Antiarrhythmic Actions of the ATP-Regulated K+ Current Activated by Pinacidil

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We tested the hypothesis that a selective increase in membrane current, as contrasted with the decreases in currents caused by most antiarrhythmic agents, would be an effective antiarrhythmic intervention. We studied models of early afterdepolarizations (EADs), delayed afterdepolarizations (DADs), and abnormal automaticity in single canine ventricular myocytes using intracellular microelectrodes or patch electrodes. EADs were induced by injected current, Bay K 8644 (0.5–1 μM), or ketanserin (1.0 μM); DADs were induced by ouabain intoxication (2×10−7 M); and abnormal automaticity was induced by exposure to barium (0.25 mM). To increase outward K+ current, we used pinacidil and the protein kinase C activator 4β-phorbol 12,13-dibutyrate (PDBu). Under control conditions, 10–100 μM pinacidil caused a concentration-dependent and reversible decrease in action potential duration and an increase in steady-state outward current; both effects were blocked by glibenclamide and thus presumably reflected changes in the ATP-regulated potassium current. Pinacidil increased the current required to induce EADs and abolished EADs caused by Bay K 8644 or ketanserin. After exposure of myocytes to ouabain, pinacidil caused a decrease in action potential duration and diminished or abolished DADs. Finally, pinacidil arrested abnormal automaticity caused by Ba2+. PDBu (30 nM) shortened action potential duration without altering plateau amplitude in some of the myocytes. In these cells the depolarizing current needed to produce an EAD was increased by over 70%; outward potassium current tails were also increased, an effect consistent with an increase of the repolarizing potassium current (I_K). These findings show that each of the mechanisms for abnormal impulse generation can be effectively antagonized by an increase in outward current and suggest to us that selective augmentation of a repolarizing current, possibly I_K, might be a reasonable antiarrhythmic intervention. (Circulation Research 1991;68:1127–1137)

Cardiac arrhythmias result from abnormalities either of impulse generation or impulse conduction.1 The basic causes of abnormal impulse generation are the development of abnormal automaticity2 or of early afterdepolarizations (EADs) or delayed afterdepolarizations (DADs).3 Abnormal automaticity is defined as automatic impulse generation from a markedly decreased maximum diastolic potential; this is the primary cause of ventricular arrhythmias 24 hours after coronary artery occlusion in the dog.4 EADs interrupt phase 3 of the transmembrane action potential and give rise to triggered impulses; EADs induced by antiarrhythmic drugs are a likely cause of rhythms called torsades de pointes.5,6 DADs cause triggered premature im-
channels. For our initial experiments we selected pinacidil, an agent that has been shown to activate the ATP-regulated K+ current (I_{KATP}) in several tissues\textsuperscript{10} and that in the heart decreases action potential duration without modifying resting potential or action potential amplitude.\textsuperscript{11,12} We based our selection on evidence that activation of I_{KATP} increases outward current at all potentials positive to \(E_K\), the potassium equilibrium potential.\textsuperscript{13} In our studies, we have found that pinacidil modifies several currents in addition to I_{KATP}, including the fast transient outward current,\textsuperscript{13} but these effects did not seem likely to confound our interpretation of results, since a reduction in the fast transient outward current would prolong the action potential. We did not assume that pinacidil was a prototype of an antiarrhythmic drug because of its prominent effects on other tissues\textsuperscript{14} and because of its potential to reduce inotropy by causing excessive shortening of the action potential. Rather, we wished to evaluate the antiarrhythmic potential of an augmented outward (largely \(K^+)\) current at potentials positive to the resting potential.

We also evaluated the effects of augmenting the normal repolarizing current, I_{Kr}, on the current threshold to produce EADs. Here we did not have an agent that acted only on I_{Kr} channels. We used the protein kinase C activator, 4β-phorbol 12,13-dibutyrate (PDBu), which enhances I_{Kr}\textsuperscript{15} but can also regulate I_{Ca,L}\textsuperscript{16-18} I_{Na}\textsuperscript{19} and I_{L} \textsuperscript{20} We had shown in a preliminary study that PDBu abolished EADs in single myocytes.\textsuperscript{21}

Our findings in this study support the proposition that increasing rather than decreasing specific sarcolemmal ionic currents can be an effective antiarrhythmic intervention.

