Reduction of ischemia-induced electrophysiologic abnormalities by glucose-insulin infusion

ARTICLE *in* JOURNAL OF THE AMERICAN COLLEGE OF CARDIOLOGY · OCTOBER 1993

Impact Factor: 16.5 · DOI: 10.1016/0735-1097(93)90440-C · Source: PubMed

JACC Vol. 22, No. 4
October 1993:1214–22

Reduction of Ischemia-Induced Electrophysiologic Abnormalities by Glucose-Insulin Infusion

SOAD BEKHEIT, MD, PHD, FACC, NIDAL ISBER, MD, HARISH JANI, MD, GHAZWAN BUTROUS, MD, PHD, MOHAMED BOUTJDIR, PHD, NABIL EL-SHERIF, MD, FACC

Brooklyn, New York

Objectives. **This study was designed to determine the effects of glucose-insulin infusion on ischemia-induced changes in extracellular potassium ($[K^+]_o$) accumulation and the associated electrophysiologic abnormalities in the canine heart.**

Background. **Although glucose-insulin-potassium infusion has been shown to limit myocardial injury in acute ischemia, its effect on ischemia-induced electrophysiologic alterations has not been investigated.**

Methods. **Recordings of $[K^+]_o$ and local electrograms from the normal, border and ischemic zones were obtained during serial (10-min) left anterior descending coronary artery occlusions in the control state and after infusion of glucose-insulin (eight dogs), glucose alone (six dogs) or insulin alone (eight dogs).**

Results. **Glucose-insulin infusion caused significant reduction in the rise of $[K^+]_o$ during the entire period of ischemia in both ischemic and border zones associated with significant improvement in the degree of intramyocardial conduction delay. At 10 min of ischemia, $[K^+]_o$ was reduced from a mean control level of 15.9 ± 3.7 to 10.1 ± 4.3 mmol/liter ($p < 0.005$) in the ischemic zone and from 6.8 ± 1.9 to 5.5 ± 1.1 mmol/liter ($p < 0.05$) in the border zone. The electrogram duration was shortened from a mean control value of 102 ± 13 to 78 ± 12 ms in the ischemic zone and from 79.2 ± 7.8 to 58.1 ± 6.6 ms in the border zone ($p < 0.005$). Glucose alone caused significant reduction in $[K^+]_o$ during the initial 6 min of ischemia, only in the ischemic zone. Conversely, insulin caused no changes in $[K^+]_o$ accumulation during ischemia. Neither glucose nor insulin alone had any effect on ischemia-induced intramyocardial conduction delay.**

Conclusions. **The present study demonstrated that the combination of glucose and insulin is essential for the salutary effect of reducing $[K^+]_o$ accumulation during ischemia and improving the associated intramyocardial conduction delay. It could be postulated that glucose in the presence of insulin increases the glycolytic flux, thereby providing adequate adenosine triphosphate for suppressing the cardiac adenosine triphosphate–sensitive potassium ion channels. The latter are, at least partially, responsible for the $[K^+]_o$ rise in the early phase of ischemia. This study highlights the antiarrhythmic potential of interventions that modulate the metabolic consequences of ischemia.**

(J Am Coll Cardiol 1993;22:1214–22)

The beneficial effects of glucose-insulin-potassium infusion in the treatment of acute myocardial infarction remain controversial (1–7) since it was initially advocated by Sodi-Pallares et al. (1) >25 years ago. Previous clinical studies have suggested that glucose-insulin infusion exerts its beneficial effects during ischemia by limiting the extent of mitochondrial damage and infarction size, thereby decreasing electrical instability and ventricular arrhythmias (3,4,8–10). Experimental studies on the mechanism of the cardioprotective effect of glucose-insulin infusion have been limited to biochemical, structural or basic electrophysiologic changes (3,8–12). It was reported that glucose-insulin-potassium significantly reduced the intracellular loss of potassium ions (K^+) and restored the potassium/sodium ratio in the ischemic myocardium (9). The effects of glucose-insulin infusion on the accumulation of extracellular K^+ ($[K^+]_o$) has never been tested during acute ischemia in the intact heart. Because cellular loss of K^+ and its extracellular accumulation are thought to contribute to the electrophysiologic changes that underlie malignant ventricular arrhythmias in the early phase of acute ischemia, we decided to study the effects of glucose-insulin infusion on ischemia-induced changes in extracellular $[K^+]_o$ and associated electrophysiologic abnormalities. Some of the data have been published in a preliminary report (13).

Methods

A total of 24 adult mongrel dogs weighing 13 to 16 kg were studied under sodium pentobarbital anesthesia (30 mg/kg body weight intravenously); supplemental doses were ad-

From the Cardiology Division, Department of Medicine, State University of New York, Health Science Center and Veterans Administration Medical Center, Brooklyn, New York. This study was supported in part by Grant RO1 HL 36680 from the National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland; by Veterans Affairs Medical Research Funds, Department of Veterans Affairs, Washington, D.C. and by a grant from Hoechst-Roussel Pharmaceuticals Inc., Somerville, New Jersey.

Manuscript received December 5, 1992; revised manuscript received March 22, 1993, accepted April 12, 1993.

Address for correspondence: Soad Bekheit, MD, Cardiology Section, Veterans Administration Medical Center, 800 Poly Place, Brooklyn New York 11209.

0735-1097/93/$6.00

ministered as required. The experimental techniques are described in detail elsewhere (14). The dogs were ventilated with 100% oxygen through an endotracheal tube by means of a positive-pressure respirator (Harvard Apparatus). Electrocardiographic (ECG) leads I and III and arterial pressure were monitored continuously on a multichannel physiologic recorder VR12 (Electronics for Medicine, PPG Biomedical Systems). A femoral vein was cannulated for intravenous infusion. The heart was exposed through a left thoracotomy and cradled in the opened pericardium. The left anterior descending coronary artery was isolated approximately 1 cm distal to its bifurcation. A snare of 1-0 silk suture was positioned around the artery distal to the first diagonal branch.

Extracellular potassium activity measurements. Measurements of extracellular potassium ion ($[K^+]_o$) activity were made with a flexible K^+-sensitive valinomycin electrode (model 201-KRPW, Biosensors Inc., Electrochemical Devices for Biology and Medicine), as previously described (14,15). Each electrode consisted of a K^+-sensitive terminal and a reference terminal glued together with cyanoacrylate. The K^+-sensitive electrodes were tested in vitro for linearity and response time before each experiment. The calibration was made with K^+ concentrations of 3, 10 and 20 mmol/liter at 37°C. The K^+ electrodes were connected to a high input impedance preamplifier and filtered at 2 Hz. Only those electrodes that had a calibration slope of 55 to 61 mV/decade change in K^+ activity and a time constant of 35 to 50 ms were used. Response time of K^+-sensitive electrodes was tested and calibrated in situ by a rapid intravenous injection of 2 mEq potassium chloride at the beginning of each experiment. Electrodes that failed to record a transient rise in $[K^+]_o$ and those that showed a baseline shift of >0.5 mmol/liter per min of K^+ during the stabilization period were discarded and replaced. At the end of each experiment, all K^+-sensitive electrodes were retested and calibrated to ensure accuracy of measurements.

