Effects of Glucose, Insulin and Potassium Infusion on Tissue Metabolic Changes Within First Hour of Myocardial Infarction in the Baboon

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SUMMARY
The effects of infusions of glucose, insulin and potassium (GIK) on the heart tissue metabolic changes found in adult baboons 60 min after coronary artery ligation were studied. Biopsies taken from 11 baboons without coronary artery ligation gave control values. A second group of 46 baboons had coronary artery ligation. A third group of 17 baboons received an infusion of KCl after coronary artery ligation. A fourth group of 26 baboons received infusions of GIK. Coronary artery ligation resulted in the expected fall of ATP, creatine phosphate, glycogen, tissue (K+ /Na+) ratio, and tissue pH, and rise of inorganic phosphate, lactate, lactate/pyruvate ratio and α-glycerophosphate in the infarction zones. Compared with ligation, additional infusions of GIK approximately doubled the contents of creatine phosphate and glycogen in the infarct zones, increased the content of ATP in the central infarct zone, and decreased the content of inorganic phosphate in the peripheral infarct zone. Other GIK effects were that the tissue (K+ /Na+) ratio rose in the peripheral infarct zone, and the content of both glycogen and lactate rose in the peri-infarct and nonischemic zones; the pH of tissue homogenates did not decrease. KCl infusions had few effects compared with the ligation group. GIK infusions exerted a beneficial effect when compared with infusions of KCl in that tissue creatine phosphate rose in the peripheral infarct and nonischemic zones; the tissue K+ /Na+ ratio rose in the peripheral infarct, peri-infarct, and nonischemic zones; and the lactate/pyruvate ratio fell in the infarct zone. It is proposed that GIK counteracted early tissue metabolic deterioration in the infarcting baboon heart.

Additional Indexing Words:
ATP, Creatine phosphate, Lactate
Glycogen, Tissue pH, Tissue K+ /Na+ ratio
Phosphofructokinase, Glycolytic flux, Potassium infusion

AN IMPORTANT CONCEPT, recently emphasized in the management of acute myocardial infarction, is that the severity of heart tissue damage might be modified by various procedures including metabolic manipulation.1-4 Although experiments on dogs have suggested that infusions of glucose, insulin and potassium (GIK) might exert a beneficial influence on the effects of coronary artery ligation by decreasing the extent of mitochondrial damage and the infarct size,5-8 yet many more experimental details are required before GIK can be recommended for general clinical use.3 Reservations in applying the findings in dog to man include 1) the major phylogenetic differences between man and the dog and 2) the more substantial collateral circulation thought to exist in dog than in the human heart,8 which is important because the therapeutic infusion presumably reaches the infarcting myocardium by collateral flow. The relatively long duration (12-24 hr) of infusions in experiments showing the beneficial effect of GIK in dogs gives no information on possible effects in the first hour of myocardial infarction, when a major portion of human mortality is thought to occur.9

For these reasons we examined the effect of GIK infusions on tissue metabolic changes found within one hour of coronary artery ligation in a subhuman primate, the Cape Chacma baboon. We found that GIK altered the metabolic response to coronary artery ligation, as assessed by the tissue values of high-energy phosphate compounds and of glycogen.

Materials and Methods
Production of Acute Myocardial Ischemia
One hundred apparently healthy, wild Cape Chacma baboons (Papio Ursinus) were captured in natural surround-
ings and used for experiments within two weeks. The majority were adult male baboons. After overnight fast, they were paralyzed by phencyclidine HCl (Sernylan, Parke-Davis) 1.25 mg/kg and thereafter given intermittent intravenous pentobarbitone (Sagatal, Burroughs Wellcome) throughout the experimental period at an average rate of 0.06 mg/kg/min. After intubation, baboons were ventilated by a Harvard respiration pump at 16 strokes/min, with a tidal volume of about 5 ml/kg body weight (initial arterial PO₂ 73 ± 3 mm Hg; final arterial PO₂ 82 ± 6 mm Hg, N = 11).

The chest was opened by midsternal thoracotomy and the heart was suspended in a pericardial cradle. Central body temperature was maintained at 37-38°C by heating of the operating room to 29°C. The anterior descending coronary artery was ligated at the junction of the middle and lower thirds to produce a well-circumscribed dark blue area involving about 10% of the heart weight. Occasionally other arteries were tied to produce comparable lesions. Full operative details are given elsewhere.10

Group A: Control Baboons

In 11 baboons, the chest was opened as above but the coronary artery was neither dissected (to avoid any coronary spasm) nor tied, and no infusions were given.