**Materials and Methods**

**Preparation of Isolated Myocytes**

Canine ventricular myocytes were isolated by collagenase dispersion using methods previously described in detail.\textsuperscript{22,23} Cells were stored at room temperature in 50% MEM-50% F12 medium containing 1.3 mM calcium and adjusted with HEPES buffer (10 mM) to pH 7.3. All studies were conducted within 12 hours of cell isolation.

A few drops of the cell suspension were added to the Lucite perfusion chamber (0.5-ml volume) placed on the stage of an inverted microscope. The cells were allowed to settle for 1-2 minutes on a glass coverslip coated with poly-L-lysine before superfusing them at a rate of 2 ml/min with a modified Tyrode’s solution containing (mM) NaCl 118, KCl 4, MgCl\textsubscript{2} 1.2, CaCl\textsubscript{2} 2.5, NaHCO\textsubscript{3} 24, NaH\textsubscript{2}PO\textsubscript{4} 1.8, and dextrose 5.5. The solution was saturated with 95% O\textsubscript{2}-5% CO\textsubscript{2} and maintained at 35-37°C (pH 7.3).

**Electrophysiological Studies**

The cells were impaled with a microelectrode containing 3 M KCl and having a tip resistance between 30 and 60 MΩ. Transmembrane potentials were recorded with an Axoclamp II voltage clamp amplifier (Axon Instruments, Inc., Burlingame, Calif.). Transmembrane potentials and the first derivative of the action potential upstroke (\(V_{\text{max}}\)) were displayed on a Tektronix 565 (Tektronix Inc., Beaverton, Ore.) and on a Gould 4072 (Gould Inc., Cleveland, Ohio) dual time-base oscilloscope. Transmembrane potentials and \(V_{\text{max}}\) were photographed with a Polaroid camera from the Tektronix oscilloscope and recorded on a Gould chart recorder. Cells were stimulated using the bridge circuit and a 10-msec rectangular depolarizing pulse. The bridge circuit also was used to inject depolarizing current during the plateau to induce EADs.

The single-electrode switched-clamp method was used in most experiments to record membrane potential and inject current for voltage-clamp experiments. Depending on the electrode resistance, the switching frequency ranged between 3 and 5 kHz. To ensure that the voltage drop across the electrode resistance had subsided after the current injection phase, the form of the voltage signal before the sample-and-hold circuit was continuously monitored.

For two experiments measuring the steady-state current-voltage (I-V) relationship, membrane currents were recorded using the whole-cell configuration of patch-clamp recording\textsuperscript{24} (Figure 2B). Patch electrodes were made from Corning 7052 glass (Corning Glass Inc., Corning, N.Y.) using a two-stage pull on a vertical puller (Narashige, Tokyo). Electrodes had resistances of 1.5–4 MΩ when filled with intracellular solution. The pipette solution contained (mM) potassium aspartate 125, KCl 15, MgATP 10, EGTA 5, and HEPES 5; pH was 7.2. The high concentration of ATP was intended to prevent opening of I_{KATP} under control conditions. The voltage was zeroed in the modified Tyrode’s solution before forming seal. All voltages reported are in reference to this zero potential. High-resistance seals (2.5–15 GΩ) were formed with 10–30 cm H\textsubscript{2}O negative pressure. After stabilization of the seal, the membrane was ruptured by a 50–100 cm H\textsubscript{2}O pulse of negative pressure. Electrical signals were amplified with a patch-clamp amplifier (model EPC-7, List Electronic, Darmstadt, Eberstadt, FRG) and low-pass-filtered at 10 kHz. The signals were digitized with a TM-100 Labmaster board (Teclam, Solon, Ohio) and stored on disk. The acquisition and analysis of data, as well as the generation of voltage-clamp protocols, were performed using an IBM PC/AT computer and version 4.0 of the PCLAMP software package (Axon Instruments). Steady-state current was measured using a depolarizing ramp from −100 to +30 mV over 8 seconds. Drug-induced currents were calculated by subtracting control currents from currents in the presence of drugs.

**Data Analysis**

Data analysis was performed using two-way analysis of variance. In the presence of a significant \(F\) value, further comparisons across groups were made
with the Scheffe’s test. The level of significance was set at \( p < 0.05 \). Data are reported as mean±SEM.

