Enhancement of Potassium-Sensitive Current in Heart Cells by Pinacidil
Evidence for Modulation of the ATP-Sensitive Potassium Channel

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Pinacidil belongs to a novel group of compounds that enhance the potassium permeability of vascular smooth muscle. Evidence also exists that this drug enhances the potassium permeability of cardiac tissue. The purpose of the present investigation was to determine if pinacidil alters potassium-channel activity in heart and, if so, which potassium channel is the target. We used the whole-cell arrangement of the patch voltage clamp to record membrane currents from isolated guinea pig ventricular cells. In solutions designed to isolate potassium currents, pinacidil enhances a time-independent current positive to the potassium equilibrium potential. Current measured at voltages negative to the potassium equilibrium potential are essentially unaltered by the drug. The potassium sensitivity of outward current indicates that the target for the drug is a potassium channel. Experiments designed to test for voltage-dependent channel gating strongly suggest that the pinacidil-sensitive current is not voltage gated. Pinacidil-sensitive current is blocked by externally applied Ba\(^{2+}\), Cs\(^{+}\), and tetraethylammonium ion. In addition, it is potently blocked after external application of 100 nM glibenclamide. Taken along with the time- and voltage-independent properties of pinacidil-sensitive current, this pharmacology strongly suggests that the target for pinacidil in heart is the ATP-sensitive potassium channel. (Circulation Research 1989;65:436–445)

Pinacidil belongs to a group of compounds that have been reported to increase the potassium permeability of vascular smooth muscle membranes.\(^1\)\(^2\) These compounds represent a new class of vasodilator antihypertensive agents that are thought to hyperpolarize smooth muscle cells and, in turn, cause relaxation. In addition to this effect on smooth muscle, there is evidence that suggests these agents alter potassium permeability of heart muscle cells.\(^3\)\(^4\) In particular, pinacidil markedly shortens the action potential duration of intact canine ventricular muscle\(^5\) and enhances outward current in isolated guinea pig ventricular cells.\(^6\) These effects are consistent with pinacidil-enhanced potassium conductance, but the channel that is the target for the drug has not been identified.

At least three types of potassium channels have been well characterized in mammalian cardiac ventricular cells. They differ in their time- and voltage-dependent properties, regulation by cytosolic factors, and pharmacology. The delayed rectifier is a voltage-gated channel that slowly activates at voltages positive to \(-30\) mV.\(^7\)\(^8\) Its activity is modulated by protein kinase A- and protein kinase C-mediated second messenger systems.\(^9\)\(^10\) The inward rectifier potassium channel is generally thought to be time independent\(^11\)\(^12\) although recent studies have indicated an inactivation process at negative voltages.\(^13\)\(^14\) The voltage-dependent properties of the inward rectifier depend on external potassium and are consistent with predictions of ion blockage of a multi-ion pore.\(^15\)\(^16\) The internal blocking particle may be Mg\(^{2+}\).\(^17\)

Two other potassium channels that are regulated by intracellular factors have more recently been identified. One of these channels is regulated by intracellular ATP and is referred to as the ATP-sensitive potassium channel. Activity of this channel is not evident unless cytosolic ATP concentrations fall below 1 mM.\(^18\) It is essentially a time- and voltage-independent channel except for rectification at extremely positive voltages that is due, most likely, to block by intracellular Mg\(^{2+}\) and Na\(^{+}\).\(^19\) Another potassium channel, activated by high lev-
els of intracellular sodium, has been briefly described. This channel is active when intracellular sodium concentration is higher than 20 mM and is also time and voltage independent.

The purpose of the present study was to determine whether, in fact, pinacidil modifies potassium-channel activity in the heart and, if so, which potassium channel it affects. Our results indicate that pinacidil does enhance potassium-sensitive current in guinea pig ventricular cells. The voltage dependence and pharmacology of the pinacidil-sensitive current provide strong evidence that this drug modulates the ATP-sensitive potassium channel.

A preliminary report of this work has appeared in abstract form.