Group B: Baboons with Ligation but No Therapeutic Infusion

In 46 baboons, coronary artery ligation was followed either by no infusion (30 baboons), or by an infusion of ½N NaCl (4.5 g/L) (16 baboons). All infusion fluids were preheated to body temperature and given at a rate of 0.2 ml/kg/min. Twenty-five baboons developed ventricular fibrillation (not defibrillated) before the planned end of the experiment 60 min after coronary artery ligation. Biopsy studies were undertaken in 12 of the survivors. Nine survivors were not biopsied but are included to calculate the incidence of ventricular fibrillation more accurately.

Group C: Baboons with Infusion of KCl

In 17 baboons KCl (40 mEq/L) in a solution of ½N NaCl, was infused at 0.2 ml/kg/min; the average volume given over the whole period was about 250 ml. KCl was given because 1) the plasma K⁺ fell in baboons in groups A and B; and 2) we wished to separate the effects of administration of K⁺ by itself from K⁺ given with glucose and insulin. Six baboons developed ventricular fibrillation and the 11 survivors were taken for biopsy studies.

Group D: Baboons with Infusions of GIK

In 26 baboons, intravenous infusions of GIK (0.2 ml/kg/min) were started 3 min after arterial ligation in one of two dosage schedules: "Low-dose" GIK infusions (dextrose 100 g/L, KCl 40 mEq/L, and soluble insulin 40 units/L) were given to 12 baboons, of whom four developed ventricular fibrillation. "High-dose" GIK infusions (dextrose 200-500 g/L, KCl 60 mEq/L and soluble insulin 60 units/L) were given to 14 baboons, of whom five developed ventricular fibrillation. Results of low and high dose schedules of GIK could not be distinguished and were pooled; thus of all GIK-infused baboons, 17 did not develop ventricular fibrillation and 9 did. NUSO (Burroughs Wellcome) soluble insulin was used because of the low content of glucagon (about 0.03 μg glucagon per unit insulin) which is important because glucagon could have independent effects on the outcome of experimental myocardial infarction.1

Only the GIK group had no Na⁺ infused; the amount of Na⁺ administered to the other groups was, however, only about 1 mEq/kg for the experimental period. The plasma Na⁺ concentration did not change during the course of the experiment in any groups.

Blood Samples

Arterial blood samples for sodium, potassium, glucose, free fatty acids and Astrup determinations were obtained immediately after the induction of anesthesia; immediately before the artery was ligated (approximately 60 min later); and 60 min later at the end of the experiment. Sodium and potassium were analyzed by flame photometer, glucose by glucose oxidase method, and free fatty acids were titrated by the Dole-Meineretz procedure.11 Initial arterial samples were also analyzed for plasma proteins, calcium, and alkaline phosphatase by a multi-channel Technicon procedure, and for hemoglobin by a Coulter counter.

Biopsy Methods

Biopsies were taken from hearts which survived for 60 min after coronary artery ligation, but those taken from hearts developing ventricular fibrillation showed severe depletion of high-energy phosphate compounds and were excluded. Biopsies for high-energy phosphate compounds, inorganic phosphate and lactate were taken in situ by the dental drill technique of Pool et al.12 using a modified drill, 3 mm in internal diameter and yielding about 0.18 g of tissue; the heart was then excised by rapid aortic transsection, arrested in ice-cold 0.25 M sucrose and further drill biopsies were taken for glycogen, K⁺ and Na⁺.

To measure glycolytic intermediates, a different procedure was used. Tissue samples were taken after aortic transsection and cardiac arrest in sucrose by rapid excision of slices (about 0.5 - 1.0 g in weight) from the heart submerged in ice-cold sucrose, taking one biopsy from each of the four biopsy areas (see next section). Such biopsies were plunged into liquid nitrogen and then analyzed for all the above biochemical parameters and also for α-glycerophosphate, pyruvate, hexose phosphates, and citrate. Whether the samples were frozen in liquid nitrogen or in freon did not change the ATP or creatine phosphate values which were obtained, and liquid nitrogen was used in most experiments.

Biopsy Sites

Biopsy sites were selected from the four areas of the free antero-lateral wall of the left ventricle (see fig. 1 in reference 13). The infarct zone comprised area 1 (center of infarct) and area 2 (periphery of infarct). Area 3 was the peri-infarct zone and area 4 was apparently normal, nonischemic tissue.

