Original Article

Contribution of Mechanical Activity to Extracellular K^+ and H^+ Accumulation in Ischemic Porcine HeartIchiro WATANABE¹⁾ and Leonard S. GETTES²⁾¹⁾ Division of Cardiology, Department of Medicine, Nihon University School of Medicine²⁾ Division of Cardiology, Department of Medicine, University of North Carolina at Chapel Hill

Myocardial ischemia induces an increase in extracellular K^+ ($[K^+]_e$) and a decrease in extracellular pH (pH_e). To clarify the role of mechanical and electrical activities in these changes, we investigated the effect of 2, 3-butanedione (BDM), a specific inhibitor of actin-myosin coupling, and pinacidil, an ATP-sensitive K^+ channel opener, on $[K^+]_e$, pH_e , action potential duration (APD) and regional contractility during 8 minutes of ischemia in porcine hearts. We inserted K^+ - and pH-sensitive electrodes and a pair of ultrasonic crystals into the mid-myocardium. We also placed a floating microelectrode in the subepicardium of the ischemic zone. The carotid artery was shunted to the LAD through a roller pump, and BDM (10 mM, $n = 7$) or pinacidil (50 μ M, $n = 8$) was infused to the LAD shunt in advance of no-flow ischemia. Regional systolic shortening became dyskinetic with the BDM infusion and decreased from $20.1 \pm 1.6\%$ to $4.9 \pm 1.6\%$ with pinacidil. APD was not affected by BDM, but it decreased from 348 ± 14 ms to 206 ± 14 ms ($P < 0.001$) with the infusion of pinacidil prior to ischemia. Both pinacidil and BDM attenuated the rise in $[K^+]_e$, but the effect of pinacidil was greater. Both agents reduced the fall in pH_e , but this effect was greater with BDM. Pinacidil-induced APD shortening was maintained during ischemia; BDM did not affect APD shortening during ischemia. The fall in pH_e during ischemia appears to be related mainly to mechanical activity but the corresponding increase in $[K^+]_e$ is more attributable to APD.

Key words: Myocardial ischemia, Extracellular K^+ , Extracellular pH, Pinacidil, 2,3-butanedione, action-potential duration

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Acute myocardial ischemia causes rapid depletion of energy-rich substrates¹⁻³⁾, an increase in extracellular K^+ ($[K^+]_e$)⁴⁻⁹⁾, and a decrease in extracellular and intracellular pH¹⁰⁻¹³⁾. The rise in $[K^+]_e$ has been attributed to enhancement of K^+ conductance via ATP-sensitive K^+ channels¹⁴⁻¹⁷⁾, anion-coupled K^+ loss¹⁸⁾, activation of Na^+ -activated or arachidonic acid-activated K^+ channels¹⁹⁾, suppression of the Na^+ pump²⁰⁾, and shrinkage of extracellular space²¹⁾. Intracellular pH (pH_i) decreases as protons are produced during hydrolysis of adenosine triphosphate (ATP) and accumulate during production of lactic acid. Likewise, extracellular pH (pH_e) decreases as intracellular protons move to the extracellular compartment by Na^+/H^+ exchange, Na^+/HCO_3^- cotransport, lactate transport, and lactic acid and CO_2 diffusion²²⁾. Changes in $[K^+]_e$ were shown previously to be reproducible by the third brief ischemic event, but changes in pH_e decreased progressively with each subsequent ischemic episode²³⁾. The rise in $[K^+]_e$ was shown to be caused by multiple factors, including K^+ conductance across the membrane channels, K^+ driving force, which is reflected by the time that the action potential remains at its plateau voltage, and the

metabolic effects of ischemia²⁴⁾. We studied effect of pinacidil, an ATP-sensitive K^+ channel opener, and that of 2,3-butanedione monoxime (BDM), a specific inhibitor of actin-myosin coupling, during 8-minute occlusion of the left anterior descending coronary artery (LAD) in an open-chest swine model. Our goal was to clarify the role of electrical and mechanical activities in the changes in $[K^+]_e$ and pH_e during the early phase of myocardial ischemia.

