EXPERIMENTAL STUDY

Effects of Verapamil and Pinacidil on Extracellular K⁺, pH, and the Incidence of Ventricular Fibrillation during 60 Minutes of Ischemia

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Summary

Ca²⁺-channel antagonist verapamil and ATP-sensitive K⁺-channel opener pinacidil are known to decrease the rise in extracellular K⁺ ([K⁺]ₑ) level and pH (pHₑ) that occurs during reversible acute myocardial ischemia and to lessen the accompanying activation delay. Verapamil is also known to decrease the incidence of ventricular tachycardia (VT)/fibrillation (VF) during acute myocardial ischemia; however, the effects of ATP-sensitive K⁺-channel opener on the incidence of VT/VF are controversial. We studied, in an in vivo pig model, the effects of verapamil and pinacidil on the changes in [K⁺]ₑ, level and pHₑ, local activation, and the incidence of VT/VF during 60 minutes of ischemia. Thirty-one pigs were divided into 2 groups: a verapamil group (9 control pigs and 8 verapamil-treated pigs) and pinacidil group (5 control pigs and 9 pinacidil-treated pigs). In the verapamil group, VF developed in 1 of the 9 control pigs, whereas no VF developed in 8 verapamil-treated pigs. In the pinacidil group, VF developed in 1 of the 9 control pigs, whereas no VF developed in 8 verapamil-treated pigs. In the pinacidil group, VF developed in 3 of the 9 control pigs and all 9 pinacidil-treated pigs. Under verapamil treatment (versus the control condition), onset of the second rise in [K⁺]ₑ level was delayed, and the maximum rise in [K⁺]ₑ level was decreased. Under pinacidil treatment (versus the control condition), time to the onset of VT/VF was shorter than that under the control condition, and VT/VF developed at lower [K⁺]ₑ level and higher pHₑ.

In conclusion, VF may develop at a lesser [K⁺]ₑ rise and pHₑ fall in the presence of pinacidil during acute myocardial ischemia.

Key words: Myocardial ischemia

Ischemia-induced changes in the myocardial extracellular K⁺ ([K⁺]ₑ) level¹⁰ and extracellular pH (pHₑ)⁴⁻¹⁰ during the initial 8-15 minutes of acute myocardial ischemia have been well characterized in animal models. This period corresponds to the early phase of ventricular arrhythmia⁴ and to the phase of reversible myocardial cell injury.¹¹ We know that irreversible cell injury occurs after prolonged ischemia; however, the changes in [K⁺]ₑ level and pHₑ during the extended periods of ischemia have not been well characterized. A triphasic rise in [K⁺]ₑ level during prolonged ischemia has been reported;¹² however, this rise has not been characterized in detail. Specifically, the exact time course, magnitude, and regional characteristics of the changes in [K⁺]ₑ level and pHₑ during prolonged ischemia have not been studied systematically. In addition, the effects of pharmacological interventions on simultaneously measured changes in [K⁺]ₑ level and pHₑ during prolonged ischemia remain unclear, as is the incidence of ventricular tachycardia/fibrillation. Because the onset of irreversible cell injury occurs during the first 60 minutes of ischemia, this time period is of considerable interest.

Previously, we and other investigators described the use of ion-selective plunge electrodes in in situ pig heart to study the changes in myocardial [K⁺]ₑ level and pHₑ and local activation during brief periods of myocardial ischemia.²⁻⁵,¹³ We used a similar method to study the changes in myocardial [K⁺]ₑ level and pHₑ, and the effects of verapamil and pinacidil on these changes during 60 minutes of ischemia, and we report our findings herein.

Methods

Experimental preparation: Thirty-one domestic pigs of both sex and weighing 30-50 kg were used in the study. Animal care conformed to the Position of the American Heart Association on the Use of Research Animals and was in accordance with the accepted guidelines for the care and treatment of experimental animals at the University of North Carolina. Approval for the study was obtained from the University of North Carolina at Chapel Hill’s Institutional Animal Care and Use Committee. The
experimental procedure was similar to those previously reported. All 31 pigs were anesthetized with sodium pentobarbital (25 mg/kg), followed by α-chloralose as needed. Mechanical ventilation and supplemental oxygen were provided via an endotracheal tube and a Harvard respirator. Arterial blood gases were monitored, and appropriate ventilator adjustments were made for the maintenance of arterial PO2 at > 80 mmHg and pH at 7.35-7.45. Femoral artery and femoral venous catheters were used for monitoring the arterial blood pressure (Millar Pressure Transducer, Millar, Inc. Houston, TX, USA) and for administering fluids and drugs. Core temperature was continuously monitored with a temperature probe (Yellow Springs Instrument Co., Yellow Springs, OH, USA). Heating blankets were used to maintain the animals’ body temperature at 36-37°C. The heart was exposed by median sternotomy and suspended in a pericardial cradle. A site midway along the left anterior descending coronary artery (LAD) that was free of branches was selected for cannulation and dissected from the surrounding tissue. The epicardial margin between the ischemic and nonischemic tissue was identified by a brief occlusion of the vessel at this site, and 4-6 ion-selective electrode groups were placed at multiple locations at the center of the ischemic zone, defined as the region >10 mm inside the visible cyanotic border, and in the normal circulation (non-ischemic zone).