Placement of K^+-sensitive electrodes. A brief (30 s) occlusion of the left anterior descending coronary artery was performed to delineate the cyanotic ischemic zone and the normally perfused (nonischemic) zone. A border zone was defined as a 5-mm region extending from the cyanotic border into the ischemic zone. A minimum of four electrodes were positioned in the border zone, spaced 5 mm apart. Two to three electrodes were positioned in the central ischemic zone, at least 10 mm from the border zone electrodes. One to two electrodes were also positioned in the normal zone 10 mm outside the cyanotic border. All electrodes were positioned in the midmyocardium approximately 4 to 6 mm from the epicardial surface by means of a 20-gauge needle specially marked to calculate the depth of insertion. The $[K^+]_o$ activity was calculated using the Nernst equation, $E = E^\circ + RT/nF \ln a_K$, where E is the measured voltage of the K^+ electrode, E° the standard electrode potential derived for each electrode from its own calibration curve, RT/nF the thermodynamic factor, and a_K the activity of K^+. The $[K^+]_o$ concentrations expressed in millimoles/liter were obtained using the equation, $a_K = y \cdot [K^+]_o$, where y is the activity coefficient (0.746).

Intramyocardial electrical activity measurements. Electrograms were recorded from the normal, border and ischemic zones using bipolar plunge electrodes made of enamel-coated stainless steel (0.2 mm in diameter). The electrodes were inserted as close as possible to the corresponding K^+ electrodes by means of a 25-gauge beveled needle similarly marked, as described earlier, for depth of insertion. The position of these electrodes was measured at postmortem examination and verified to be within 2 mm of the corresponding K^+ electrodes. Electrograms were filtered between 30 and 500 Hz and continuously monitored along with the surface ECG leads and arterial pressure. Recordings at paper speeds of 100 and 150 mm/s were made at 1-min intervals. Activation delay was defined as the time from the onset of the QRS complex in the surface ECG to the peak of the largest high frequency deflection in each local electrogram. Further details of measurements have been described elsewhere (14). After placement of all electrodes, the chest cavity was closed. Intrathoracic temperature was monitored continuously by a temperature probe (Yellow Springs Instrument Co., Inc.) and maintained at 37°C by a heating blanket or a heating lamp, or both. A period of 60 min was allowed for stabilization of the K^+ electrodes in situ.

Reproducibility of changes in $[K^+]_o$ during serial ischemia in the dog model. To design the experimental protocol, the changes in $[K^+]_o$ and intramyocardial electrical activity measurements were recorded during four consequent 10-min periods of left anterior descending artery occlusion separated by a 60-min reperfusion period in six dogs (14). The serial occlusions were analyzed as control ligation CL1 to CL4.

Experimental protocols. A 10-min period of left anterior descending coronary artery occlusion was performed by abruptly tightening the snare around the artery. Continuous recordings of $[K^+]_o$ activity and electrograms were made during this period and analyzed as control ischemia I. Release of occlusion was performed slowly, over 60 to 90 s, to minimize reperfusion ventricular arrhythmias. After 60 min of reperfusion, another 10-min period of occlusion was performed, and continuous recordings of $[K^+]_o$ activity and electrograms were analyzed as control ischemia II. For each experiment, two control periods of ischemia were performed, but only data from the second control period were used for comparison with the experimental intervention.

Protocol I. Eight dogs were studied in this protocol. After control ischemia II, 30 min of reperfusion was allowed, after which an infusion of 300 g of glucose + 50 U of regular insulin (Humulin Regular Insulin, Eli Lilly and Company) was administered at a rate of 0.2 ml/kg per min for 60 min. A repeat 10-min period occlusion of the left anterior descending artery was performed at the end of the infusion, and continuous recordings of $[K^+]_o$ and electrograms were ob-

JACC Vol. 22, No. 4
October 1993:1214–22

Table 1. Changes in Extracellular Potassium Ions During Serial Occlusions of the Left Anterior Descending Coronary Artery

Time (min)	Border Zone (n = 6)				Ischemic Zone (n = 6)			
	CL1 (mmol/liter)	CL2 (mmol/liter)	CL3 (mmol/liter)	CL4 (mmol/liter)	CL1 (mmol/liter)	CL2 (mmol/liter)	CL3 (mmol/liter)	CL4 (mmol/liter)
0	3.8 ± 0.3	3.9 ± 0.2	3.9 ± 0.1	3.8 ± 0.1	3.7 ± 0.4	3.8 ± 0.6	3.8 ± 0.2	3.8 ± 0.3
1	5.3 ± 1.1	5.4 ± 0.8	5.3 ± 0.8	5.1 ± 0.9	7.6 ± 2.2	7.6 ± 2.5	8.0 ± 2.6	8.1 ± 2.2
2	5.4 ± 1.4	6.2 ± 1.0	6.3 ± 1.1	5.9 ± 1.1	9.4 ± 3	9.2 ± 3.0	10.0 ± 2.1	10.1 ± 2.8
3	5.6 ± 1.4	6.7 ± 1.4*	6.9 ± 0.8	6.8 ± 1.0	11.0 ± 2.8	10.8 ± 2.6	11.1 ± 2.3	11.7 ± 2.6
4	6.0 ± 1.4	6.7 ± 1.4	6.9 ± 1.3	7.4 ± 0.8	11.5 ± 2.6	11.4 ± 2.4	12.0 ± 2.1	12.3 ± 2.0
5	6.1 ± 1.4	7.0 ± 1.0*	6.9 ± 1.1	7.0 ± 0.9	12.3 ± 2.6	12.7 ± 2.1	12.7 ± 1.5	12.9 ± 1.8
6	6.0 ± 1.4	7.3 ± 1.3*	7.3 ± 0.8	7.2 ± 1.0	13.4 ± 2.6	13.4 ± 2.4	13.2 ± 2.1	13.5 ± 1.8
7	6.4 ± 1.4	7.3 ± 1.3	7.6 ± 1.4	7.3 ± 1.1	13.2 ± 2.4	14.4 ± 2.3	14.1 ± 2.2	14.0 ± 2.4
8	6.4 ± 1.3	7.5 ± 1.4*	7.4 ± 1.1	7.2 ± 1.3	13.2 ± 2.8	14.3 ± 2.8	14.4 ± 2.7	14.0 ± 2.8
9	6.4 ± 1.1	7.4 ± 1.4*	7.1 ± 1.1	7.0 ± 0.8	13.4 ± 2.9	14.5 ± 3.2*	14.6 ± 2.6	14.7 ± 2.1
10	6.2 ± 1.0	7.1 ± 1.5	7.1 ± 1.3	6.9 ± 1.1	13.0 ± 3.2	14.4 ± 3.3*	14.2 ± 3.1	14.4 ± 2.2

*$p < 0.05$. Values presented are mean value ± SD. CL = control ligation (occlusion).

tained as described previously and analyzed as ischemia + glucose-insulin.

Protocol II. Six dogs were subjected to the same procedure as described in protocol I, except that glucose alone was infused. After 60 min of glucose infusion, a repeat 10-min occlusion of the left anterior descending coronary artery was performed, and continuous recordings of $[K^+]_o$ and electrograms were obtained as previously described in protocol I and analyzed as ischemia + glucose.