Materials and Methods

Cell Isolation and Recording Procedures

Single myocytes were isolated from either ventricle of adult male or female guinea pigs (Charles River, Charles River, Massachusetts) weighing approximately 200–350 g. The isolation procedure is a modification of that of Mitra and Morad, which has been previously described.

Recording methods were as described by Hamill et al for the whole-cell configuration. Pipettes were made from Gold Seal Accu-fill 90 Micropets (Clay Adams, Inc., Parsippany, New Jersey). The resistance of the pipette was typically 1–3 MΩ when filled with pipette solution (see below). Series-resistance compensation was used in all experiments and was adjusted to give the fastest possible capacity transients without producing ringing. Voltages were corrected for liquid junction potential as described by Hagiwa and Ohmori.

Recordings were made at room temperature (20–24°C) from a Plexiglas chamber mounted on the stage of an Olympus IMT inverted microscope (Lake Success, New York). Solutions in the chamber were changed with voltage-controlled valves (General Valve Corp., Fairfield, New Jersey) connected to syringe reservoirs. Solution was removed with suction. The chamber volume of 1 ml was completely exchanged 1 minute after initiating a solution change. Approximately 15 ml solution was washed through for a solution change. In some experiments, cells were perfused at a rate of 1 ml/min; in others, the solution remained static between solution changes. Results were similar in both cases.

Solutions and Drugs

Isolated cells were initially placed in a standard Tyrode’s solution consisting of (mM) NaCl 132, KCl 4.8, MgCl₂ 3, CaCl₂ 1, glucose 5, and HEPES 10 at pH 7.4. After establishment of whole-cell voltage clamp, the external solution was changed to one that isolates current flow through potassium channels. This sodium-free solution contains (mM) tris(hydroxymethyl)aminomethane (TRIS) HCl 140, TRIS base 20, KCl 4.8, MgCl₂ 3, CaCl₂ 1, dextrose 5, tetrodotoxin (TTX) 10–50 (µM), and nisoldipine 200–500 (nM) at pH 7.3. In some experiments, TRIS was replaced by NaCl (150 mM). The experimental results were similar in both solutions. Extracellular potassium concentration ([K]₀) was changed by varying the ratio of KCl to TRIS while maintaining extracellular chlorine concentration constant. [K]₀ was 5 mM unless otherwise indicated.

The pipette solution consisted of (mM) KCl 50, potassium glutamate 60, MgCl₂ 2, CaCl₂ 1, HEPES 10, EGTA 11, and K₂ATP 5 and was buffered to pH 7.3 with KOH (final potassium concentration 140 mM). The free Ca²⁺ and Mg²⁺ concentrations of these solutions were 10 nM and 150 µM, respectively.

Pinacidil (see Figure 1 for structure), a gift of Lilly Research Laboratories (Indianapolis, Indiana), was dissolved as a stock solution in 1.5% 2N HCl and diluted appropriately before study. Nisoldipine, a gift from Miles Laboratories (New Haven, Connecticut), which was dissolved in polyethylene glycol as a 1 mM stock solution, was diluted for individual experiments. TTX was purchased from Behring Diagnostics (La Jolla, California) and dissolved as a stock solution in water. Glibenclamide (Sigma, St. Louis, Missouri) was dissolved as a 1-mM stock solution in 0.1 N NaOH.

Voltage Protocols

For most experiments, brief (40–100 msec) voltage steps were applied from the holding potential to eliminate overlap from the delayed rectifier current, which activates with time constants on the order of seconds at room temperature. Currents measured with this protocol consist predominately of time-independent inward rectifier current plus leak current, which is minimal under the conditions of our experiments. Unless otherwise indicated, holding potential was −40 mV.

Control data were taken 5 minutes after changing to the appropriate extracellular solution. The bath was then changed to pinacidil-containing solution (100 µM unless otherwise indicated), and currents were measured 5–10 minutes later. The bath was then changed back to drug-free solution, and protocols were repeated 5–10 minutes after washout. Only experiments with clear reversal of drug effect were used for data analysis. Pinacidil-sensitive cur-

![Pinacidil](image-url)
current was obtained by subtracting the average of control and washout data from drug-containing data.