After electrode placement, systematic heparin (a bolus of 10,000 U followed by infusion at 2000 U/hour) was administered. A carotid artery-to-LAD shunt was created by placing a polyethylene catheter (Intramedic, ID 1.77 mm) in the aortic arch through the right carotid artery, routing the catheter through a Masterflex roller pump, and connecting the distal end to a cannula (Insul-Tab 7444 AWG 19, 0.070OD) that was placed in the LAD at the previously selected site. Placement of the shunt in the LAD took approximately 2-3 minutes. To ensure that the duration of ischemia during the shunt placement was consistent, the ischemia was maintained for a total of 5 minutes in each pig. Perfusion to the distal LAD was provided by the shunt and maintained at 1.2 mL/kg body weight/minute, which was previously known to yield a flow of 1.2-1.5 mL/g heart tissue/minute. Atrial pacing was used to increase the heart rate. Arterial blood pressure and a lead II electrocardiogram were recorded on a 12-channel Graphtec Linearcorder (Graphtec, Yokohama, Kanagawa, Japan) and were continuously monitored throughout the experiments.

**Ion-selective electrodes:** Ion-selective plunge electrodes were fashioned and calibrated as previously described. Briefly, one end of a Teflon-coated silver wire (0.007 in diameter) was chloridized by soaking it in sodium hypochlorite; it was then covered with a cellulose acetate-titanium dioxide sponge. K+- and H+-ion-sensitive electrodes were made by covering the sponge with a polyvinylchloride (PVC)-valinomycin- or PVC-tridodecylamine-based membrane. Reference electrodes were fashioned in an analogous manner but lacked the ion-selective membrane. A K+-sensitive electrode, H+-sensitive electrode, and reference electrode constituted an electrode group. Electrodes were calibrated before each experiment in the standard solution (3 and 10 mmol/L KCl for the K+-sensitive electrodes and pH 8 and pH 6 for the H+-sensitive electrodes). Only electrodes with a stable baseline drift (< 1 mV/hour) and 95%-105% of the predicted Nernstian slope (56-62 mV shift per decade change in K+ activity at room temperature) were used. The electrode group was threaded into a 20-gauge hypodermic needle, which was used to insert the electrodes into the midmyocardium to a depth of 4-6 mm. We placed 4-6 K+- and pH-sensitive electrodes in the ischemic midmyocardium. The *in vivo* performances of these electrodes were tested by the methods previously described. At the end of each experiment, the electrodes were removed from the heart and were retested *in vitro* to confirm their stable function throughout the experiment. The needle was then withdrawn, leaving the electrodes imbedded in the myocardium.

**Experimental protocol:** Pigs were randomly assigned to a verapamil group [control pigs (n = 9) or verapamil-treated pigs (n = 8)] or pinacidil group [control pigs (n = 5) or pinacidil-treated pigs (n = 9)]. In both groups, myocardial ischemia was induced by abrupt cessation of flow through the LAD shunt 50 minutes after cannulation of the LAD. Five% ethanol, a pinacidil solvent, were given as a placebo in the 5 pigs of the pinacidil group as the same speed as pinacidil administration pigs. Verapamil-treated pigs were administered an intravenous bolus of 0.2 mg/kg verapamil, infused over a 20-minute period beginning 35-40 minutes prior to the LAD occlusion. The verapamil bolus was followed by a maintenance infusion of 0.0065 mg/kg/hour. Pinacidil-treated pigs were administered 500 μM/L of pinacidil infused through the side arm of the shunt at a rate calculated to produce a concentration of 10 μM/L (n = 2) or 25 μM/L (n = 7) in the blood shunted to the LAD. Pinacidil infusion was initiated after 30 minutes of reperfusion and maintained for 20 minutes. In both the verapamil and pinacidil groups, right atrial pacing at 120 beats/second was initiated 10 minutes prior to the LAD occlusion and was maintained throughout the remainder of the experiment.