Protocol III. Ten dogs were studied in this protocol. After the second control occlusion, a period of 45 min of reperfusion was allowed. Regular insulin (0.05 U/kg) was administered intravenously; 30 min later, during peak effect (16), a repeat 10-min period of occlusion of the left anterior descending coronary artery was performed. Recordings of $[K^+]_o$ and electrograms were made as described in the previous protocols. The results were analyzed as ischemia + insulin.

Blood samples were obtained to determine the serum levels of glucose and K^+ before and immediately after completion of the infusions in protocols I and II and at 30 min after the administration of regular insulin in protocol III.

Statistical analysis. The Student *t* test for paired and unpaired data was used to compare heart rate, blood pressure, serum glucose and K^+, $[K^+]_o$ and the duration of intramyocardial electrograms in the normal, border and ischemic zones during control ischemia II and ischemia + glucose-insulin or ischemia + glucose or ischemia + insulin. Analysis of variance and a multiple comparison, Scheffé F test were used for analysis of changes in $[K^+]_o$ during serial occlusions of the left anterior descending coronary artery. Data are presented as mean value ± SD. A confidence level of 95% was considered statistically significant.

Results

Of the 24 dogs studied, 2 were excluded from the analysis because of the development of ventricular fibrillation during the control occlusion periods. The mean arterial pressure showed no significant change during control ischemia and during any of the protocols (122 ± 6 mm Hg before ischemia, 124 ± 10 mm Hg during control ischemia II, 120 ± 8 mm Hg during ischemia + glucose-insulin, 121 ± 8 mm Hg during ischemia + glucose and 123 ± 4 mm Hg during ischemia + insulin).

The mean heart rate did not change significantly during control ischemia II and during ischemia with glucose-insulin infusion or with glucose infusion. The mean heart rate during control ischemia was 145 ± 16 beats/min and was 142 ± 14 and 146 ± 14 beats/min during ischemia after glucose-insulin infusion and glucose infusion, respectively. However, the heart rate after insulin increased from a mean of 142 ± 14 beats/min during control ischemia to a mean of 161 ± 6 beats/min ($p < 0.05$).

Serum levels of glucose and K^+. The serum glucose level was reduced from a mean control level of 103 ± 6 to 55 ± 13 mg/dl ($p < 0.001$) after insulin and was increased to 510 ± 14 and 582 ± 13 mg/dl after glucose-insulin and glucose, respectively ($p < 0.000$). Although the serum K^+ was not significantly reduced after glucose or insulin, it was significantly reduced from a mean control level of 4.0 ± 0.3 to 3.4 ± 0.4 mmol/liter after glucose-insulin infusion ($p < 0.05$).

Effect of serial occlusions on $[K^+]_o$ and intramyocardial conduction. Table 1 shows the effects of four serial control occlusions of the left anterior descending coronary artery on changes in $[K^+]_o$ in the border and ischemic zones in six dogs. There was a slight increase in the degree of rise of $[K^+]_o$ in both the border and the ischemic zones during the second control ligation period ($p < 0.001$). The multiple comparison test showed no significant differences in the degree of $[K^+]_o$ accumulation between the second, third and fourth occlusions. No significant change in the degree of intramyocardial conduction delay was noted during serial occlusions, and the delay in the electrograms rapidly normalized after each release of occlusion. Figure 1 shows the effect of four serial control occlusions on changes in $[K^+]_o$ in the border and ischemic zones.

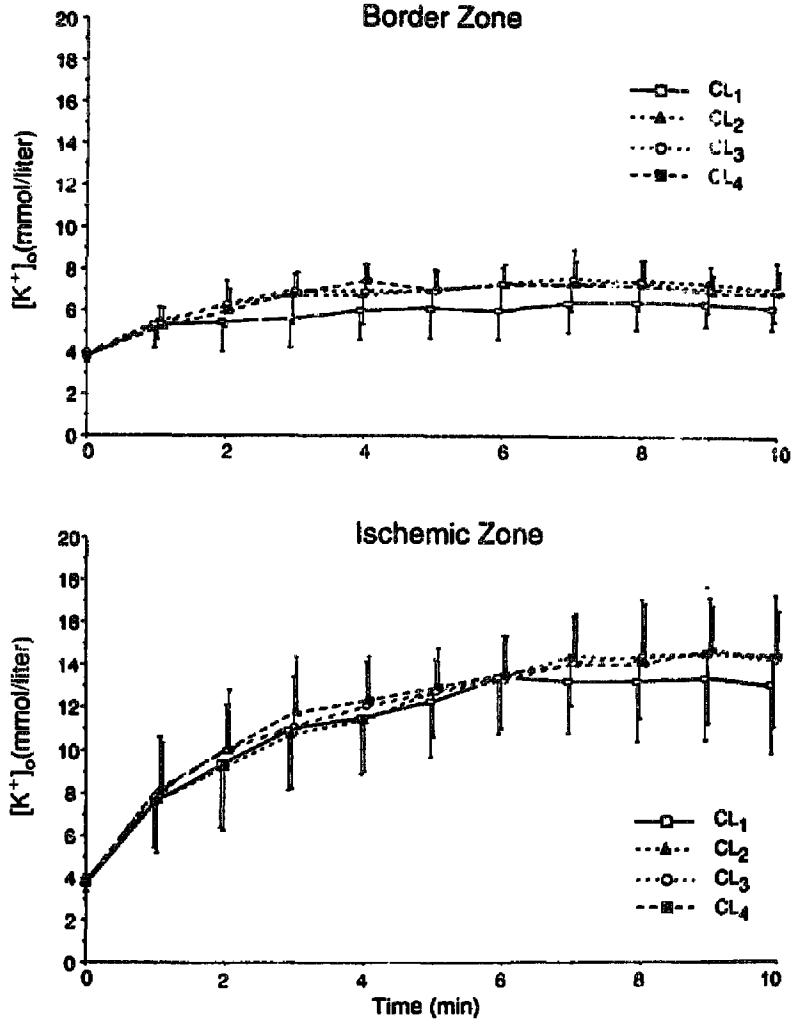

Figure 1. Effect of four serial (10-min period) left anterior descending coronary artery occlusions on extracellular potassium ($[K^+]_o$) in the border and ischemic zones. There was a slight increase in the degree of accumulation of $[K^+]_o$ in the border and ischemic zones during the second control occlusion (CL2). There were no significant differences in degree of $[K^+]_o$ accumulation between the second, third and fourth occlusions. CL = control ligation (occlusion).

Effect of glucose-insulin on ischemia-induced changes in $[K^+]_o$. The effects of glucose-insulin on $[K^+]_o$ are demonstrated in Figures 2 to 4. Glucose-insulin caused a reduction in the degree of rise of $[K^+]_o$, which was significant during the entire period of ischemia in the ischemic and border zones (Fig. 3 and 4). At 10 min of ischemia, $[K^+]_o$ decreased from a mean control level of 15.9 ± 3.7 to 10.1 ± 4.3 mmol/liter in the ischemic zone ($p < 0.005$) and from 6.8 ± 1.9 to 5.5 ± 1.1 mmol/liter in the border zone ($p < 0.05$). Although glucose-insulin caused a reduction in the $[K^+]_o$ in the normal zone, the $[K^+]_o$ did not change during the course of ischemia (Fig. 2). This reduction was attributed to the effect of glucose-insulin on the serum level of K^+.