To study blockers of the pinacidil-sensitive current, the following sequence of solution changes was used: drug free, pinacidil-containing, pinacidil plus blocker (10 minutes), pinacidil-containing, and drug free.

Data Analysis

All averaged data that are included in text or figures are mean±SEM. The concentration-effect curves were fit by applying a nonlinear least-squares fitting program to a modified Michaelis-Menten equation:

\[ \% \text{ of maximal effect} = \frac{1}{1 + \left(\frac{[D]}{K_d}\right)^n} \]

where \([D]\) is the drug concentration, \(K_d\) is the drug concentration for half-maximal effect, and \(n\) is the Hill coefficient.

Results

Pinacidil Enhances Outward Current

Figure 2 shows the effects of pinacidil on membrane currents under ionic and voltage conditions designed to isolate rapidly activating potassium channel currents (see "Materials and Methods"). In the absence of drug, currents measured under these conditions are dominated by inward rectifier currents. This results in a control current-voltage relation \((I/V)\) that favors inward current negative to but limits outward current positive to the Nernst potential for a potassium-selective electrode \((V_K)\). 6,11,14 In the presence of pinacidil, inward rectification is reduced. There is a clear increase in outward current at all voltages positive to \(V_K\) \((-81 mV\) in this cell). Despite this marked increase of outward currents, the drug has little effect on currents measured negative to \(V_K\).

The effects of pinacidil on membrane current develop slowly after cells are exposed to the drug. Outward-current increases are not immediately measured after changing to a pinacidil-containing solution but occur with a slight delay \((2.3±1.1\) minutes, \(n=16\)) and reach steady-state levels in \(5.4±2.2\) minutes. This is a relatively slow time course because solutions can be changed completely within 30 seconds in our system. Reversal of the effects of pinacidil is complete but also slow \((5.8±1.9\) minutes after initiating washout).

The current traces shown in the inset to Figure 2 show that the drug does not produce marked changes in the kinetics of currents measured at this time resolution. Outward currents are time independent immediately after the decay of the capacity transient. Pinacidil also consistently promotes current fluctuations at positive voltages. This reversible increase in current noise can be seen in the inset to Figure 2 (see also Figures 3 and 7) by comparing the traces recorded at \(+10 mV\) in the absence and presence of the drug.

Properties of the pinacidil-sensitive current can be seen more clearly in Figure 3 in which the average of control and recovery data has been subtracted from currents measured in the presence of drug. The difference \(I/V\) shows little or no inward current at voltages negative to \(V_K\) as discussed above. In 10 out of 16 cells, we observed no change in currents measured at voltages negative to \(V_K\) in four cells there was a slight reduction, and in two there were slight increases in current measured over this voltage range. There were marked increases in currents measured at voltages positive to \(V_K\) in all 16 cells. Results were similar in five cells in which external TRIS was replaced with Na+.
The outward current induced by pinacidil is linear over the voltage range of -70 to 0 mV (Figure 3) but becomes nonlinear at voltages positive to +25 mV. At positive potentials, outward pinacidil-sensitive current saturates and even becomes smaller, which is consistent with the possibility that an internal blocking particle inhibits this current at very positive voltages. In [K]o = 5 mM, we typically observed saturation or reduction of pinacidil-sensitive current over the voltage range of +25 to +60 mV.

Outward current is enhanced by pinacidil in ventricular cells in a concentration-dependent manner as is shown in Figure 4. The concentration-effect curve for pinacidil-sensitive current shown in this figure was determined by measuring currents at -10 mV in control, submaximal drug concentration, maximal drug concentration (500 μM), and recovery. Data recorded at a test concentration in each cell were normalized to the current induced by 500 μM pinacidil in that cell. The normalized current is plotted against test concentration in the figure. The data show a steep dependence on concentration with a Kd of 65 μM. The Hill coefficient is 1.9. This value indicates that there may be cooperativity of multiple binding sites.