We limited data collection in the following ways. Only myocytes that were quiescent, rod-shaped, and free of blebs and that possessed clear sarcolemmal striations were used. Data were collected only from cells that maintained a resting potential more negative than \(-80 \text{ mV}\) and had a value of \( V_{\text{max}} \) greater than \( 200 \text{ V/sec} \). Impalement in such cells could be maintained for 2 hours or more without modification of transmembrane potential parameters.

Pinacidil was a gift from Eli Lilly and Company, Indianapolis, In. The stock solution was made using ethanol (35% vol/vol) and 1N HCl (3.5% vol/vol). Other drugs were Bay K 8644, ouabain, ketanserin, PDBu, and glibenclamide; all were from Sigma Chemical Co., St. Louis. Drugs were added to the Tyrode’s solution to provide the desired final concentrations.

**Results**

**Effects of Pinacidil on Control Cells**

We evaluated the effects on action potential parameters of concentrations from 1 to 300 \( \mu \text{M} \) in a series of control cells. The effects we observed were like those reported by others for different types of myocytes and are summarized in Table 1 and Figure 1. Pinacidil caused no change in the resting potential, action potential upstroke, or \( V_{\text{max}} \). It did cause a concentration-dependent decrease in action potential duration. The effect of 1.0 \( \mu \text{M} \) pinacidil was usually evident but very small, whereas the effect of 300 \( \mu \text{M} \) pinacidil was not much greater than that seen with 100 \( \mu \text{M} \) pinacidil. Repeated exposure of the same cell to concentrations of 3–30 \( \mu \text{M} \) pinacidil caused changes of similar magnitude in action potential duration, and effects were reversed with 10 minutes of washout. Because we had shown through studies with patch-clamp methods that the intensity of the effect of pinacidil varied as a function of intracellular ATP concentration, for each group of experiments we tested the response of each myocyte to a low concentration of pinacidil, usually 10 \( \mu \text{M} \).

The effects on the steady-state I-V relationship were determined in 12 cells with a slow depolarizing ramp that carried transmembrane potentials from \(-100 \text{ to } +30 \text{ mV}\). Drug-induced currents were obtained by subtracting control currents from the I-V relationship of a single myocyte. We did not normalize data in relation to cell capacitance. In Figure 2A, the switched-clamp method was used to obtain I-V plots for the same cell that provided the action potential in Figure 1; the measurement of the I-V relationship was made immediately after the action potential was recorded. Pinacidil did not change the zero current intercept but increased outward current at potentials positive to it. At potentials negative to the zero current intercept, pinacidil had no significant or consistent effects on the I-V relationship. Figure 2B shows I-V relationships obtained with a patch electrode for a range of concentrations. The pinacidil-induced current was reasonably linear between \(-60 \text{ and } +30 \text{ mV}\) (Figure 2C). Results were not influenced by the type of recording electrodes used.

To obtain evidence that the effects of pinacidil were mediated through \( I_{\text{K,ATP}} \), we studied interactions between glibenclamide and pinacidil in 10 cells by recording action potentials and steady-state I-V relationships. Glibenclamide at 10 \( \mu \text{M} \) had no effect on resting potential, action potential amplitude, or \( V_{\text{max}} \). Also, it had no consistent or significant effect on action potential duration. In some cells it caused a very slight prolongation, but since action potential duration tended to increase slightly with time, this change could not necessarily be attributed to drug effect. When action potential duration had been shortened by pinacidil in concentrations of 10 or 30 \( \mu \text{M} \), the change was abolished by glibenclamide at 10 \( \mu \text{M} \). In one experiment, the action potential shortening caused by 100 \( \mu \text{M} \) pinacidil was only partially reversed by 3 \( \mu \text{M} \) glibenclamide. Prior exposure of a cell to glibenclamide (10 \( \mu \text{M} \)) prevented a change in action potential duration by pinacidil at 10–30 \( \mu \text{M} \) (data not shown). The block or reversal of effects of pinacidil on action potential duration by glibenclamide presumably was due to abolition of the effects of pinacidil on the steady-state I-V relationship (Figure 3).
Effects on Early Afterdepolarizations

We studied the effect of pinacidil on EADs in a total of 14 cells; in five we used applied current to induce EADs, in two we used current during superfusion with isoproterenol (10^{-8} M), in three we used Bay K 8644 to induce EADs, and in two we used ketanserin. Bay K 8644 presumably causes EADs by augmenting the L-type calcium current; the mechanism by which ketanserin causes this abnormality is uncertain, although there is evidence that it blocks I_k in rabbit sinus node.