Effect of glucose on ischemia-induced changes in $[K^+]_o$. Glucose infusion alone caused a significant reduction in $[K^+]_o$ accumulation during the initial 6 min of ischemia in the ischemic zone and no significant change in the border or normal zone (Fig. 2 to 4). At 6 min of ischemia, the $[K^+]_o$ was reduced from a mean control level of 12.4 ± 3 to 10.8 ± 1.2 mmol/liter in the ischemic zone ($p < 0.05$) and from 8.7 ± 0.8 to 8.3 ± 1.1 mmol/liter in the border zone (p = NS).

Effect of insulin on ischemia-induced changes in $[K^+]_o$. Insulin caused no significant changes in the degree of rise of $[K^+]_o$ in the normal, border or ischemic zone (Fig. 2 to 4).

Changes in intramyocardial activation. During control ischemia, local electrograms in both the ischemic and the border zone showed progressive delay associated with a decrease in amplitude and an increase in duration with frequent fractionation. The degree of myocardial delay was greater in the ischemic zone. The increase in electrogram duration in both the ischemic and the border zone reached a maximum at 6 to 7 min, followed by a gradual decrease. This decrease in intramyocardial delay occurred while $[K^+]_o$ remained close to peak levels in both the ischemic and the border zone. Glucose-insulin significantly reduced the degree of intramyocardial conduction delay in both of these zones. Figure 5 compares the electrogram duration during control ischemia and ischemia after glucose-insulin recorded from the normal, border and ischemic zones. Figure 6 shows selected electrograms recorded from the normal, border and ischemic zones during ischemia before and after glucose-insulin in one of the experiments. Glucose alone caused a

JACC Vol. 22, No. 4
October 1993:1214–22

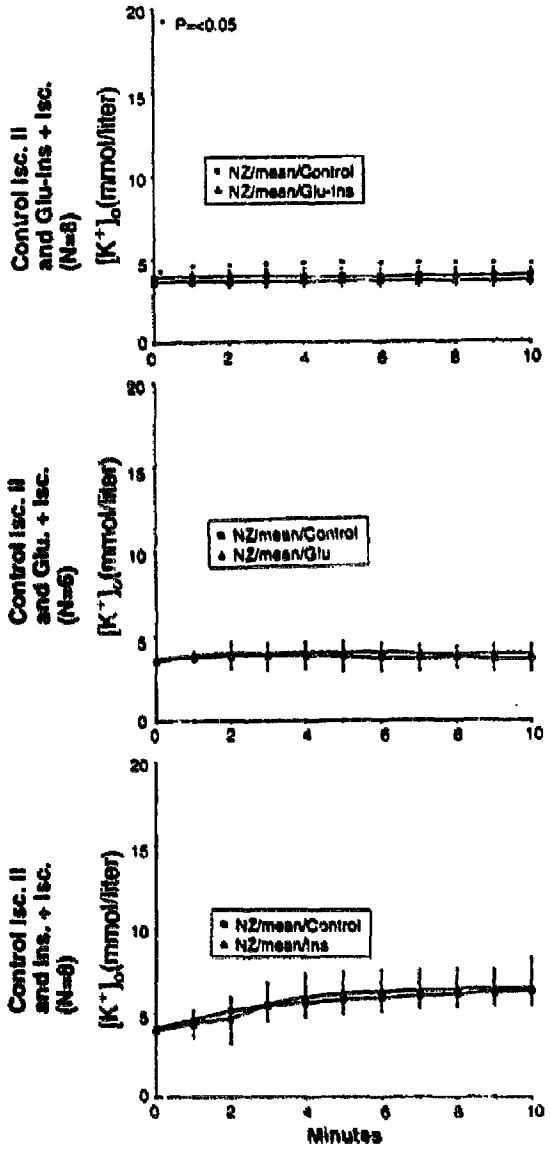

Figure 2. Time course of changes in extracellular potassium $[K^+]_o$ during 10 min of control ischemia II (Control Isc. II) and 10 min of ischemia after glucose-insulin (Glu-Ins + Isc.), after glucose (Glu. + Isc.) and after insulin (Ins. + Isc.) recorded in the normal zone (NZ).

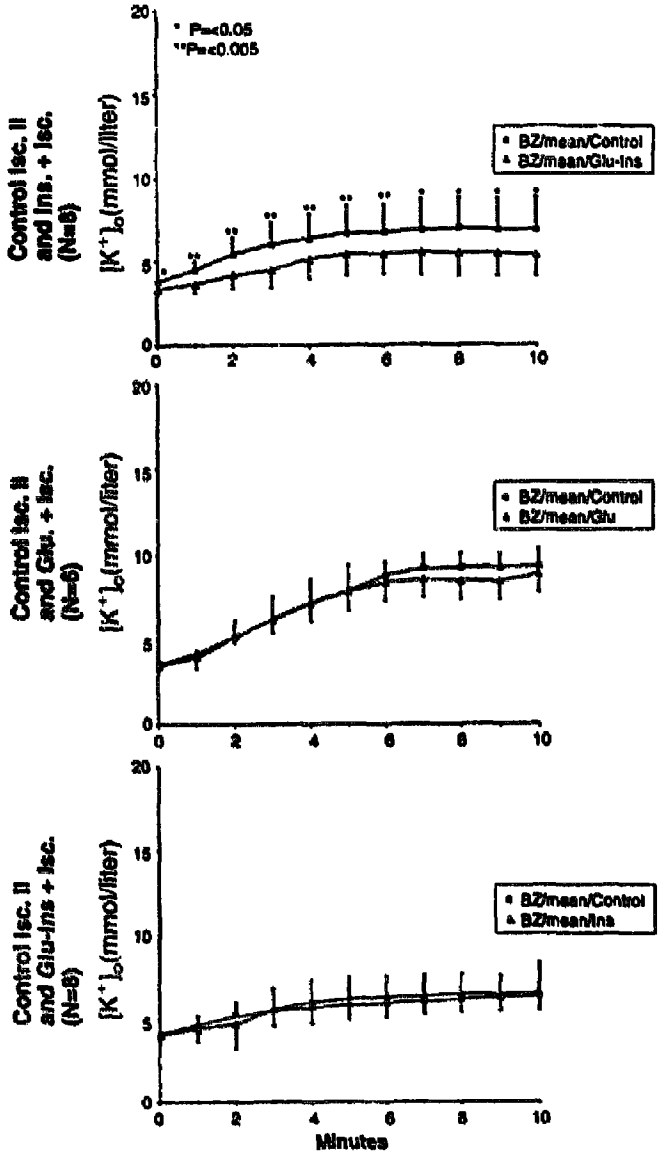

Figure 3. Time course of changes in extracellular potassium $[K^+]_o$ during 10 min of control ischemia II (Control Isc. II) and 10 min of ischemia after glucose-insulin (Glu-Ins + Isc.), after glucose (Glu. + Isc.) and after insulin (Ins. + Isc.) recorded in the border zone (BZ).

nonsignificant reduction in the degree of intramyocardial activation delay in the ischemic zone (Fig. 7). There was no change in the degree of intramyocardial activation delay in the ischemic or border zone after insulin (Fig. 8).