Methods

Experimental preparation: Fifteen domestic pigs of either sex and weighed 30–50 kg, were used in the study. Care of the animals conformed to the Position of the American Heart Association on the Use of Research Animals and was in accordance with accepted guidelines for the care and treatment of experimental animals at the University of North Carolina at Chapel Hill. Approval for the study was obtained from the University of North Carolina at Chapel Hill's Institutional Animal Use and Care Committee. The experimental procedure was similar to that reported previously (Fig. 1)²⁵⁾. The 15 animals were

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Fig. 1 Schematic of the experimental procedure. The carotid artery is shunted to the left anterior descending coronary artery through a roller pump, and 4–6 K⁺- and pH-sensitive electrodes are inserted into the mid-myocardium of the ischemic zone. A pair of ultrasonic crystals are placed in the ischemic zone. A floating glass microelectrode is inserted into the epicardium of the ischemic zone.

BDM: 2,3 butanedione monoxime, LCX: left circumflex coronary artery, LVP: left ventricular pressure.

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 maintenance of arterial PO₂ at > 80 mmHg and pH at 7.35–7.45. Femoral artery and femoral venous catheters were used for monitoring 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 blanket was 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 ischemic and non-ischemic tissue was identified by brief occlusion of the vessel at this site, and 4–6 ion-selective unipolar and bipolar electrode groups were placed at multiple locations in 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 2,000 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, 0.07OD) 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 shunt placement was consistent, the ischemia was maintained for a total of 8 minutes in each animal²⁶. Perfusion to the distal LAD was provided by the shunt and maintained at 1.2 mL/kg body weight/min, which was previously shown to provide 1.2–1.5 mL/g heart tissue/minute²⁷. Atrial pacing was used to increase heart rate. Arterial blood pressure and a lead II electrocardiogram were recorded on a 12-channel Graphtec Linearcorder (Graphtec, Yokohama, Kanagawa, Japan) and monitored continuously throughout the experiments.

Ion-selective electrodes: Ion-selective plunge electrodes were fashioned and calibrated as described previously²⁸. 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⁺ ion- and H⁺ ion-sensitive electrodes were made by covering the sponge with a polyvinyl chloride (PVC)-valinomycin- or PVC-tridodecylamine-based membrane. Reference electrodes were fashioned in an analogous manner but lacked the ion-selective membrane. A K⁺- or H⁺-sensitive electrode plus a reference electrode constituted 1 electrode group. Electrodes were calibrated before each experiment in standard solution (3 and 10 mmol/L KCl for the K⁺-sensitive electrode and pH 8 and pH 6 for the H⁺-sensitive electrode). Only electrodes with a stable baseline drift (< 1 mV/hour) and 95–105% of the predicted Nernstain slope (56–62 mV shift per decade change in K⁺ activity at room temperature) were used. We placed 4–6 K⁺- and

pH-sensitive electrodes in the ischemic mid-myocardium. The in vivo performances of these electrodes were tested by methods described previously²⁷⁾. At the end of each experiment, the electrodes were removed from the heart and retested in vitro to confirm stable function throughout the experiment. The electrode group was threaded into a 19-gauge hypodermic needle, which was used to insert the electrodes into the mid-myocardium to a depth of 4–6 mm. The needle was then withdrawn, leaving the electrodes imbedded in the myocardium. Four to 6 electrode groups were used in each experiment.

Microelectrodes: Transmembrane potentials in the subepicardial tissue layers were recorded with micropipettes having a flexible, long tip; the micropipettes were pulled with a standard microelectrode puller and filled with 3M KCl solution. Tip resistance was in the range of 15–35 M Ω . The electrode was mounted on a tungsten wire (0.002 diameter), and the wire was connected to a high-input resistance buffer amplifier. The microelectrode had a reference electrode consisting of silver/silver chloride wire placed as close as possible to it. Signals were differentially DC-amplified, continuously monitored on an oscilloscope, and recorded (Graphtec Linearcorder) at 50 mm/second.

Myocardial segment length: A pair of ultrasonic crystals, 8–15 mm apart, were placed 6 ± 2 mm below the epicardial surface of the left ventricular wall in the center of the ischemic zone.

Experimental protocol: Fifty minutes after cannulation of the LAD, the first in a series of myocardial ischemia events was induced by abrupt cessation of flow through the LAD shunt. We first induced two 8-minute ischemic episodes because our earlier studies showed that the increase in $[K^+]_e$ in the first ischemic episode differed from increases that occurred during subsequent ischemic episodes²³⁾. Second ischemia was defined as control ischemia. We then infused 500 μ M/L pinacidil or 100 mmol/L BDM through the sidearm of the shunt at a rate calculated to produce a pinacidil concentration of 50 μ M/L or BDM concentration of 10 mmol/L in blood shunted to the LAD²⁴⁾. The pinacidil infusion was initiated after 30 minutes of reperfusion and maintained for 20 minutes, and the BDM infusion was initiated after 40 minutes of reperfusion and maintained for 10 minutes, at which time coronary flow was discontinued for 8 minutes (Pinacidil or BDM ischemia).