**Pinacidil Enhances Potassium-Sensitive Current**

As a first test of the identity of the charge carrier for the pinacidil-induced current, we measured the influence of [K]o on the drug-sensitive current. In this set of experiments, we measured pinacidil-sensitive current over a K<sub:o</sub> concentration range of 0-140 mM, and Figure 5 illustrates results near the extremes of this range. The figure presents current-voltage relations in the absence and presence of pinacidil under conditions of 1 mM (Figure 5A) and 140 mM (Figure 5B) [K]o. Shown in Figure 5C are the pinacidil-sensitive currents determined from Figures 5A and 5B.

The pinacidil-sensitive current is clearly affected by changes in [K]o. External potassium influences the magnitude and voltage-range of the currents induced by pinacidil. Although the data obtained in Figure 5C were recorded in different cells, the pinacidil-induced currents measured in 140 mM K<sub:o</sub> are much greater than those recorded in 1 mM K<sub:o</sub>. This difference suggests that the conductance of the pinacidil-sensitive channel might be affected by potassium. In other experiments (data not shown), we confirmed the K dependence of the conductance of the pinacidil-sensitive channel by measuring cur-
rents in 5 and 50 mM $[K_o]$ in the same cell. Using the linear portions of the difference I/V curve to calculate slope conductance, we found a 3.6±0.5-fold ($n=3$) increase in slope conductance when $[K_o]$ was changed from 5 to 50 mM.

As has been shown for 5 mM $[K_o]$, pinacidil did not affect currents measured at voltages negative to $V_K$ in either concentration of potassium. Consequently, it was not possible to determine the reversal potential for the drug-sensitive current to probe the ionic basis of this effect. Instead, we defined the first voltage where outward current was observed (arrows in Figure 5C) as the "threshold voltage" for pinacidil-sensitive current. Figure 6 plots the dependence of threshold voltage on $[K_o]$. The solid line is a linear regression through these points and indicates a strong dependence on $[K_o]$, with a 52.2 mV change per 10-fold change in $[K_o]$. The dashed line is that predicted for a potassium-selective electrode. The agreement between the potassium-electrode predictions and the threshold voltage measurements strongly suggests that pinacidil enhances a potassium-sensitive pathway, but one in which inward ionic movement is very limited under the conditions of our experiments.

**Pinacidil Does Not Alter Delayed Rectifier Potassium Current**

One explanation for the lack of pinacidil-sensitive inward current is that the drug interacts with a channel that opens on depolarization but is not activated at negative voltages. The data presented in Figure 5 argue against this possibility because the "activation threshold" for pinacidil-sensitive outward current shifts with $[K_o]$. A voltage-gated channel would be expected to activate over the same voltage range under these conditions, but the amplitude and direction of the drug-induced current would change with driving force as external potassium is varied.

Although the evidence summarized above suggests that pinacidil does not act on a voltage-gated channel, we tested directly for possible modification of the voltage-gated delayed rectifier channel. Figure 7 presents the results of this experimental test. Here, time-dependent currents were measured with 2-second voltage steps to +40 mV. Current through delayed rectifier channels is identified as the time-dependent current during the 2-second pulses. Time-dependent current is revealed after subtraction of time-independent currents. The inset
shows delayed rectifier currents recorded before and during exposure to pinacidil. For comparison, the time-independent current at +40 mV was also measured and plotted with delayed rectifier changes during the exposure and washout of pinacidil. As expected from our previous experiments, pinacidil caused a reversible increase in outward time-independent current at +40 mV but did not affect the delayed rectifier amplitude or time course. We also found that the activation voltage range of delayed rectifier was not affected by pinacidil (data not shown). Similar results were obtained in three additional experiments. From these results, we conclude that pinacidil does not modulate the delayed rectifier channel.