Discussion

The results of this study indicate that the infusion of glucose-insulin before occlusion of the left anterior descending coronary artery significantly reduced the degree of $[K^+]_o$ accumulation during ischemia in both the ischemic and the border zone of the canine heart. This was associated with an improvement in the degree of intramyocardial conduction delay during the entire period of ischemia. The beneficial effects of glucose-insulin infusion in this study were not related to changes in heart rate or to lack of reproducibility of the changes in either $[K^+]_o$ or intramyocardial electrograms during serial occlusions.

After the infusion of glucose-insulin, there was an initial (0 min), slight reduction in serum K^+ level. This may account in part for the salutary effect of glucose-insulin infusion on ischemic loss of $[K^+]_o$. However, it could not explain the marked and consistent reduction in $[K^+]_o$ during the entire period of ischemia in both the ischemic and the border zone.

Glucose infusion alone administered at the same concentration and rate caused a significant reduction in the degree

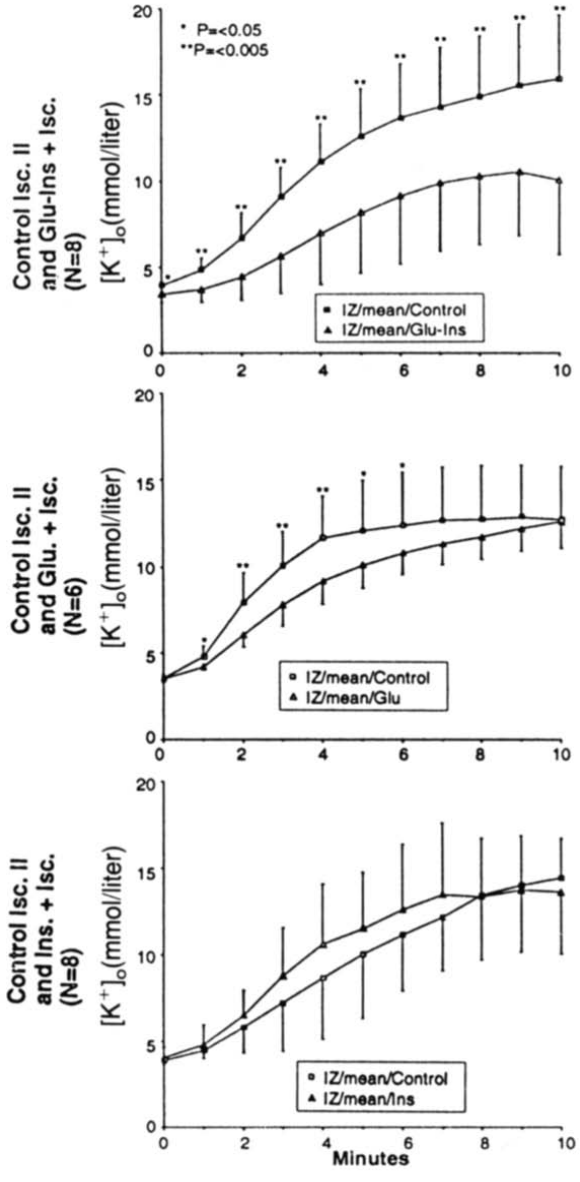

Figure 4. Time course of changes in extracellular potassium $[K^+]_o$ during 10 min of control ischemia II (Control Isc. II) and 10 min of ischemia after glucose-insulin (Glu-Ins + Isc.), after glucose (Glu. + Isc.) and after insulin (Ins. + Isc.) recorded in the ischemic zone (IZ). Note insignificant increase in degree of $[K^+]_o$ accumulation after insulin.

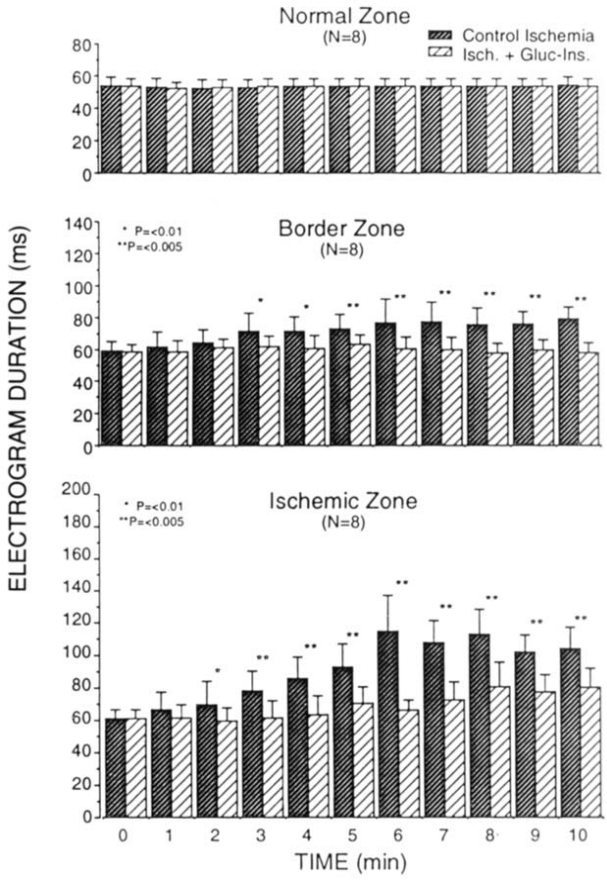

Figure 5. Time course of changes in intramyocardial electrogram duration during 10 min of control ischemia and 10 min of ischemia after infusion of glucose-insulin (Isch. + Gluc-Ins.) in the normal, border and ischemic zones.

of $[K^+]_o$ accumulation during the initial 6 min of ischemia only in the ischemic zone, with no improvement in the border zone and no change in the degree of intramyocardial activation delay. Insulin alone did not alter ischemia-induced changes in $[K^+]_o$ or the duration of electrograms in either the ischemic or the border zone. It therefore appears that insulin in the presence of glucose caused the most prompt and most persistent reduction in ischemia-induced $[K^+]_o$ accumulation and improvement in the degree of intramyocardial conduction delay.

Metabolic and cardioprotective effects of glucose-insulin infusion. Several postulated protective effects of glucose-insulin-potassium infusion on the metabolism of the ischemic myocardium have been reported. Sodi-Pallares et al. (1,17) suggested that glucose-insulin infusion acts as a "polarizing" agent to restore K^+ loss from the ischemic area and hence results in preservation of mitochondrial function. Other workers (3,9,10) have shown that glucose-insulin infusion increased the adenosine triphosphate level and tissue glycogen in the ischemic zone. During anoxia in the isolated perfused rat heart, glucose transport is accelerated, the rate of anaerobic glycolysis is increased and the supply of exogenous glucose may facilitate these changes (18,19). A similar mechanism may occur after coronary artery ligation if residual collateral circulation is present, thereby allowing the increase in supply of glucose (20). Other studies (21) have suggested that the glycogen-sparing effect of glucose-insulin-potassium infusion is responsible for myocardial protection by prolonging the availability of glycolytic substrate necessary for the production of high energy phosphate.