Data collection and analysis: Signals from all electrodes were individually amplified by high-impedance amplifiers. The amplified signals from all electrodes, along with a lead II electrocardiogram, were digitized

(Phoenix Data analog-to-digital converter) and simultaneously sampled (1,000 samples per second) every 15 seconds during myocardial ischemia and stored on a Micro-VAX II/GPX computer (Digital Equipment Corporation, Maynard, MA, USA). $[K^+]_e$ and pH_e values were calculated from measured millivolt changes that were based on the calibration curve for each electrode and the systemic $[K^+]$ and pH determined from the arterial blood sample obtained immediately before the event. An activity coefficient of 0.746 was used in calculating the $[K^+]_e$ concentration. The number of acceptable electrodes in each experiment ranged from 3 to 5 for $[K^+]_e$ and from 2 to 3 for pH_e . Action potential duration (APD) was measured manually at 90% (APD₉₀) repolarization. End-diastolic length (EDL) and end-systolic length (ESL) were measured as described by Theroux et al.,²⁹⁾ and shortening was expressed as a percentage (% Δ L) calculated as $(EDL - ESL) \times 100/EDL$.

Statistics: Values are shown as mean $\pm$ SEM unless otherwise indicated. Between-group differences in serum K^+ , arterial pH, heart rate, pH_e , $[K^+]_e$, % Δ L, and APD₉₀ during control and post pinacidil ischemia, were analyzed by Wilcoxon signed-rank test or Mann-Whitney U test. All statistical analyses were performed with Stat View 5.0 (SAS Institute, Cary, NC, USA), and $P < 0.05$ was considered significant.

Results

Eight-minute no-flow ischemia was achieved in all 15 animals, and no ventricular fibrillation occurred. The pre-ischemia serum K^+ concentration, arterial pH, and heart rate did not differ from the corresponding values observed during control ischemia and post-pinacidil ischemia (K^+ : 3.84 ± 0.22 vs. 3.93 ± 0.40 mM; arterial pH: 7.41 ± 0.04 vs. 7.42 ± 0.03 ; heart rate: 112 ± 16 vs. 113 ± 18 /minute). Serum K^+ , arterial pH, and heart rate before ischemia did not differ in control ischemia as compared with post-pinacidil ischemia (3.57 ± 0.17 vs. 3.53 ± 0.23 mM, 7.38 ± 0.03 vs. 7.40 ± 0.03 , 104 ± 13 vs. 103 ± 14 /minute, respectively). With pinacidil administration, % Δ L decreased from $20.1 \pm 1.6\%$ to $4.9 \pm 1.6\%$ ($P < 0.0001$), and with BDM administration, % Δ L decreased from 23.8 ± 2.2 to $-1.2 \pm 1.3\%$ ($P < 0.0001$). Also with BDM administration, serum Ca^{2+} decreased from 1.537 ± 0.04 to 1.020 ± 0.05 mM ($P < 0.05$).

Extracellular K^+ : No change was seen in $[K^+]_e$ or pH_e with administration of pinacidil or BDM before the induction of ischemia. Changes in $[K^+]_e$ that occurred every 30 seconds with pinacidil administration during the control and post-pinacidil occlusions are shown in Fig. 2A. With pinacidil administration, the rise in $[K^+]_e$ decreased significantly from 2 to 8 minutes of ischemia ($P < 0.01$).

Fig. 2A Effect of pinacidil on the increase in extracellular potassium ($[K^+]_e$) during 8 minutes of no-flow ischemia. Values are mean $\pm$ SEM. $**P < 0.01$, $^\dagger P < 0.001$.

Fig. 2B Effect of pinacidil on the decrease in extracellular pH (pH_e) during 8 minutes of no-flow ischemia. Values are mean $\pm$ SEM. [Dashed line indicates changes in pH_e anticipated during the subsequent episodes of ischemia induced in the absence of the drug (derived from earlier studies under identical control conditions; see Reference 23). $*P < 0.05$, $^\dagger P < 0.001$.