Pharmacology of Pinacidil-Induced Current: Evidence for Modulation of ATP-Sensitive Channel

Because pinacidil enhances potassium-sensitive current that is not carried via a voltage-gated channel, it is possible that this drug modifies either the ATP-regulated potassium channel or the inward rectifier channel. To distinguish between these two possibilities, we tested the sensitivity of pinacidil-induced currents to pharmacological probes that are known to block potassium-channel currents in heart, as probes of the pinacidil-sensitive current. We found that externally applied Ba²⁺ (5 mM) as well as Cs⁺ (20 mM) completely blocked the inward rectifier and pinacidil-enhanced currents. However, these cations also block the delayed rectifier, ATP-sensitive, and most other potassium-channel currents in heart cells. Thus, the results with Cs⁺ and Ba²⁺ provide additional evidence that pinacidil enhances a potassium-channel current, but they are not helpful in identifying the channel type.

On the other hand, our results with organic potassium-channel blockers are very helpful in distinguishing the potassium-channel target of pinacidil. Figure 8 summarizes our findings with externally applied tetraethylammonium ion (TEA). Figure 8A shows that TEA (20 mM) has little effect on inward rectifier current. Currents measured positive to V_K are unaffected by the blocker, and currents measured negative to V_K are only slightly reduced (18.0±9.0% at -100 mV, n=3). These data are consistent with block of the channel pore as a consequence of inward movement of TEA ions. Figure 8B shows that the same concentration of

![Figure 7. Plot showing that pinacidil does not alter the time dependence of delayed rectifier current. Currents in response to 2-second voltage steps from -40 to +40 mV were measured every 15 seconds before, during, and after washout (Wash) of pinacidil (Pin). The plot shows the time-independent current at +40 mV (○) and the delayed rectifier current (●) measured as the time-dependent current during the pulse. Insets show delayed rectifier currents obtained after subtraction of time-independent current. Currents before (●) and during (○) pinacidil are superimposed. Solid line indicates zero current. Calibrations are 100 pA and 200 msec. [K]₀, 0 mM.](image)

Table 1. Comparison of Pharmacology of Pinacidil-Sensitive Current to Inward Rectifier and ATP-Sensitive Potassium Currents

<table>
<thead>
<tr>
<th>Blocker</th>
<th>Pinacidil-sensitive current</th>
<th>Inward rectifier current</th>
<th>ATP-sensitive channel current</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ba²⁺ (5 mM)</td>
<td>100%</td>
<td>100%</td>
<td>Kakai and Noma [27]</td>
</tr>
<tr>
<td>Cs⁺ (20 mM)</td>
<td>100%</td>
<td>100%</td>
<td>Quayle et al [28]</td>
</tr>
<tr>
<td>TEA (20 mM)</td>
<td>100%</td>
<td>&lt;10%</td>
<td>Spruce et al [29]</td>
</tr>
<tr>
<td>glibenclamide (100 nM)</td>
<td>100%</td>
<td>0%</td>
<td>Schmid-Antomarchi et al [30]</td>
</tr>
</tbody>
</table>

Percent values are a result of the present study and indicate percent blockage of the inward rectifier current and pinacidil-sensitive current by various blocking agents. Blockage of the ATP-sensitive channel current has been demonstrated in studies by the above-referenced investigators. TEA, tetraethylammonium ion.
TEA is very effective in blocking the pinacidil-induced currents that are measured at voltages positive to $V_K$. Thus, at a concentration and voltage range over which inward rectifier current is unaffected, TEA completely blocks the current induced by pinacidil.

Glibenclamide is a sulfonylurea compound that has been shown to potently inhibit the ATP-sensitive channel in pancreatic islet cells.\(^30\) We find that glibenclamide does not affect inward rectifier current, even at concentrations as high as 1 $\mu$M (Figure 9A). However, at 10 times lower concentrations, this compound completely blocks the pinacidil-induced current. This result is illustrated in Figure 9B in which we show pinacidil-sensitive current recorded in the presence and absence of glibenclamide in a single cell. Glibenclamide completely blocks the pinacidil-induced current at this concentration (100 nM). Similar results were obtained in three other cells.

Because glibenclamide and TEA block the ATP-sensitive channel but not the inward rectifier at these concentrations, our results suggest strongly that pinacidil modulates the ATP-sensitive potassium channel.