**Data collection and analysis:** Signals from all electrodes were individually amplified by high-impedance amplifiers. The amplified signals from all electrodes, along with the lead II electrocardiogram, were digitized with the use of an analog-to-digital converter (Phoenix Data) and were simultaneously sampled (1000 samples per second) every 15 seconds during myocardial ischemia and stored on a MicroVAX II/GPX computer (Digital Equipment Corporation, Maynard, MA, USA). [K+]e level and pH were calculated from the measured millivolt changes that were based on the calibration curve for each electrode, and the systemic [K+] level and pH were determined from the arterial blood sample obtained immediately before the event. An activity coefficient of 0.746 was used in calculating the [K+]e level. The number of acceptable electrodes in each experiment ranged from 3 to 5 for [K+] and from 2 to 3 for pH. [K+] level during the 60-minute ischemia period were monitored to assess the following: 1) the [K+] level during the plateau period, 2) the time to onset of the [K+] plateau, 3) the time to onset of the second rise in [K+] level, and 4) the maximum [K+] level attained. pH,
during the 60-minute ischemia period was monitored to determine 1) the maximum \( [K^+e] \) fall, 2) \( \text{pH}_e \) at the onset of the second rise in \( [K^+]e \) level at the same site in the myocardium, and 3) the minimum \( \text{pH}_e \) attained. In 3 pigs that were administered pinacidil, the transmembrane action potentials were recorded from the epicardial surface of the ischemic zone, as previously described.16,17

**Statistical analysis:** Values are presented as mean ± SEM unless otherwise indicated. Between-group differences in \( [K^+]e \) level and \( \text{pH}_e \) were analyzed by the Mann-Whitney U test or Fisher’s exact probability test, as appropriate. All statistical analyses were performed with StatView 5.0 software (SAS Institute, Cary, NC), and \( P < 0.05 \) was considered as statistically significant.

**Results**

Changes in \( [K^+]e \) level and \( \text{pH}_e \) that occurred in the center of the ischemic zone during the 60-minute occlusion period under the control condition during an experiment are presented in Figure 1. The \( [K^+]e \) trajectory was characterized by an initial, rapid rise during the first 5 minutes of ischemia, followed by a plateau phase during which \( [K^+]e \) level increased minimally. A second, slower rise in \( [K^+]e \) level then occurred after approximately 20 minutes of ischemia. In contrast to \( [K^+]e \), with its triphasic rise, \( \text{pH}_e \) revealed no transient plateau phase. \( \text{pH}_e \) fell rapidly during the first 5 minutes of ischemia and reached a plateau of approximately 5.80.

In the verapamil group, the \( [K^+]e \) level did not rise as quickly as it did in the control group, and the time to onset of the \( [K^+]e \) plateau and time to onset of the second rise in \( [K^+]e \) level were markedly longer in the verapamil group; however, there was no significant difference in the maximum rise in \( [K^+]e \) level between the verapamil and control groups (Table I). The fall in \( \text{pH}_e \) did not occur as rapidly in the verapamil group as it did in the control group, and neither the minimum \( \text{pH}_e \), nor the \( \text{pH}_e \) at the onset of the second rise in \( [K^+]e \) level differed significantly from their respective levels in the control group (Table II).

In the verapamil group, VF occurred in 1 of the 9 control pigs at 22 minutes of ischemia, and no VF occurred in the 8 verapamil-treated pigs. In the pinacidil group, VF occurred in 3 of the 5 (60%) pigs in the control group at 27.5, 24.0, and 33.6 minutes of ischemia, respectively, but VF occurred in all 9 pigs in the pinacidil group (100%) at 18.1 ± 2.6 (5.5-32.2) minutes of ischemia (\( P = 0.110 \)); however, the time to onset of VF was significantly shorter with pinacidil than with verapamil (\( P = 0.0332 \); Figure 2, Table III). The \( [K^+]e \) level during VF was significantly lower in the pinacidil group than in the control group (9.1 ± 0.41 versus 12.0 ± 0.88 mM, \( P = 0.0126 \), Table III). The \( \text{pH}_e \) during VF was higher in the pinacidil group than in the control group (6.99 ± 0.08 mM versus 6.29 ± 0.58, \( P = 0.0093 \), Table III). Epicardial action potentials were recorded from the ischemic zone in each of the 3 pinacidil-treated pigs, and action potential

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**Figure 1.** Time course of changes in the mid-myocardial extracellular \( [K^+]e \) (open symbols—left axis) and \( \text{pH}_e \) (closed symbols—right axis) recorded from the center of the ischemic zone following ligation of the left anterior descending coronary artery in the open-chested, anesthetized pigs.