For tests with current-induced EADs, we injected a rectangular pulse of depolarizing current that started shortly after the action potential was evoked. We determined the current needed to cause one or more clear and typical EAD (Figure 4). We evaluated the effect of pinacidil by noting if, during exposure to the drug, the same current induced the

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**FIGURE 2.** Graphs showing effects of pinacidil on the steady-state current-voltage relationship. Panel A: Increase in outward current induced by 10 μM pinacidil (+) and the return to control values (○) after drug washout (●). Panel B: Control plot (○) and the dose-dependent increase in outward current caused by pinacidil at concentrations of 10 (●), 30 (△), and 100 (○) μM. The change in inward current seen here was not typical. Panel C: Pinacidil-induced current at 20 μM (○, pinacidil-control; ●, washout-control).
same EAD; if it did not, we determined the minimum current needed to cause an EAD like those seen under control conditions. Pinacidil typically reduced the amplitude of or abolished the EAD caused by the control current and increased the current needed to duplicate the control EAD by a factor of two to eight (Figure 4). There were no other consistent changes in the EAD that could be attributed to the action of pinacidil. On exposure to Bay K 8644 (0.5–1.0 μM) during pacing at cycle lengths of 2,000–3,000 msec, action potential duration increased to approximately twice the control value, and then each action potential was followed by a series of two or more EADs. Addition of pinacidil (10 μM) to the superfusate abolished EADs and shortened the action potential duration but not to the control value, whereas at 100 μM, pinacidil caused action potential duration to decrease to considerably less than control values.

The effects of ketanserin (1 μM) were much like those of Bay K 8644 (Figure 5). Ketanserin prolonged the action potential markedly and induced EADs. During continued exposure to ketanserin, pinacidil (10 μM) abolished EADs and greatly shortened the action potential. On washout of pinacidil, EADs returned, and on washout of ketanserin, the action potential returned to the control duration and configuration. We also studied two cells that were abnormal in that, at long (<3,000-msec) cycle lengths, they demonstrated EADs in the absence of any experimental intervention. Pinacidil (10 μM) shortened the action potential and abolished EADs in both.

**FIGURE 3.** Graph showing effects of glibenclamide on the increase in outward current induced by pinacidil. ○, Control; □, 10 μM pinacidil; Δ, 10 μM glibenclamide in the presence of pinacidil.

**FIGURE 4.** Effects of pinacidil on the early afterdepolarizations induced by inward current pulses. The upper tracing shows the duration and strength of the depolarizing pulses; the lower tracing shows the transmembrane action potential recorded at a fast and slow paper speed. Under control conditions, early afterdepolarizations are induced by a current of 0.02 nA. During exposure to increasing concentrations of pinacidil (1, 3, and 10 μM), the current required to cause comparable early afterdepolarizations increased to 0.05, 0.125, and 0.175 nA. On washout of pinacidil, the current requirement decreases to 0.035 nA.

**FIGURE 5.** Tracings showing effects of pinacidil on early afterdepolarizations induced by ketanserin. Panel A: The control action potential. Panel B: Ketanserin (1 μM) increases action potential duration and induces an early afterdepolarization. Panel C: Pinacidil (10 μM) abolishes the early afterdepolarization and greatly shortens the action potential. Panel D: Return of early afterdepolarization on washout of pinacidil. Panel E: Restoration of control action potential configuration on washout of ketanserin.
Effects on Delayed Afterdepolarizations

We studied effects of pinacidil on ouabain-induced DADs in 11 cells. After one or more control exposures to pinacidil, we superfused the cells with Tyrode's solution containing $2 \times 10^{-7}$ M ouabain for 30 minutes while stimulating at a cycle length of 500–800 msec. The drive stimulus was interrupted periodically, and the amplitude of the DAD was measured. Within 20–30 minutes of exposure to ouabain, DADs of at least 5 mV were recorded. At this point, we began ouabain washout with control Tyrode's solution. Earlier studies have shown that this protocol induces relatively stable DADs in canine Purkinje fibers; the DADs have a constant amplitude for at least 60 minutes. After recording the DAD amplitude, we added pinacidil in concentrations ranging from 10 to 25 µM and repeated the measurements. Finally, we washed out the pinacidil and determined the extent to which effects of ouabain persisted by noting action potential duration and DAD amplitude. This sequence was completed in 10 of the 11 cells; in one cell, we failed to obtain data after pinacidil washout.