Areas 1 and 2 were identified by 1) the dark blue-brown color of the epicardium; 2) bulging during systole; and 3) the marked ST elevation on the epicardial unipolar ECG recordings. Area 1 was separated from area 2 by means of epicardial ECG mapping or by continuous sweeping ECG recordings over the epicardium of the left ventricle and the infarct area (see next section). In the center of the infarct (area 1) there was maximum epicardial ST elevation. Blood flow to area 1 measured with radioactive microspheres was about 5-10% of the preligation values.13 Area 2 surrounded area 1 and showed a progressive centrifugal decrease of the epicardial ST-segment elevation. Blood flow in area 2 was up to 30% of the preligation value.13 Biopsy cylinders from area 1 and 2 showed the most severe biochemical tissue and blood flow changes.
Area 3 was the apparently normal peri-infarct tissue which surrounded area 2 and had the usual red epicardial color. There was no systolic bulging in area 3. The ST segment was isoelectric or more frequently depressed. Area 3, which was about 1 cm in width, corresponded with the area in which blood flow was increased if there were the subsequent development of ventricular fibrillation, but with a 90% decrease in blood flow in baboons surviving for one hour. The well-defined difference between area 2 (blue) and area 3 (red) corresponded with the narrow transition band from ST-segment elevation (area 2) to ST-segment depression (area 3).

Area 4 was never in contiguity with area 3 and was chosen as far away from the center of the infarct and area 3 as possible. The color of the epicardium was normal, the ST segment was isoelectric, there was never systolic bulging and the blood flow was unchanged throughout the whole experiment.

Biopsies were taken in order from areas 4, 3, 2, and 1 and usually again from area 4.

Electrocardiographic Monitoring

Continuous rhythm recordings were obtained either from a Holter recording system or from conventional limb leads. Intermittent epicardial records were taken from twelve epicardial sites by the method of Becker et al. and recorded on a Corbin-Farnsworth two-channel recorder LGGR. Intermittent sweeping movements of the epicardial electrode were made from base to apex of the heart, through the infarct area and back again and also at right angles from the exposed right ventricle through the infarction to the border of the left ventricle. Sweeping traces were recorded either on the Corbin-Farnsworth machine with a wire-electrode, or with a pH Calomel electrode on a 549 Tektronix oscilloscope with a 1A7A differential amplifier and a band width DC to 1 MHz.

Approximate Size of Visible Infarct

After biopsies were taken, the transition in color from area 3 to area 2 was still visible and the infarction zone (areas 1 and 2) was cut along the visible border, weighed and compared with the weight of the whole heart. Appropriate allowances were made for the weights of the biopsies.

Tissue Analyses

Frozen tissue was deproteinized and analyzed for ATP, phosphocreatine, ADP, AMP and inorganic phosphate by methods previously described except that inorganic phosphate was determined by the method of Seraydarian et al., which gave values about half those obtained by the previously used method of Berenblum and Chain. Analyses of fructose 1, 6-diphosphate, pyruvate, and dihydroxyacetone phosphate were carried out on the day of the experiment, hexosemonophosphate, a-glycerophosphate, lactate and citrate were analyzed within ten days after storage in a deep-freeze. Enzymatic methods previously described were used. Aliquots of the biopsy tissue were taken for 1) digestion in KOH for analysis of glycogen by the method of Good et al. with estimation of glucose by the glucose 6-phosphate dehydrogenase method; 2) digestion in nitric acid for analysis of sodium and potassium by flame photometry; and 3) pH determinations which were done after homogenizing the whole tissue in sodium fluoride-iodoacetate, as described by Newbold and Lee.

Expression of Results

Results are expressed in terms of wet weight (mean values ± SEM for number of observations). Using two-tailed Student's t-test, P values of 0.05 or less were regarded as significant.

Results

Characterization of Animals

The mean body weight in all groups of baboons was 20.5 kg. The mean heart weight was 5% of the body weight (compared with 7% for our mongrel dog hearts and 4.5% for adult man). Mean arterial blood plasma protein values were 7.1 gr/100 ml (albumin 2.9); calcium 9.1 mg/100 ml; cholesterol 130 mg/100 ml; and hemoglobin 14.6/ml. Heart rates after thoracotomy and pericardiectomy were: 11 control baboons, 125 ± 6; 11 ligation baboons, 118 ± 10; nine ligation + KCl, 105 ± 9; and 16 ligation + GIK, 112 ± 7. Final heart rates were: 11 control baboons, 136 ± 8; 14 ligation baboons, 125 ± 9; ten ligation + KCl, 119 ± 10; and 17 ligation + GIK baboons, 121 ± 7. No significant differences were found.