Another proposed mechanism (12,22,23) for the beneficial effect of glucose-insulin infusion in acute ischemia is the alteration in free fatty acid metabolism. It was suggested (22–24) that glucose-insulin may inhibit lipolysis and reduce circulating free fatty acid levels as well as intracellular free fatty acid accumulation, thus preventing ischemic conse-

JACC Vol. 22, No. 4
October 1993:1214–22

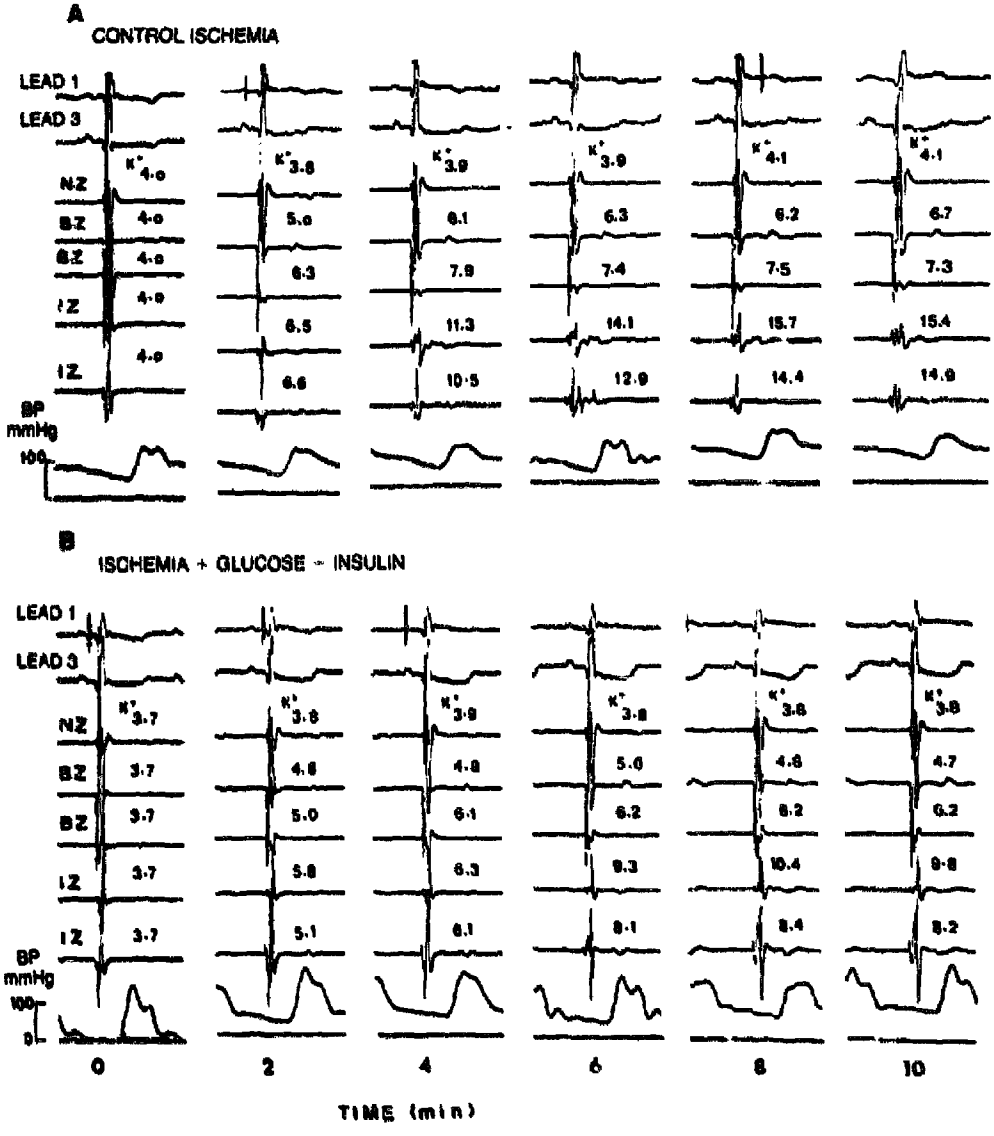

Figure 6. Effects of glucose-insulin infusion on ischemia-induced changes in intramyocardial electrograms from the normal (NZ), border (BZ) and ischemic (IZ) zones: **A**, Control ischemia; **B**, Ischemia + glucose-insulin. Control ischemia was associated with a marked increase in extracellular potassium (K^+) and a decrease in amplitude and fractionation of intramyocardial electrograms at the border and ischemic zones. After glucose-insulin infusion, ischemia-induced changes in K^+ and intramyocardial electrograms were markedly lessened. BP = blood pressure.

quences, such as arrhythmias. It was further suggested (25) that glucose-insulin-potassium infusion during global ischemia and reperfusion enhances the scavenging of free radicals, thus protecting the excitation-contraction coupling system. Other diverse mechanisms, such as the increase in blood osmolarity and plasma volume (26,27) and the direct polarizing effect of insulin on the hypoxic tissue (28,29), have also been postulated.

Possible mechanisms for glucose-insulin effect on ischemia-induced increase in $[K^+]_o$. Most of the increase in $[K^+]_o$ during the early phase of ischemia results primarily from an increase in the net efflux of K^+ from the intracellular compartment rather than from a decreased K^+ influx (30–32); however, the mechanism of loss of K^+ in early ischemia is not yet well defined. Recently it has been shown (33–38) that the activation of cardiac adenosine triphosphate–sensitive K^+ channels is a possible cause of K^+ efflux during ischemia.

The reported discrepancy between intracellular adenosine triphosphate concentration and the activation of adenosine triphosphate–sensitive K^+ channels has been explained by the alteration in the K^+ channel sensitivity to adenosine triphosphate during metabolic inhibition (37–42). It is possible that the effects of glucose-insulin on ischemia-induced intracellular K^+ loss are due to its salutary effects on the rate of depletion of intracellular adenosine triphosphate and the consequent opening of adenosine triphosphate–sensitive K^+ channel; however, the degree of contribution of this channel to ischemia-induced K^+ loss is still controversial. For example, it was shown (14,43) that in the presence of glibenclamide, a specific blocker of the adenosine triphosphate–sensitive K^+ channel, there was only partial prevention of K^+ loss. Conversely, some studies have shown that K^+ channel–opening agents, such as cromakalim and pinacidil, may have beneficial effects on the degree of ischemic damage (44).