The results of these experiments are summarized in Table 2 and demonstrated in Figure 6. Ouabain caused no consistent change in resting potential but, in all but one case, slightly decreased action potential duration. The average change was a decrease of 13%. In all experiments, ouabain induced DADs that ranged in amplitude from 5 to 18 mV. On exposure to pinacidil, there was no change in resting potential, but a significant decrease in action potential duration was recorded from each cell. With respect to the effect of ouabain alone, the decrease in action potential duration amounted to 20%. This decrease in action potential duration was less than when pinacidil was applied under control conditions (compare Tables 1 and 2). After washout of pinacidil, action potential duration increased as did DAD amplitude. In isolated canine ventricular myocytes, the interval between the action potential upstroke and the peak of a DAD induced by ouabain may vary by 10–15%. In 10 myocytes, pinacidil increased this interval in three, decreased it in two, caused no change in two, and abolished the DAD in three cases. In only one instance was the change greater than 10%. Figure 6 illustrates graphically the changes in action potential duration and DAD amplitude caused by pinacidil. During exposure to ouabain alone, average DAD amplitude was 8.7 mV; pinacidil decreased this to 3.2 mV, and on washout of pinacidil, average DAD amplitude increased to 5.7 mV. Ouabain did not much change the control I-V relationship, although in some experiments there was an increase in outward current at positive voltages and in many there was an increase in oscillatory current at positive voltages. Because of these oscillations, we were able to obtain stable I-V relationships throughout the experiment in only three cells: after ouabain, the current induced by pinacidil (20 µM) at 0 mV was reduced by 90%, 10%, and 57% in these three cells.

Effects on Abnormal Automaticity

We studied effects of pinacidil on abnormal automaticity in eight cells. Under control conditions, all demonstrated a stable resting potential of −80 mV or greater. When BaCl$_2$ was added to the superfusate in a concentration of 0.25 mM, all cells partially depolarized and began to fire spontaneously. Maximum diastolic potentials ranged from −58 to −42 mV at cycle lengths from 1,300 to 800 msec. On addition of pinacidil in a concentration of 10 µM, the rate of the automatic rhythm decreased, and spontaneous activity ceased. Slowing was due to a progressive decrease in the slope of phase 4 depolarization and an apparent positive shift in the transition between phase 4 and phase 1 of the action potential. In some cells, the resting potential that was recorded after pinacidil had stopped the automatic activity was 4–8 mV positive to the maximum diastolic potential; in others, resting potential was 2–6 mV more negative than maximum diastolic potential. On washout of pinacidil the automatic rhythm was restored with characteristics like those noted before pinacidil. Figure 7 demonstrates these effects of pinacidil on one cell.
Effects of PDBu on Early Afterdepolarizations

The protein kinase C activator PDBu can, under certain circumstances, selectively enhance $I_{K^{-}}$.$^{15,16}$ The drawback of using this compound is that it can have a biphasic effect on $I_{Ca}$ (initial stimulation followed by inhibition), alter $I_{Na}$ kinetics, and inhibit the transient outward current.$^{19,20}$ In spite of these complications, short exposures (1–2 minutes) to 30 nM PDBu resulted in shortening of the action potential duration at 90% repolarization with no significant effect on plateau potential. These changes in action potential configuration are consistent with activation of $I_{K^{-}}$. In a subset of cells (approximately 45%), this action potential configuration was maintained in the continuous presence of PDBu (Figure 8A). The remainder of cells responded in a biphasic manner with a secondary plateau elevation and increase in action potential duration that developed within 5 minutes of exposure to PDBu (Figure 8B).