Incidence of Ventricular Fibrillation and Approximate Size of Infarction

The apparent size of the infarct was the same in all groups (9-12% of the whole heart) and the average time between coronary artery ligation and the onset of ventricular fibrillation (about 35 min) was also the same in all groups. In both KCl and GIK-treated hearts the incidence of ventricular fibrillation was 35% compared with 54% in group B ligation baboons (difference not significant).

Blood Concentrations of K+, Na+, Glucose and Free Fatty Acids

In both group A (no ligation) and group B (ligation) plasma K+ fell (initial, 3.9-4.0 mEq/L; final 3.2-3.7 mEq/L), blood glucose rose (initial, 77-94 mg%; final 103-134 mg%) and plasma FFA increased (initial, 304-362 µEq/L; final, 592-698 µEq/L) during the experimental period. In baboons infused with KCl (group C), plasma K+ rose from an initial value of 3.8 µEq/L to a final value of 4.3 µEq/L but the changes in glucose and FFA were similar to those in baboons with ligation alone. In GIK-infused animals (group D) plasma K+ was steady from the time of ligation (4.0 mEq/L) to the end (3.8 mEq/L), but the blood sugar rose to over 380 mg/100 ml and the plasma FFA (319 µEq/L) had fallen to about half the final value in the other groups (P < 0.05). In all groups, initial and final plasma sodium concentrations were the same.

Biopsy Procedures

A baboon heart without coronary artery ligation was subjected to ten consecutive slice biopsies. Values...
for ATP, inorganic phosphate and creatine phosphate in the first four slices were similar to those obtained with drill biopsies, whereas CP values decreased in biopsies from the 5th to the 10th slice. In another baboon heart, six consecutive drill biopsies were taken in rapid succession; ATP, inorganic phosphate and CP values were similar in all six biopsies. Glycogen values were constant (29.9 ± 1.5 μmole glucose equivalent/g, first four slices; 28.5 ± 1.5 μmole glucose equivalent/g, last three slices). Lactate/pyruvate ratios were 39 ± 6 in the first four slices, 105 ± 15 in the next three slices and 318 ± 53 in the last three slices; however, the ratio α-glycerophosphate/dihydroxyacetone phosphate stayed constant, being 18 ± 2 in the first four slices, and 16 ± 5 in the last three slices. Lactate/pyruvate ratios in tissue biopsies taken with the drill method were 26 ± 7 (N = 4). It was concluded that four slice biopsies or six in situ drill biopsies could be taken from a baboon heart without substantial changes in the measured biochemical parameters. Slice biopsies were used for analysis of glycolytic intermediates which required large amounts of tissue for assay by a conventional spectrophotometer; the values of high-energy phosphate compounds, lactate and glycogen obtained from slice biopsies were added to the drill biopsy results.

Regional Variations in Nonligated Hearts

It could be that there are significant regional variations in contents of electrolytes and of glycogen, as suggested for the dog heart; hence multiple drill biopsies from areas 1-4 (apex-base) were taken from six control hearts (no ligation), were divided into epicardial and endocardial halves and analyzed for ATP, ADP, AMP, CP, inorganic phosphate, lactate, glycogen, K⁺ and Na⁺. In no case was there a significant regional variation in the baboon heart, or between epicardial and endocardial halves in these nonligated hearts.

Error Introduced by Sampling Techniques after Coronary Artery Ligation

Our conclusions are based on transmural biopsies. In the dog there is thought to be more endocardial than epicardial damage following coronary artery ligation; hence analysis of transmural biopsies could obscure changes in one layer of the myocardium. Following coronary artery ligation in six baboons, drill biopsies were split into endocardial and epicardial halves and results from infarct areas compared. Values of phosphocreatine, inorganic phosphate, ADP and lactate were similar in endocardial and epicardial halves. In the infarct areas, values for ATP were lower in the epicardial tissue (1.58 ± 0.52 μmole/g, N = 9) compared with endocardial tissue (2.11 ± 0.42 μmole/g; P < 0.05); values for glycogen in epicardial infarct tissue (7.1 ± 1.2 μmole glucose equivalent, N = 11) were also lower than endocardial values (12.3 ± 2.5 μmole/g, P < 0.05). Thus an error could have been introduced in the case of ATP and glycogen by taking endocardial and epicardial halves together.