Discussion

Our results indicate that pinacidil increases potassium-sensitive current in isolated guinea pig ventricular myocytes. Under the conditions of our experiments, the most prominent characteristic of pinacidil-sensitive current is its marked outward rectification. We rarely found drug-induced enhancement of currents measured at voltages negative to $V_K$. The potassium sensitivity of the outward current induced by pinacidil strongly suggests that the drug interacts with a potassium channel, and the pharmacology of the pinacidil-induced current provides evidence that this channel is the ATP-sensitive potassium channel that has been reported in heart\(^35,36\) and other cells.\(^18,29,37,38\)

Evidence Supporting Interaction With ATP-Regulated Potassium Channel

Pinacidil-sensitive current is time and voltage independent. These properties, taken along with the
data illustrated in Figure 7, rule out the time- and voltage-dependent delayed rectifier channel as a target for the drug. Consistent with this view are the results of Iijima and Taira, who have reported that pinacidil does not affect delayed rectifier tail currents in isolated guinea pig ventricular cells. The sodium-activated potassium channel is also not a likely target for pinacidil because our experiments were carried out in sodium-free internal and external solutions. The two possibilities that remain are that pinacidil alters the permeation properties of the inward rectifier channels and that it modulates ATP-regulated potassium channels.

It is possible that pinacidil binds to a site within or near the inward rectifier channel that prevents the indigenous blocking ion from binding to this site and inhibiting outward current flow through the channel. Such a scheme would predict pinacidil-enhanced outward but not inward current. It would also be consistent with the potassium sensitivity of the threshold voltage and conductance of the pinacidil-induced current. However, despite the attractive simplicity of this model, it cannot easily explain the pharmacological results we have obtained.

We have found that externally applied TEA and glibenclamide do not block outward current through inward rectifier channels but completely block outward currents induced by pinacidil. This occurrence can be explained by assuming that pinacidil-bound inward rectifier channels have different sensitivities to these compounds, but we suggest a more direct reason.

The voltage-dependence of the pinacidil sensitive current resembles that of the ATP-sensitive current. The current-voltage relation of the pinacidil-sensitive current has many properties in common with that reported for the ATP-sensitive channel. In a study of whole-cell currents in guinea pig ventricular cells, Noma and Shibasaki perfused cells with low ATP solutions that contained cyanide to induce current through the ATP-sensitive potassium channel. The currents promoted by this perfusion were time independent in the outward direction, but they were small or nonexistent at voltages negative to V_K, as is the case for pinacidil-induced current. Although some of the gating characteristics of the ATP-sensitive channel revealed by single-channel studies support outward rectification of the macroscopic currents, the mechanism that underlies this property of whole-cell currents remains to be unraveled.

Another parallel between pinacidil-sensitive current and whole-cell ATP-channel currents is the pinacidil-induced current fluctuations observed at very positive voltages (see Figures 2, 3, and 7). Noma and Shibasaki found these fluctuations to be consistent with the fluctuations observed for ATP-sensitive single-channel currents. Finally, the current-voltage relation for cyanide-induced current becomes nonlinear at positive voltages in a manner that parallels the current-voltage relation for pinacidil-sensitive current. This rectification is consistent with single-channel current records reported for the ATP-sensitive channel. The saturation and inhibition of currents at extreme positive voltages is due to channel block by Mg^2+ applied to the internal face of the membrane. Although Na^+ ions can also block the channel from the inside, it is likely that the saturation and inhibition we report for the pinacidil-sensitive current is due to Mg^2+ block because our intracellular solution was Na^+ free with a free Mg^2+ concentration of 150 μM.

Additional evidence for this mechanism is that the voltage range over which rectification occurs shifts in the depolarizing direction as [K]_o is increased (e.g., Figure 5C). This is expected in the blocking scheme proposed by Horie et al in which elevated [K]_o increases the influx of K^+ through the channel that can knock out Mg^2+ from its binding site. In high [K]_o, very positive voltage steps are necessary for Mg^2+ to reach its binding site within the channel.

