP by glibenclamide.²⁵ Although the properties of Iₖ_ATP between cardiac and smooth muscle cells were not exactly the same, the present results obtained from cardiac myocytes may help to solve the mechanism of a vasodilatory action by K⁺ channel openers. Study in this area continues,²⁶²⁷ and it is an interesting area for future studies.

The physiological and clinical significance of the present finding is not known. It would be interesting to know why pinacidil, cromakalim, and nicorandil consistently are found to activate Iₖ_ATP even though their chemical structures differ. Information regarding the structure-function relation of these compounds may be helpful in clarifying the regulatory mechanism of Iₖ_ATP itself and may lead to an understanding of the basic mechanism of the drug action on the heart. Pinacidil would be expected to be more effective in the ischemic heart than in the normal heart, because the ischemic heart would have less [ATP], than the normal. Whether or not the action of pinacidil could bring about beneficial effects for the function of the ischemic heart is to be evaluated further.

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References


**Key Words** • ATP-sensitive K+ current • intracellular ATP • K+ channel opener • pinacidil
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