Effect of Coronary Artery Ligation on Tissue Biopsy Values

The results of tissue biopsy tables are given in tables 1 and 2.

The results of arterial ligation in group B baboons (arterial ligation but no therapeutic infusions) were: a fall in values of ATP and phosphocreatine in central and peripheral infarct areas; a rise in inorganic phosphate; increased values of AMP from 0.13 ± 0.02 (N = 13) in nonligated hearts to 0.53 ± 0.11 (N = 4) in infarct tissue; a rise in the ratio lactate/pyruvate; a rise in contents of lactate and α-glycerophosphate; a fall in glycogen; a fall in tissue K⁺/Na⁺ ratio; a fall in homogenate pH. The contents of inorganic phosphate rose in the peri-infarct and in nonischemic zones (areas 3 and 4). In KCl-infused baboons the patterns of change were similar to those found in ligation baboons except that the tissue K⁺/Na⁺ ratio in the nonischemic zone fell.

Comparing GIK-infused baboons with ligated baboons, ATP rose threefold in the central infarct area. Creatine phosphate and glycogen increased about twofold in central and peripheral infarct areas. There was a decreased content of AMP (0.35 ± 0.06 μmole/g, N = 17 for infarct tissue; P < 0.05 versus ligation or ligation + KCl). ADP did not change after ligation or KCl or GIK. Inorganic phosphate also decreased in the peripheral infarct area. The inorganic phosphate content in biopsies from peri-infarct and nonischemic areas in GIK-infused baboons was similar to that in the ligated group, and increased when compared with nonligated controls. That this was a real finding was shown by the sum of the values for ATP, ADP, Pi and CP which were, respectively, in areas 3 and 4 (μmole/g) 14.99 and 15.80 in nonligated controls; 15.09 and 15.91 in ligated hearts; 15.22 and 15.53 in KCl-infused hearts; and 17.27 and 17.23 in GIK-infused hearts. Increased tissue uptake of phosphate is known to occur after insulin administration.

Comparing GIK- with KCl-infused baboons, values of CP and the K⁺/Na⁺ ratio were higher in the peripheral infarct zone and values of AMP, lactate/pyruvate ratio, and α-glycerophosphate were lower (central and peripheral infarct tissue grouped) in the GIK group. The K⁺/Na⁺ ratio was also higher in peri-infarct and nonischemic zones in GIK- than in KCl-infused hearts.

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### Table 1

**Baboon Heart Contents of ATP, Creatine Phosphate, Inorganic Phosphate; Tissue K⁺/Na⁺ Ratios; and Homogenate pH Values. Effects of Infarction and of Additional Infusions of KCl or GIK Within 60 Minutes of Coronary Artery Ligation**