Ischemia-induced increase in $[K^+]_o$ and its subsequent improvement after glucose-insulin infusion may also be partly related to mechanisms other than modulation of

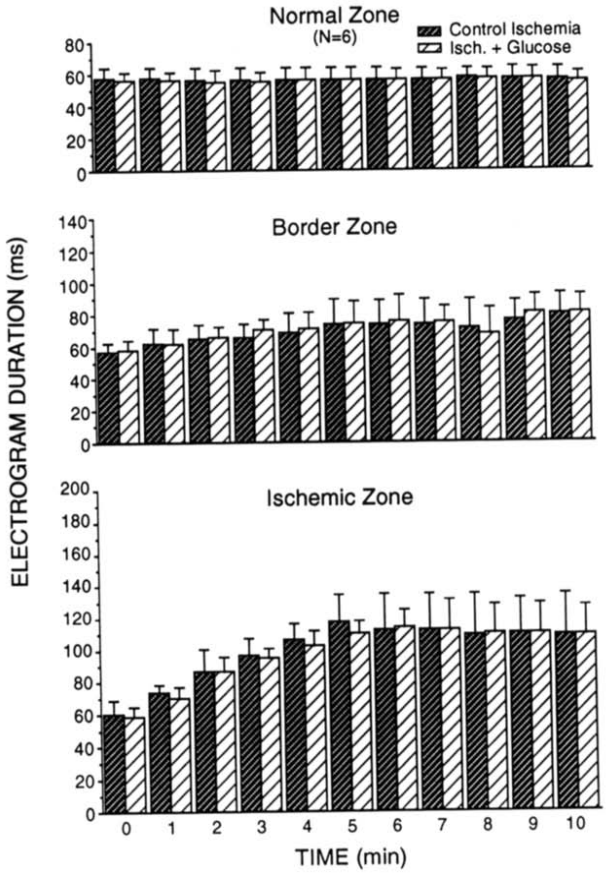

Figure 7. Time course of changes in intramyocardial electrograms during 10 min of control ischemia and 10 min of ischemia after infusion of glucose (Isch. + Glucose) in the normal, border and ischemic zones. There were no significant changes in the duration of electrograms during control ischemia and during ischemia + glucose.

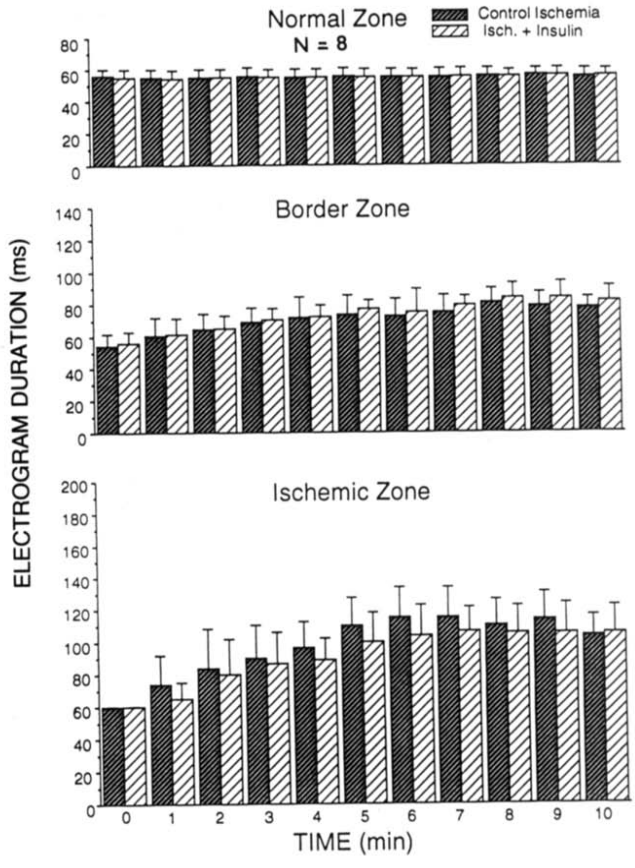

Figure 8. Time course of changes in intramyocardial electrograms during 10 min of control ischemia and 10 min of ischemia after insulin (Isch. + Insulin) in the normal, border and ischemic zones. There were no significant changes in the duration of electrograms during control ischemia and during ischemia + insulin.

adenosine triphosphate–regulated K^+ channels. Reduced sodium ion/potassium ion pump function, increased K^+ efflux as a consequence of intracellular acidosis and increase of K^+ efflux through calcium-activated K^+ channels have been mentioned (30) as possible explanations of ischemia-induced increase in $[K^+]_o$.

Although this study has shown that glucose infusion alone caused an improvement in ischemia-induced increase in $[K^+]_o$ accumulation, this effect was not persistent and was evident only in the ischemic zone. In addition, there was no associated improvement in the degree of intramyocardial conduction delay. These findings are in agreement with those of other workers (45), who reported that maximal and complete restoration of action potentials in nonoxygenated human papillary muscle occurred with glucose-insulin infusion and only partially with glucose alone. The superiority of glucose-insulin to glucose alone when both infusions were administered at the same rate and volume also suggests that acute hypervolemia or an increase in osmolarity did not play a major role in the reduction of ischemic loss of K^+. Our study has excluded the possibility that insulin by itself is responsible for the salutary effect of glucose-insulin infusion.

Study limitations. Measurements of intramyocardial conduction delays during ischemia with or without glucose-insulin infusions were obtained from analysis of bipolar electrograms recorded from relatively few sites in the ischemic and border zones. Recording from more sites would have allowed a more accurate determination of changes in intramyocardial activation patterns and conduction abnormalities. The proposed hypothesis that glucose-insulin infusion exerts its beneficial effects through decreased opening of adenosine triphosphate–regulated K^+ channels was not substantiated in the present study by direct measurement of pertinent metabolic or electrophysiologic changes.

References

1. Sodi-Pallares D, Testelli MR, Fishleder BL, et al. Effects of an intravenous potassium glucose-insulin solution on the electrocardiographic signs of myocardial infarction. A preliminary clinical report. Am J Cardiol 1962;9:166–81.
2. Whitlow PL, Rogers WJ, Smith LR, et al. Enhancement of left ventricular