We measured the current threshold required to produce an EAD in cells exhibiting a maintained shortening of the action potential duration at 90% repolarization in response to 30 nM PDBu. In this subset of cells, PDBu increased the current threshold to produce an EAD. One of these experiments is shown in Figure 9. Cells were paced at a basic cycle length of 2 seconds; a 1.2-second pulse of outward current initiated at the end of the basic stimulus was injected every fifth cycle. The current required under control conditions to induce an EAD was 53±11 pA (mean±SEM, $n=7$). During PDBu exposure, the current threshold increased to 94±16 pA ($p<0.05$). On removal of PDBu, the current threshold returned to control levels (55±14 pA). Measurement of $I_{K}$ tail currents on repolarization to −20 mV after a 1.5-second step at +60 mV (single electrode switch clamp) confirmed that PDBu (30 nM) enhanced this current. In four myocytes, peak tail current increased from 197±102 to 292±102 pA ($p<0.05$). After washout, the peak tail current returned to 196±59 pA. Control experiments employing 4α-phorbol 12,13-

![Figure 7](image_url)

**Figure 7.** Panel A: Tracing showing automatic rhythm caused by superfusion with Tyrode's solution containing 0.25 mM BaCl$_2$. Panel B: Tracing showing arrest of spontaneous activity by pinacidil (10 μM). Panel C: Tracing showing resumption of spontaneous firing on washout of pinacidil.

![Figure 8](image_url)

**Figure 8.** Tracings showing effects of 30 nM 4β-phorbol 12,13-dibutyrate (PDBu) on the action potential. Panel A: PDBu shortens action potential duration without altering plateau amplitude. Panel B: The initial response of another cell to PDBu was similar to that shown in panel A. Further superfusion increased plateau amplitude and prolonged action potential duration.

![Figure 9](image_url)

**Figure 9.** Tracings showing effect of 4β-phorbol 12,13-dibutyrate (PDBu) on current threshold for early afterdepolarization production. The top tracing in each panel represents the transmembrane potential; the bottom tracing shows duration and strength of depolarizing current pulses. Five minutes of superfusion with PDBu increased the current threshold for the induction of early afterdepolarizations (control, 60 pA; PDBu, 105 pA).
dibutryrate, the biologically inactive stereoisomer of PDBu, did not reveal any effect on action potential duration.

**Discussion**

We set out to test the assumption that each of the three electrophysiological mechanisms for abnormal impulse generation—EADs, DADs, and abnormal automaticity—might be controlled by increasing outward current at membrane potentials positive to the resting potential. We assumed that added outward current would restore normal repolarization to cells developing EADs, would decrease action potential duration and thus attenuate or abolish DADs, and would hyperpolarize abnormally automatic cells and thus slow or arrest impulse generation. Although it is obvious that a sufficient change in net membrane current would have the desired effect for each arrhythmogenic mechanism, it was not obvious that the agents we used, pinacidil and PDBu, would bring about the needed changes in net current. We also speculated that the induction of the desired change in net current with these agents would indicate that it might be desirable to develop antiarrhythmic agents that increased, rather than decreased, current in membrane ion channels. Since these are somewhat different postulates, it seems reasonable to discuss first the results of our studies and then their general implications with respect to treatment of arrhythmias.

We studied single canine ventricular myocytes with intracellular microelectrodes and whole-cell patch electrodes. We induced EADs by several means (Bay K 8644,27 ketanserin,28,29 and applied current) and found that pinacidil in all cases decreased the amplitude of EADs or abolished them. We induced DADs by exposing myocytes to ouabain30; pinacidil decreased action potential duration and, in association with this change, decreased the amplitude of or abolished DADs. We induced abnormal automaticity by exposing the myocytes to barium31; pinacidil consistently terminated the automatic rhythm but did not cause an appreciable increase in maximum diastolic potential. In the cells that responded to PDBu with a maintained shortening of action potential duration, the current needed to induce an EAD was increased by more than 70%. We obtained evidence indicating that the effects of pinacidil were accompanied by, and presumably caused by, an increase in outward current, that this current was $I_{K,ATP}$, and that the effects of PDBu were due to an increase in $I_{K,ATP}$.

We used pinacidil, an agent known to increase the conductance of the ATP-regulated K+ channel,11 because we had shown in canine ventricular myocytes13 that it induces an outward current that increases linearly with voltage at membrane potentials positive to the resting potential and that the magnitude of the added current is a function of the concentration of pinacidil. Others have studied the effects of pinacidil11,12 and other agents32 that increase $I_{K,ATP}$ and have found a dose-dependent decrease in action potential duration in the absence of changes in resting potential or action potential amplitude. Clearly, pinacidil has multiple effects on the membrane ionic currents of cardiac cells,13 but the dominant effect is on $I_{K,ATP}$.