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Condition</th>
<th>No. of baboons</th>
<th>Center Zone</th>
<th>Periphery Zone</th>
<th>Peri-infarct zone</th>
<th>Nonischemic zone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Zone 1</td>
<td>Zone 2</td>
<td>Zone 3</td>
<td>Zone 4</td>
</tr>
<tr>
<td>ATP</td>
<td>Control</td>
<td>(7)</td>
<td>4.5 ± 0.5</td>
<td>5.4 ± 0.2</td>
<td>5.4 ± 0.2</td>
<td>4.2 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>Ligation</td>
<td>(6)</td>
<td>0.4 ± 0.1*</td>
<td>1.5 ± 0.4*</td>
<td>3.8 ± 0.1</td>
<td>4.2 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>+ KCl</td>
<td>(9)</td>
<td>0.8 ± 0.2*</td>
<td>1.9 ± 0.6*</td>
<td>3.6 ± 0.4</td>
<td>4.0 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>+ GIK</td>
<td>(16)</td>
<td>1.0 ± 0.2*, b</td>
<td>2.2 ± 0.3*</td>
<td>4.0 ± 0.2</td>
<td>4.3 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>Ligation</td>
<td>(8)</td>
<td>8.6 ± 0.8</td>
<td>9.1 ± 0.6</td>
<td>7.9 ± 0.5</td>
<td>8.9 ± 0.4</td>
</tr>
<tr>
<td>Creatine phosphate</td>
<td>Control</td>
<td>(10)</td>
<td>2.5 ± 0.3</td>
<td>2.3 ± 0.2</td>
<td>2.1 ± 0.2</td>
<td>2.1 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>Ligation</td>
<td>(6)</td>
<td>13.0 ± 1.7*</td>
<td>11.4 ± 0.9*</td>
<td>5.2 ± 1.1*</td>
<td>3.7 ± 0.6*</td>
</tr>
<tr>
<td></td>
<td>+ KCl</td>
<td>(5)</td>
<td>10.0 ± 0.6*</td>
<td>8.4 ± 0.9*</td>
<td>5.1 ± 0.7*</td>
<td>3.5 ± 0.5*</td>
</tr>
<tr>
<td></td>
<td>+ GIK</td>
<td>(15)</td>
<td>9.1 ± 1.1*</td>
<td>6.7 ± 0.7*, b</td>
<td>5.2 ± 0.5*</td>
<td>3.5 ± 0.2*</td>
</tr>
<tr>
<td>K⁺/Na⁺ ratio</td>
<td>Control</td>
<td>(10)</td>
<td>1.8 ± 0.11</td>
<td>1.9 ± 0.16</td>
<td>1.9 ± 0.11</td>
<td>1.9 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>Ligation</td>
<td>(11)</td>
<td>1.4 ± 0.09*</td>
<td>1.5 ± 0.10*</td>
<td>1.7 ± 0.26</td>
<td>1.8 ± 0.07</td>
</tr>
<tr>
<td></td>
<td>+ KCl</td>
<td>(8)</td>
<td>1.5 ± 0.12</td>
<td>1.5 ± 0.10*, b</td>
<td>1.5 ± 0.10*</td>
<td>1.6 ± 0.07*, b</td>
</tr>
<tr>
<td></td>
<td>+ GIK</td>
<td>(15)</td>
<td>1.5 ± 0.11</td>
<td>1.8 ± 0.08*, c</td>
<td>1.9 ± 0.09*</td>
<td>1.9 ± 0.09*</td>
</tr>
<tr>
<td>Homogenate pH</td>
<td>Control</td>
<td>(3)</td>
<td>7.1 ± 0.07</td>
<td>7.1 ± 0.04</td>
<td>7.1 ± 0.04</td>
<td>7.3 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>Ligation</td>
<td>(8)</td>
<td>6.6 ± 0.06*</td>
<td>6.7 ± 0.10*</td>
<td>7.1 ± 0.06</td>
<td>7.1 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>+ KCl</td>
<td>(6)</td>
<td>6.5 ± 0.11*</td>
<td>6.7 ± 0.09*</td>
<td>6.9 ± 0.07*</td>
<td>7.1 ± 0.04*</td>
</tr>
<tr>
<td></td>
<td>+ GIK</td>
<td>(12)</td>
<td>6.4 ± 0.10*</td>
<td>6.6 ± 0.09*</td>
<td>6.6 ± 0.06*</td>
<td>7.1 ± 0.04*</td>
</tr>
</tbody>
</table>

Abbreviations: a = P < 0.05 vs control (no ligation); b = P < 0.05 vs ligation; c = P < 0.05 vs ligation + KCl.
Number of biopsies in each group equal to number of baboons except in area 4 (two biopsies from each baboon). Mean values = x ± s.e.m. ATP, CP, and inorganic phosphate expressed as μmol/g wet wt; pH as pH units.

* = P 0.59 vs ligation + KCl.

### Table 2

**Baboon Heart Contents of Glycogen, Lactate, α-Glycerophosphate and Hexosemonophosphates, and Ratios of Lactate/Pyruvate. Effects of Infarction and of Additional Infusion of KCl or GIK Within 60 Minutes of Coronary Artery Ligation**