function by glucose-insulin-potassium infusion in acute myocardial infarction. Am J Cardiol 1982;49:811–20.
3. Opie LH, Bruyneel K, Owen P. Effect of glucose-insulin-potassium infusion on metabolic changes within first hour of myocardial infarction in the baboon. Circulation 1975;52:49–75.
4. Maroko PR, Libby P, Sobel BE, et al. Effects of glucose-insulin-potassium infusion on myocardial infarction following experimental coronary artery occlusion. Circulation 1972;45:1160–75.
5. Pentecost BL, Mayne NM, Lamb P. Controlled trial of intravenous glucose, potassium and insulin in acute myocardial infarction. Lancet 1968;1:946–8.
6. Medical Research Council Working Party on the Treatment of Myocardial Infarction. Potassium, glucose, and insulin treatment for acute myocardial infarction. Lancet 1968;2:1355–60.
7. Heng MK, Norris RM, Peter T, Nisbet HD, Singh BN. The effects of glucose-insulin-potassium on experimental myocardial infarction in the dog. Cardiovasc Res 1978;12:429–35.
8. Sybers HD, Maroko PR, Ashraf M, Libby P, Braunwald E. The effect of glucose-insulin-potassium infusion on cardiac ultrastructure following acute experimental coronary artery occlusion. Am J Pathol 1973;70:401–20.
9. Dalby AJ, Bricknell OL, Opie LH. Effect of glucose-insulin-potassium infusions on epicardial ECG changes and on myocardial metabolic changes after coronary artery ligation in dogs. Cardiovasc Res 1981;15:588–98.
10. Opie LH, Owen P. Effect of glucose-insulin-potassium infusion on arterio-venous differences of glucose and of free fatty acids and on tissue metabolic changes in dogs with developing myocardial infarction. Am J Cardiol 1976;31:310–21.
11. Kinoshita K, Yatani A. Aftereffects of perfusion with glucose-insulin-potassium solution on the membrane current and tension components of frog atrial muscle. Jpn Heart J 1982;23:791–804.
12. Kobayashi A, Kamiya J, Yamashita T, et al. Effects of glucose-insulin-potassium solution on free fatty acid metabolism in ischemic myocardium. Jpn Circ J 1984;48:591–5.
13. Bekheit S, Isber N, Jani H, et al. Protective electrophysiological effects of glucose-insulin during acute ischemia (abstr). Circulation 1991;84(suppl II):II-550.
14. Bekheit S, Restivo M, Boutjdir M, et al. Effects of glyburide on ischemia-induced changes in extracellular potassium and local myocardial activation: a potential new approach to the management of ischemia-induced malignant ventricular arrhythmia. Am Heart J 1990;114:1025–33.
15. Hill J, Gettes L, Lynch M. Flexible valinomycin electrodes for on line determination of intravascular and myocardial K^+. Am J Physiol 1978;235:455–9.
16. Rosak C, Boehm BO, Althoff PH, Schoffling K. Biosynthetic human proinsulin, a new therapeutic compound for diabetics? A comparative study of biosynthetic human proinsulin with biosynthetic human insulin. Horm Metab Res Suppl 1988;18:16–21.
17. Sodi-Pallares D, Bisteni A, Medrano A, Testelli R, Micheli DE. The polarizing treatment of acute myocardial infarction: possibility of its use in other cardiovascular conditions. Dis Chest 1963;43:424–32.
18. Morgan HE, Randle PJ, Regen DM. Regulation of glucose uptake by muscle—the effects of insulin, anoxia, salycilates and dinitrophenol on membrane transport and intracellular phosphorylation of glucose in the isolated rat heart. Biochem J 1959;73:573–9.
19. Weissler AM, Kruger FA, Baba N. Role of anaerobic metabolism in the preservation of functional capacity and structure of anoxic myocardium. J Clin Invest 1968;47:403–16.
20. Opie LH, Owen P, Riemersma RA. Relative rates of oxidation of glucose and free fatty acids by ischemic and non-ischemic myocardium after coronary artery ligation in the dog. Eur J Clin Invest 1973;3:419–35.
21. Hoekenga, Brainard Jr, Hutson JY. Rate of glycolysis and glycogenolysis during ischemia in glucose-insulin-potassium treated perfused hearts: a ^{13}C ^{31}P nuclear magnetic resonance study. Circ Res 1988;62:1065–74.
22. Opie LH. Metabolism of free fatty acids, glucose and catecholamines in acute myocardial infarction. Am J Cardiol 1975;36:938–50.
23. Opie LH. Effects of regional ischemia on metabolism of glucose and fatty acid. Relative rates of aerobic and anaerobic energy production during myocardial infarction and comparison with effects of anoxia. Circ Res 1973;38(suppl I):1–52, I–74.
24. Wood JM, Hutchings AE, Brachfeld N. Lipid metabolism in myocardial cell-free homogenates. J Mol Cell Cardiol 1972;4:97–111.
25. Hess ML, Okabe E, Poland J, Warner M, Stewart JR, Greenfield LJ. Glucose insulin, potassium protection during the course of hypothermic global ischemia and reperfusion: a new proposed mechanism by the scavenging of free radicals. J Cardiovasc Pharmacol 1983;5:35–43.
26. Powell WJ, Dibona DR, Flores J, Frega N, Leaf A. Effects of hyperosmotic mannitol in reducing ischemic cell swelling and minimizing myocardial necrosis. Circulation 1976;53(suppl I):I-145–55.
27. Burke WM, Asokan SK, Moschos CB, Oldewurtel HA, Regan TJ. Effect of glucose and non-glucose infusions on myocardial, potassium ion transfers and arrhythmias during ischemia. Am J Cardiol 1969;24:713–22.
28. Zierler KL. Effect of insulin on membrane potential and potassium content of rat muscle. Am J Physiol 1959;179:515–23.
29. Hiatt N, Sheinkoph JA, Warner NE. Prolongation of survival after circumflex artery ligation by treatment of massive doses of insulin. Cardiovasc Res 1971;5:48–53.
30. Kleber AG. Extracellular potassium accumulation in acute myocardial ischemia. J Mol Cell Cardiol 1986;16:389–94.
31. Boyle PJ, Conway EJ. Potassium accumulation in muscle and associated changes. J Physiol (Lond) 1941;100:1–3.
32. Case RR. Ion alteration during myocardial ischemia. Cardiology 1971/1972;56:245–62.
33. Noma A. ATP regulated K channels in cardiac muscle. Nature 1983;305:147–8.
34. Trube G, Hescheler J. Inward-rectifying channels in isolated patches of the heart cell membrane: ATP-dependence and comparison with cell-attached patches. Pflugers Arch 1984;401:178–84.
35. Noma A, Shibasaki T. Membrane currents through adenosine-triphosphate-regulated potassium channels in guinea-pig ventricular cells. J Physiol (Lond) 1985;363:463–80.
36. Nichols CG, Ripoll C, Lederer WJ. ATP sensitive potassium channel modulation of the guinea pig ventricular action potential and contraction. Circ Res 1991;68:280–7.
37. Weiss JN, Venkatesh N, Lamp ST. ATP-sensitive K^+ channels and cellular K^+ loss in hypoxic and ischemic mammalian ventricle. J Physiol (Lond) 1992;447:649–73.
38. Lederer WJ, Nichols CG. Nucleotide modulation of the activity of rat heart ATP-sensitive K^+ channels in isolated membrane patches. J Physiol (Lond) 1989;419:193–211.
39. Elliott AC, Smith GL, Allen DG. Simultaneous measurements of action potential duration and intracellular ATP in isolated ferret hearts exposed to cyanide. Circ Res 1989;64:583–91.
40. Kakei M, Noma A, Shibasaki T. Properties of adenosine-triphosphate-regulated potassium channels in guinea pig ventricular cells. J Physiol (Lond) 1985;363:441–62.
41. Kakei M, Kelly AP, Ashcroft SJH, Ashcroft FM. The ATP sensitivity of K^+ channels in rat pancreatic B-cells is modulated by ADP. FEBS Lett 1986;208:63–6.
42. Corky BE, Tornheim K, Deeny JT, et al. Linked oscillations of free $Ca2^+$ and the ATP/ADP ratio in permeabilized RINm5F insulinoma cells supplemented with a glycolysing cell-free muscle cell extract. J Biol Chem 1988b;263:4254–8.
43. Kantor PF, Coetzee WA, Carmeliet E, Dennis SC, Opie LH. Reduction of ischemic K^+ loss and arrhythmias in rat hearts. Effect of glibenclamide a sulfonylurea. Circ Res 1990;66:478–85.
44. Grover GJ, McCullough JR, Henry DE, Conder ML, Sleph PG. Anti-ischemic effects of the potassium channel activators pinacidil and cromakalim and the reversal of these effects with the potassium channel blocker glyburide. J Pharmacol Exp Ther 1989;251;1:98–104.
45. Prasad K, Callaghan JC. Electrophysiologic bases of use of a polarizing solution in the treatment of myocardial infarction. Clin Pharmacol Ther 1971;12:666–75.