Because the intensity of effects of pinacidil may be a function of intracellular ATP concentration and because of its multiple effects, we performed some control studies. We found that, with our cells, concentrations of pinacidil between 3 and 30 μM produced moderate to marked shortening of the action potential without modifying resting potential or action potential amplitude. The effect of pinacidil on action potential duration was promptly reversed on washout of the drug, and repeated exposures to the same concentrations caused changes of similar magnitude. The decrease in action potential duration was accompanied by a dose-dependent increase in outward current but no consistent change in inward current in the steady-state I-V relationship. Both the change in action potential duration and the augmented outward current were prevented or reversed by glibenclamide, and so it seemed most likely that the effects we observed were due to drug-induced changes in $I_{K,ATP}$.

We also studied the effects of the phorbol ester PDBu on EADs to determine if it was feasible to sufficiently augment $I_{K}$ to modify this arrhythmogenic mechanism. PDBu leads to phosphorylation of several classes of channels, including $I_{K}$ and $I_{Ca,L}$, but in some experiments the major effect was an increase in delayed rectifier current.

We used three different means to induce EADs. This abnormality can result from an increase in inward current, from a decrease in the outward current, or from changes in both that result in a small increase in net inward current. When EADs were caused by inward current pulses, pinacidil consistently increased the current needed for them to appear. When we used Bay K 8644 to augment $I_{Ca,L}$ and cause EADs, pinacidil shortened the action potential and abolished the EADs. We also used ketanserin because it is known to cause EADs28 and probably does so by reducing $I_{K}$.29 Pinacidil was effective in abolishing EADs caused by ketanserin.

DADs can be caused by a number of interventions and result from an increase in intracellular calcium activity that causes oscillatory calcium release from and reuptake by the sarcoplasmic reticulum.33 The oscillatory changes in calcium activity induce a transient inward current either through the sodium-calcium exchanger34 or through a nonselective cation channel.35 The transient inward current causes DADs. Ouabain has been used to induce DADs in a variety of tissues, and the dose–response relationship is reproducible. Thus, we used ouabain and a standard protocol to induce DADs. Our assumption was that, since pinacidil decreases action potential duration and since the amplitude of the DAD is a function of the duration of the preceding depolarization, pinacidil should attenuate or abolish the DAD.
The effects of ouabain on our canine ventricular myocytes were, for the most part, what would be expected. After exposure to ouabain, there was a modest decrease in action potential duration, and after a brief period of relatively rapid pacing, DADs appeared. The unexpected finding was the constancy of the resting potential, since in most other experiments there is an accompanying moderate depolarization when ouabain induces DADs. This finding suggests that the depolarization noted in studies on multicellular preparations is caused mainly by accumulation of K⁺ in interfiber spaces or by depletion of intracellular K⁺ and not by a significant decrease in pump current or a direct effect of ouabain on membrane ion channels. In our studies in single myocytes, a decrease in intracellular K⁺ concentration probably is prevented by diffusion of K⁺ from the microelectrode. When ouabain induced DADs, pinacidil in low concentration caused moderate shortening of the action potential and either the disappearance of or a decrease in the amplitude of DADs. On washout of pinacidil, DADs reappeared or increased in amplitude, thus demonstrating that the toxic effect of ouabain had persisted.

The means we used to induce abnormal automaticity—exposure to Tyrode’s solution containing barium—was not well suited to our test with pinacidil, since barium ions block Iₖ,ATP channels as well as other K⁺ channels in a voltage-dependent manner. However, the ability of Ba²⁺ to induce abnormal automaticity in canine ventricular fibers is consistent and well characterized, and the method is one with which we were familiar. Also, it seemed quite likely that the block induced by Ba²⁺ would be incomplete. As in multifiber preparations of canine ventricular muscle, Ba²⁺ induced typical abnormal automaticity in our isolated myocytes. This arrhythmia was slowed or arrested by pinacidil.

The results obtained through these experiments indicate clearly that an augmentation of outward current at potentials positive to the resting potential is an effective means of influencing the three mechanisms for abnormal impulse generation and that a change in outward current of the sort induced by pinacidil can be an effective antiarrhythmic intervention in the case of arrhythmias caused by EADs, DADs, or abnormal automaticity.