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Condition</th>
<th>No. of baboons</th>
<th>Infarct zones Zones 1 &amp; 2</th>
<th>Peri-infarct zone Zone 3</th>
<th>Nonischemic zone Zone 4</th>
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</thead>
<tbody>
<tr>
<td>Glycogen</td>
<td>Control</td>
<td>(12)</td>
<td>44 ± 4</td>
<td>42 ± 6</td>
<td>41 ± 4</td>
</tr>
<tr>
<td></td>
<td>Ligation</td>
<td>(12)</td>
<td>10 ± 2*, b</td>
<td>33 ± 7</td>
<td>40 ± 3</td>
</tr>
<tr>
<td></td>
<td>+ KCl</td>
<td>(2)</td>
<td>12 ± 1*</td>
<td>42 ± 3*</td>
<td>46 ± 4</td>
</tr>
<tr>
<td></td>
<td>+ GIK</td>
<td>(7)</td>
<td>21 ± 4*, b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactate</td>
<td>Control</td>
<td>(8)</td>
<td>1.9 ± 0.3</td>
<td>2.2 ± 0.5</td>
<td>1.9 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>Ligation</td>
<td>(8)</td>
<td>21.1 ± 2.4*, b</td>
<td>3.2 ± 0.4</td>
<td>2.1 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>+ KCl</td>
<td>(10)</td>
<td>23.5 ± 3.1*</td>
<td>5.5 ± 1.3*, b</td>
<td>2.4 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>+ GIK</td>
<td>(16)</td>
<td>17.8 ± 1.6*</td>
<td>5.9 ± 1.2*, b</td>
<td>3.5 ± 0.4*, b</td>
</tr>
<tr>
<td>α-glycerophosphate</td>
<td>Control</td>
<td>(4)</td>
<td>0.6 ± 0.009</td>
<td>0.5 ± 0.13</td>
<td>0.5 ± 0.07</td>
</tr>
<tr>
<td></td>
<td>Ligation</td>
<td>(4)</td>
<td>1.9 ± 0.24*, b</td>
<td>0.4 ± 0.26</td>
<td>0.2 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>+ KCl</td>
<td>(7)</td>
<td>2.5 ± 0.21*, b</td>
<td>1.0 ± 0.19</td>
<td>0.5 ± 0.07</td>
</tr>
<tr>
<td></td>
<td>+ GIK</td>
<td>(7)</td>
<td>1.5 ± 0.19*, c</td>
<td>0.7 ± 0.14</td>
<td>0.4 ± 0.06</td>
</tr>
<tr>
<td>Hexosemonophosphates</td>
<td>Ligation + KCl</td>
<td>(5)</td>
<td>599 ± 125</td>
<td>421 ± 80</td>
<td>188 ± 80</td>
</tr>
<tr>
<td></td>
<td>Ligation + GIK</td>
<td>(7)</td>
<td>547 ± 83</td>
<td>499 ± 97</td>
<td>387 ± 55</td>
</tr>
<tr>
<td>Ratio L/P</td>
<td>Control</td>
<td>(4)</td>
<td>38 ± 14</td>
<td>32 ± 11</td>
<td>40 ± 14</td>
</tr>
<tr>
<td></td>
<td>Ligation</td>
<td>(4)</td>
<td>533 ± 122*, b</td>
<td>58 ± 7</td>
<td>51 ± 12</td>
</tr>
<tr>
<td></td>
<td>+ KCl</td>
<td>(3)</td>
<td>586 ± 117*, b</td>
<td>53 ± 8</td>
<td>42 ± 13</td>
</tr>
<tr>
<td></td>
<td>+ GIK</td>
<td>(4)</td>
<td>315 ± 60*, c</td>
<td>124 ± 38*</td>
<td>66 ± 12</td>
</tr>
</tbody>
</table>

Abbreviations: a = P < 0.05 vs control (no ligation); b = P < 0.05 vs ligation; c = P < 0.05 vs KCl. Number of biopsies = 2 × number of baboons for infarct and non-ischemic zones.
Units: μmol/g wet wt (glycogen = μmol glucose equivalent); hexosemonophosphates = nmol/g wet wt.
Mean = S.E.M (number of biopsies).
In both GIK- and KCl-infused animals contents of glucose 6-phosphate and fructose 6-phosphate rose in the infarct area, while contents of fructose diphosphate and citrate fell. Values of hexosephosphates (glucose 6-phosphate and fructose 6-phosphate) and of citrate were similar in GIK- and in KCl-infused animals, except that the contents of hexosemonophosphates approximately doubled in non-ischemic tissue (area 4) after GIK infusion. The value of fructose 6-phosphate also doubled from 27 ± 6 (N = 8) to 69 ± 10 (N = 9) nmole/g.