**Table I.** Rise in \( [K^+]e \), During the 60-Minute Ischemia Period in the Control and Verapamil-Treated Pigs

<table>
<thead>
<tr>
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<th>Control</th>
<th>Verapamil treated</th>
<th>( P )</th>
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<tbody>
<tr>
<td>Plateau ( [K^+]e ), (mM)</td>
<td>9.8 ± 1.1</td>
<td>10.3 ± 1.3</td>
<td>0.420</td>
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<tr>
<td>Maximum ( [K^+]e ), (mM)</td>
<td>31.2 ± 7.7</td>
<td>25.2 ± 8.9</td>
<td>0.171</td>
</tr>
<tr>
<td>Onset of second ( [K^+]e ), rise (minutes)</td>
<td>9.8 ± 2.6</td>
<td>23.9 ± 5.9</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Onset of ( [K^+]e ), plateau (minutes)</td>
<td>6.0 ± 2.0</td>
<td>13.0 ± 4.0</td>
<td>&lt; 0.001</td>
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**Table II.** Fall in \( \text{pH}_e \), during the 60-Minute Ischemia Period under the Control and Verapamil Conditions

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Verapamil treated</th>
<th>( P )</th>
</tr>
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<tbody>
<tr>
<td>( \text{pH}_e ) at onset of second ( [K^+]e ), rise</td>
<td>6.20 ± 0.15</td>
<td>6.27 ± 0.22</td>
<td>0.469</td>
</tr>
<tr>
<td>Minimum ( \text{pH}_e )</td>
<td>5.67 ± 0.34</td>
<td>5.87 ± 0.30</td>
<td>0.120</td>
</tr>
<tr>
<td>( \text{pH}_e ) slope (pH unit/minute)</td>
<td>0.18 ± 0.07</td>
<td>0.10 ± 0.04</td>
<td>0.014</td>
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Figure 2. Effect of 25 μmol/L pinacidil on the rise in extracellular K⁺ ([K⁺]ₑ) and pH (pHe), and the action potential duration (APD) at 90% repolarization during ischemia. Notably, ventricular fibrillation (VF) occurred at 18 minutes 37 seconds after cessation of the coronary flow and APD just before the occurrence of VF was markedly shortened.

**Discussion**

Our core study findings were as follows: under verapamil treatment, the time to onset of the [K⁺]ₑ plateau and time to onset of the second rise in [K⁺]ₑ level were markedly delayed during the 60-minute ischemia period, and the time to the fall in pH was markedly increased; however, no change was observed in the maximum rise in [K⁺]ₑ level or the minimum pH. In addition, no VF occurred. Under pinacidil treatment, however, VF occurred in all pigs. In addition, the [K⁺]ₑ level was lower, the VF occurred sooner, and the pH, at the time of VF occurrence was higher than that under the control condition.

**Reported effects of verapamil:** Fleet et al. reported that verapamil reduced the rise in [K⁺]ₑ level and fall in pH after LAD occlusion in the center and margin of the ischemic zone. Using an isolated papillary muscle preparation, Cascio et al. reported that verapamil also postpones cell-to-cell uncoupling, the secondary rise in [K⁺]ₑ level, and the ischemic contracture. Johnson et al. reported that verapamil lowers the rate at which the inhomogeneities in change in [K⁺]ₑ level develop. Whether these observations represent an important mechanism underlying the known antifibrillatory effect of verapamil cannot be determined because the calcium channel blockade exerts several additional effects that may be of equal or greater significance. These include suppression of action potentials dependent on the calcium inward current, preservation of energy stores, and mitochondrial protection. In the present study, we did not record action potentials during ischemia. A previous report revealed that verapamil enhanced the ischemia-induced action potential shortening in vitro.