The effects of PD Bu varied among cells and with time in a single cell. In some cases, changes in the action potential configuration suggested that we had augmented Iₒ,Cl and, in this manner, had shifted the plateau to more positive potentials and increased action potential duration. In other cases, there was a clear and maintained decrease in action potential duration and, associated with this, an increase in the amplitude of Iₖ tail current and an increase in the current needed to induce EADs. This finding suggests that selective augmentation of Iₖ would be an effective antiarrhythmic intervention for arrhythmias caused by EADs and also by DADs, since the amplitude of the latter is a function of action potential duration.

Clearly, there are many reasons why pinacidil might not be a good antiarrhythmic agent. Among other problems, it will cause relaxation of vascular smooth muscle and decrease systemic arterial pressure. Further, its mechanism of action is not yet certain. Finally, since the pinacidil-induced current is time independent and since the intensity of action of pinacidil may depend on intracellular ATP concentration, there always will be a possibility of excessive shortening of the action potential and consequent weakening of contraction. In addition to the negative inotropic effect, an excessive shortening of action potential duration will cause a proportional decrease of refractoriness, and this action may increase the likelihood of certain types of arrhythmias. Tachyarrhythmias have been observed in dog Purkinje fibers superfused with high concentrations of pinacidil. Intravenous administration of pinacidil and cromakalim, another activator of Iₖ,ATP, to anesthetized dogs at doses reducing atrial refractory period by more than 20% and arterial pressure by 40% was accompanied by frequent episodes of supraventricular tachyarrhythmia when a premature stimulus was used to determine refractoriness. This arrhythmogenic effect resembles that of vagal stimulation. Contrasting results were obtained by Chi et al, who administered pinacidil to normal dogs and dogs studied 3–5 days after infarction induced by ligation of the left anterior descending coronary artery. They found only a trivial decrease in refractory period duration in both normal areas and areas damaged by ischemia, and in neither case was there any increase in the incidence or number of multiple responses induced by programmed stimulation. They did find, however, that pinacidil clearly increased the incidence of ventricular fibrillation when circumflex occlusion was superimposed on a preexisting anterior wall infarct. These results emphasize the fact that different arrhythmogenic mechanisms respond differently to antiarrhythmic intervention. Another study on the isolated ischemic rat heart showed that pinacidil and other agents enhancing Iₖ,ATP shortened the time to fibrillation from 30 to 15 minutes. However, in the presence of pinacidil, the heart rate was more than double the control rate; thus, the cause of the decreased time to fibrillation is difficult to determine.

In sum, although pinacidil brought about the desired changes in transmembrane action potentials in cells demonstrating EADs or DADs, it clearly is not a prototype of a new antiarrhythmic drug. For this reason, we evaluated the effects of increasing delayed rectifier current. Since this current develops slowly during the plateau, its increase is not likely to cause excessive shortening of the action potential and concomitant weakening of contraction. Further, results of earlier studies showed that a relatively selective increase in Iₖ brought about by exposure of single ventricular myocytes to isoproterenol after block of calcium channels by nisoldipine, caused a
significant decrease in action potential duration. The agent we chose to augment \( I_{K} \), PDBu, was not ideal; it lacked selectivity in many instances. Nevertheless, when changes in action potential duration and configuration suggested a predominant effect on the delayed rectifier, PDBu increased the current threshold for EADs by more than 70%, and this effect was accompanied by a significant increase in \( I_{K} \) tail amplitude (+44%).

Currently, much effort is being spent in the development of novel class III antiarrhythmic agents that specifically block \( I_{K} \). Thus, it might seem contradictory to advocate the development of agents that enhance outward current and, in particular, \( I_{K} \). However, we judge that both types of agents should have therapeutic usefulness in different types of arrhythmias. One might expect, for example, that class III agents, by prolonging refractoriness, would be effective in some reentrant arrhythmias. On the other hand, they might be ineffective or even arrhythmogenic in conditions in which a decrease of repolarizing current will facilitate the generation of afterdepolarizations. In contradistinction, agents that increase outward current during repolarization might provide a therapeutic advantage in treating arrhythmias caused by EADs, DADs, or abnormal automaticity. Recent results showing that low doses of pinacidil suppress cesium-induced arrhythmias in rabbits provide support for this hypothesis.

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KEY WORDS  •  pinacidil  •  ATP-regulated K+ current  •  early afterdepolarizations  •  delayed afterdepolarizations  •  abnormal automaticity  •  antiarrhythmic interventions
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Circ Res. 1991;68:1127-1137
doi: 10.1161/01.RES.68.4.1127

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