Pharmacological evidence that pinacidil acts on the ATP-sensitive channel. Perhaps the most convincing evidence in favor of pinacidil modulation of the ATP-sensitive channel comes from our pharmacological data (see Table 1). We find that the pinacidil-induced current, but not inward rectifier current, is blocked by externally applied TEA and glibenclamide.

Glibenclamide is a sulfonylurea compound that potently (10–20 nM) blocks the ATP-sensitive potassium channels of pancreatic B cells and the insulin-producing cell line RINm5F. In this cell line, correlation of binding data, Rb^+ efflux data, and pharmacological data has suggested that the glibenclamide (sulfonylurea) receptor is closely associated with the ATP-sensitive potassium-channel protein. In our experiments, glibenclamide (100 nM) blocked the pinacidil-sensitive current but did not affect the inward rectifier.

Our experimental results obtained with TEA also support the hypothesis that pinacidil acts on the ATP-sensitive potassium channel. We find that externally applied TEA has little effect on the inward rectifier, particularly at voltages positive to V_K. Although there have been few detailed studies of the effects of TEA on inward rectifier current, other studies in heart cells have suggested that inward rectifier is insensitive to externally applied TEA.

In contrast, Spruce et al have reported that externally applied TEA blocks the ATP-sensitive channel in frog skeletal muscle. Additionally, other investigators have shown the ATP-sensitive potassium channel is sensitive to internally applied TEA (for review, see Aschroft). We find that external TEA (20 nM) completely blocks pinacidil-sensitive current. Thus, our results with TEA and glibenclamide suggest that the target for pinacidil is the

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ATP-sensitive potassium channel, and not the inward rectifier channel.

Relation to Other Potassium-Channel Openers

The results reported here are very similar to those reported for two other potassium-channel "openers," BRL 34915 and nicorandil, in isolated guinea pig ventricular cells. In particular, nicorandil produced an increase in time-independent outward current with no change or slight block of current measured at voltages negative to $V_K$. In addition, the current induced by these two drugs saturates and rectifies at positive voltages and is accompanied by enhanced membrane noise.5,4,6

Pinacidil, BRL 34915, and nicorandil relax vascular smooth muscle. This action is probably caused by the enhanced potassium conductance of the cell membrane that shifts the membrane resting potential toward $V_K$.2,4 The channel responsible for this effect has yet to be identified. For pinacidil, relaxation of vascular smooth muscle is observed at submicromolar levels two to three orders of magnitude less than necessary for increased outward currents in this study. Interestingly though, the Hill coefficient for the concentration-response curve of inhibition of spontaneous activity in guinea pig portal vein5 is similar (1.7) to that reported here (1.9) for enhancement of outward current in heart. Although many explanations for the discrepancy in $K_H$ for heart and smooth muscle exist (such as temperature, species differences, and assay used), an intriguing one is that ATP-sensitive channels are also the target channel in smooth muscle and that the regulation of these channels by pinacidil and cytosolic factors, such as the ATP/ADP ratio and/or GTP,18 is different in heart and smooth muscle. This represents an interesting area for future studies. However, it is important to note that ATP-sensitive channels have yet to be reported in vascular smooth muscle.18,48

Conclusions

We have shown that pinacidil increases outward current through a potassium-sensitive pathway in isolated guinea pig ventricular myocytes. The voltage and time dependence as well as the pharmacological profile of the current induced by pinacidil strongly suggest that the target for this drug is the ATP-sensitive potassium channel. The activation of a metabolically regulated channel by an organic compound is an exciting prospect that will, in future studies, no doubt provide insight into the mechanism by which this widespread channel is regulated.

Note added in proof. After submission of this manuscript, Sangiunietti et al (Proc Natl Acad Sci USA 1988;85:8360–8364) and Escande et al (Biochem Biophys Res Commun 1988;154:620–625) have reported similar results with BRL 34915.

References


25. Fabiato A: Computer programs for calculating total from specified free or from specified total ion concentra-
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