**Reported effects of pinacidil:** We previously reported that 10 μmol/L pinacidil caused a slight but significant lessening in the rise in [K⁺]ₑ level in an *in vivo* pig model of ischemia and that with 25 μmol/L pinacidil, the rise in duration (APD) at 90% repolarization before the occurrence of VF was 130, 80, and 80 msec, respectively (Table III). Representative changes in [K⁺]ₑ level and pH during ischemia from a control, a 10 μM/L pinacidil-treated pig, and a 25 μM/L pinacidil-treated pig are presented in Figures 3, 4. VF did not develop in one control pig during 60 minutes of ischemia. Despite a similar rise in [K⁺]ₑ level and pH, fall between the control and 10 μM/L pinacidil-treated pig, VF occurred in the pinacidil-treated pigs at 13 minutes of ischemia, and VF occurred at 32.2 minutes of ischemia despite the lesser rise in [K⁺]ₑ level and lesser fall in pH, in the 25 μM/L pinacidil-treated pig.
pinacidil affected the rise in [K⁺]e level and pH e fall only in pigs because we had previously reported that 10 μmol/L pinacidil. We then tested 10 μmol/L pinacidil in 2 minutes of ischemia. VF occurred in all 7 pigs treated with 25 μmol/L pinacidil, the rise in [K⁺]e level was significantly less than that under the control condition. In the pinacidil group, the fall in pH e after administration of 25 μmol/L was less than that anticipated; however, with both 10 μmol/L and 25 μmol/L pinacidil in the pig and rabbit models of ischemia, APD was significantly shortened (in comparison to that under control conditions). In these experiments, the ischemia time was limited to 6-8 minutes, and the incidence of arrhythmias was not assessed. Therefore, the effects of pinacidil on the incidence of VF during ischemia were controversial; Chi et al. demonstrated the proarrhythmic actions of pinacidil in a canine model of sudden coronary death; Wolleben et al. revealed that the time to onset of serious ventricular rhythm disturbances was shortened; Vegh et al. reported that levocromakalim did not significantly modify the arrhythmia severity during ischemia; and D’Alonzo et al. revealed potential antiarrhythmic activity during ischemia. The discrepant results may be due to 1) drug dosage, 2) route of drug administration, 3) drug specific effect, and 4) experimental design (in vivo versus in vitro, use of different animal species, and different ischemia duration). In the present study, VF occurred at a mean time of 18.1 ± 2.6 minutes of ischemia in the pinacidil group associated with marked shortening of APD. Therefore, it is possible that pinacidil may indicate proarrhythmic effects during acute myocardial ischemia through marked shortening of the APD despite the lesser rise in [K⁺], level and lesser pH e fall.

**Study limitations:** The number of experiment in each group was small, and the action potential was recorded from only 3 experiments in the pinacidil group; moreover, the activation time from the ischemic myocardium was not evaluated. Furthermore, verapamil was administered intravenously and pinacidil was administered directly from the coronary artery, and the pinacidil dose was higher than the clinically effective concentration (1-2 μmol/L). Therefore, the effects of pinacidil on the rise in [K⁺], level and pH e fall and the incidence of ventricular arrhythmias during acute myocardial ischemia at the clinically relevant dose remain unclear.

**Conclusion**

VF may develop at a lesser rise in [K⁺], level and pH e fall in the presence of pinacidil during acute myocardial ischemia.

**Disclosures**

**Conflicts of interest:** The authors declare no conflict of interest.
Figure 3. Representative experiments from a control (closed circle), a 10 μmol/L pinacidil-treated pig (open triangle), and a 25 μmol/L pinacidil-treated pig (open circle) on the rise in extracellular K⁺ ([K⁺]e) after cessation of the coronary flow. Notably, the rise in [K⁺]e level in a 10 μmol/L pinacidil-treated pig was similar to that in the control pig, but ventricular fibrillation occurred at 13 minutes of ischemia. The rise in [K⁺]e level in the 25 μmol/L pinacidil-treated pig was less than that in the control group, but ventricular fibrillation occurred at 32.2 minutes of ischemia.

Figure 4. Same experiment as Figure 3. Notably, the fall in pHₑ in a 10 μmol/L pinacidil-treated pig was similar to that in the control pig, but the fall in pHₑ in the 25 μmol/L pinacidil-treated pig was less than that in the control group. Closed circle indicates control pig; open triangle, 10 μmol/L pinacidil pig; and open circle, 25 μmol/L pinacidil-treated pig.
References

28. Watanabe I, Gettes LS. Effects of pinacidil on ST-T wave alternans during acute myocardial ischemia in the in-situ pig heart. Accepted for publication in Journal of Nihon University Medical Association.