Fig. 2C Effect of pinacidil on the changes in action potential duration at 90% repolarization (APD_{90}) during 8 minutes of no-flow ischemia. Values are mean $\pm$ SEM. $**P < 0.01$, $^\dagger P < 0.001$.

Fig. 3A Effect of 2,3 butanedione monoxime (BDM) on the increase in extracellular potassium ($[K^+]_e$) during 8 minutes of no-flow ischemia. Values are mean $\pm$ SEM. $*P < 0.05$, $**P < 0.01$, $^\dagger P < 0.001$.

Fig. 3B Effect of 2,3 butanedione monoxime (BDM) on the decrease in extracellular pH (pH_e) during 8 minutes of no-flow ischemia. Values are mean $\pm$ SEM. [Dashed line indicates changes in pH_e anticipated during the subsequent episodes of ischemia induced in the absence of the drug (derived from earlier studies under identical control conditions; see Reference 23). $*P < 0.05$, $**P < 0.01$, $^\dagger P < 0.001$.

Fig. 3C Effect of 2,3 butanedione monoxime (BDM) on the changes in action potential duration at 90% repolarization (APD_{90}) during 8 minutes of no-flow ischemia. Values are mean $\pm$ SEM. $*P < 0.05$.

The effects of BDM on the changes in $[K^+]_e$ that occurred during control and post-BDM occlusions are shown in Fig. 3A. With BDM administration, the rise in $[K^+]_e$ decreased significantly from 1 minute 30 seconds to 8 minutes of ischemia.

Extracellular H^+ : The effects of pinacidil on the changes in pH_e that occurred every 30 seconds during the control and post-pinacidil occlusions are shown in Fig. 2B. With pinacidil administration, the fall in pH_e significantly decreased beyond that expected solely as a result of serial occlusions²³⁾ from 2.5 minutes to 8 minutes of ischemia. The effects of BDM on the changes in pH_e that occurred every 30 seconds during the control and post-pinacidil occlusions are shown in Fig. 3B. With BDM administration, the fall in pH_e decreased significantly beyond that expected solely as a result of serial occlusions²³⁾ from 3 to 8 minutes of ischemia.

Epicardial APD: The minute-by-minute changes in APD_{90} during control and post-pinacidil occlusions are shown in Fig. 2C. With pinacidil administration, the APD_{90} decreased significantly from 348.3 ± 8.1 to 206.4 ± 14.0 ms before ischemia and throughout the ischemic event. The minute-by-minute changes in APD_{90} that occurred during the control and post-BDM occlusions are shown in Fig. 3C. With BDM administration, the APD_{90} did not change before the ischemia (350.8 ± 41.2 vs. 339.2 ± 38.0 ms, $P = 0.0684$), but the APD_{90} was significantly increased at 4 minutes of ischemia (269 ± 20 ms vs. 317 ± 39 ms, $P < 0.05$).

Comparison of pinacidil and BMD on changes in $[K^+]_e$, pH_e , and APD_{90} during ischemia: Effects of pinacidil and BDM on the changes in $[K^+]_e$, pH_e , and APD_{90} at 4 and 8 minutes of ischemia are shown in Table I. The increase in $[K^+]_e$ was significantly smaller at 8 minutes of ischemia after pinacidil administration than that after BDM administration. The decrease in pH_e was significantly smaller at 4 minutes of ischemia after BDM

administration than after pinacidil administration. The APD_{90} at both 4 and 8 minutes of ischemia was significantly shorter after pinacidil administration than after BDM administration.

Discussion

Our study revealed a marked pinacidil-induced decrease in ADP_{90} before the initial and during the subsequent ischemic events but that BDM had no effect on ADP_{90} before or during ischemia. Regional myocardial contraction, measured by changes in myocardial segment length, was depressed by pinacidil and became dyskinetic in the presence of BDM. The rise in $[K^+]_e$ during ischemia was lessened by pinacidil and BDM, but the degree of lessening was significantly greater with pinacidil. The decrease in pH_e was lessened by both pinacidil and BDM, but a significantly greater degree of lessening occurred with BDM than with pinacidil.

The increase in $[K^+]_e$ during ischemia has been attributed to enhancement of K^+ conductance via ATP-sensitive K^+ channels¹⁴⁻¹⁷⁾, anion-coupled K^+ loss¹⁸⁾, activation of Na^+ -activated or arachidonic acid-activated K^+ channels¹⁹⁾, suppression of the Na^+ pump²⁰⁾, and shrinkage of the extracellular space²¹⁾. We have shown that the increase in $[K^+]_e$ is due to multiple factors, including K conductance across the cell membrane channels, K driving force which is reflected by the amount of time at which the action potential is at its plateau voltage, and the metabolic effects of ischemia on the cell membrane²²⁾. We have proposed that the unanticipated lessening of the increase in $[K^+]_e$ by the ATP-sensitive K^+ channel opener, pinacidil reflects the balance of its effects on these several parameters. In the present study, the more significant lessening of the rise in $[K^+]_e$ induced by pinacidil vs. BDM further supports the notion that the increase in $[K^+]_e$ during ischemia is determined in part by the amount of time at which action potential is at its plateau voltage. The fact that BDM lessened the fall in pH_e during ischemia more than did pinacidil might be explained in part by the metabolic protection afforded by BDM, due to complete suppression of

Table I Changes in $[K^+]_e$, pH_e , and APD_{90} After 4 and 8 minutes of Ischemia in the Control Conditions and Under Pinacidil and BDM Administration

	Control ischemia		Pinacidil ischemia		Control ischemia		BDM ischemia	
	(n = 8)		(n = 8)		(n = 7)		(n = 7)	
	4 min	8 min	4 min	8 min	4 min	8 min	4 min	8 min
$[K^+]_e$ (mM)	7.0 ± 1.5	10.4 ± 2.1	$4.7 \pm 0.7^\dagger$	$5.9 \pm 1.0^{\dagger, \ddagger}$	7.3 ± 1.0	9.3 ± 1.1	$4.9 \pm 0.7^\dagger$	$7.3 \pm 0.8^*$
pH_e	7.11 ± 0.07	6.96 ± 0.09	$7.26 \pm 0.08^{\dagger, \ddagger}$	$7.18 \pm 0.07^\dagger$	7.12 ± 0.08	6.89 ± 0.07	$7.35 \pm 0.13^{**}$	$7.24 \pm 0.08^\dagger$
APD_{90} (msec)	270 ± 14	250 ± 12	$139 \pm 13^{\dagger, \ddagger}$	$113 \pm 21^{\dagger, \ddagger}$	269 ± 20	255 ± 15	$317 \pm 39^*$	273 ± 29

Mean $\pm$ SD values are shown.

BDM: 2, 3-butanedione

$[K^+]_e$: extracellular K^+ , pH_e : extracellular pH, APD_{90} : action potential duration at 90% repolarization.

* $P < 0.05$ vs. control ischemia, ** $P < 0.01$ vs. control ischemia, $^\dagger P < 0.001$ vs. control ischemia.

$^\ddagger P < 0.05$ vs. BDM ischemia, $^{**} P < 0.01$ vs. BDM ischemia.

contraction before ischemia. BDM produces dyskinesia by decreasing myofibrillar sensitivity to calcium, and intracoronary BDM has been shown to decrease infarct size after reperfusion³⁰. However, regional oxygen consumption has been shown to persist in dyskinetic canine myocardium³¹. Furthermore, rapidly activated delayed rectifier current and slowly activated delayed rectifier current are significantly blocked by BDM at concentrations needed for excitation-contraction decoupling³². In the present study, BDM did not affect APD before ischemia, and it slightly reduced shortening of the APD during ischemia. This effect may be related to suppression of activation of ATP-sensitive K⁺ channels during ischemia.

Therefore, the effect of any drugs on the increase in [K⁺]_e during ischemia will be determined by 1) the balance of its effects on K⁺ conductance via the various voltage- and ligand-gated K⁺ channels, 2) K⁺ driving force which is reflected by the time during which the left ventricular action potential is at its plateau voltage, and 3) the metabolic changes by acute myocardial ischemia.

Study limitation

In the present study, we used regional administration of high-dose of pinacidil and BDM as reagent to investigate the pathophysiologic mechanism of action potential duration and regional contraction on the rise in [K⁺]_e and the fall in pH_e during acute myocardial ischemia. Therefore, further studies are needed to determine the cardioprotective effects of ATP-sensitive K⁺ channel activator and E-C uncoupling drug in the clinically relevant setting experiments.

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