Epicardial ECG

In baboons with ligation only (group B), the peak epicardial ST-segment elevation was 13 ± 4 (N = 7) mV at 15 min and 24 ± 2 (N = 11) mV at 60 min. Values in KCl-infused baboons were 20 ± 4 (N = 4) mV at 15 min and 20 ± 5 (N = 7) mV at 60 min. In GIK-infused baboons values were 14 ± 2 (N = 10) at 15 min and 22 ± 2 (N = 16) at 60 min postligation. Mean ST-segment elevation (i.e., total ST elevation in all areas divided by number of areas recorded) was similar in all groups. The only detectable effect of GIK infusions was that the peri-infarct ST-segment depression stayed constant over 60 min (−0.4 ± 0.1 at 15 min postligation, N = 10; −0.3 ± 0.1 at 60 min, N = 16) whereas in other groups the ST-segment depression doubled (−0.4 ± 0.1 at 15 min, N = 9; −0.9 ± 0.2 at 60 min, N = 12).

Discussion

Metabolic Changes in Infarct Zones

Major metabolic changes which develop in ischemic tissue within the first minutes of coronary artery ligation in dogs include depletion of high-energy phosphate compounds and glycogen, lactate accumulation, and probably, intracellular acidosis. An increased ratio of NADH/NAD+ (nicotinamide adenine dinucleotide) is reflected in increased tissue ratios of lactate/pyruvate.

Tissue K+ depletion and impaired mitochondrial function develop within the first hour but become more clearly evident after several hours. Even later visible tissue necrosis occurs. It is not yet known how these sequential changes are linked but a significant parallel irreversible damage occurs within 20-60 min of arterial occlusion in severely ischemic tissue in the dog. If a given therapeutic intervention were to improve the outcome of coronary artery ligation, some effect might be expected within the first 60 min; thus we studied the effect of GIK (glucose, insulin, potassium) infusions on early tissue metabolic changes of regional ischemia in the baboon. However, our model has definite limitations.

Limitations of the Model

These open-chest baboons were not in a steady metabolic state in that both blood glucose and fatty acid concentrations rose even in the control non-ligated group. Thoracotomy in the baboon increases circulating catecholamine concentrations, and increased sympathetic activity could explain the metabolic changes as well as the marked tendency to develop ventricular fibrillation in ligated baboons. In man there are marked metabolic changes within the first hour of myocardial infarction, including hyperglycemia and increased concentrations of circulating FFA and of catecholamines; hence another defect of our model was that systemic metabolic changes were similar in nonligated and ligated baboons. In addition, plasma K+ decreased during the experimental period in both control (nonligated) and ligated animals, whereas it remained constant in GIK-infused animals. Infusions of KCl were also given with the aim of keeping the blood K+ constant; however, KCl infusion had no major effects on tissue metabolic changes.

GIK Effects on Infarct and Peri-infarct Tissue

When compared with the ligated group, GIK infusions resulted in lessening of the severity of tissue metabolic damage in the infarct zones. GIK infusion reduced the loss of ATP in the infarcting tissue by about 15%, while glycogen loss was reduced by about 25%. Compared with KCl, GIK increased CP and the ratio K+/Na+ in the peripheral infarct zone, and the ratio K+/Na+ in the peri-infarct zone.

An increased rate of anaerobic glycolysis and of anaerobic synthesis of ATP has been thought to be a desirable effect of GIK infusions on ischemic tissue. Increased flow through glycolysis and, hence, through the enzyme phosphofructokinase during GIK infusions would require either altered increased concentrations of hexosemonophosphates (substrates of phosphofructokinase) or else altered concentrations of effectors of the enzyme such as ATP, citrate and H+(which inhibit the enzyme) or of ADP, AMP, inorganic phosphate and fructose 1,6-diphosphate (which accelerate the activity of the enzyme).

Under the influence of GIK, tissue ATP rose and inorganic phosphate fell; AMP fell; ADP, citrate and hexosemonophosphates were unchanged within the infarct. The over-all effect of these factors would be to limit rather than stimulate glycolytic flux through phosphofructokinase in the infarct zones. Reservations are that 1) in no case could the actual concentrations of the above chemicals available to the enzyme in the cell be tested, but only over-all tissue contents were measured; and 2) not all known effectors of phosphofructokinase were measured, particularly sul-
fate\textsuperscript{22} and cyclic AMP.\textsuperscript{33} However, the absence of increased tissue content of lactate and α-glycerophosphate, and the unchanged tissue pH values after GIK also argue against the possibility of the increased glycolytic flow in the infarcting myocardium. We could not exclude an increased glycolytic flux occurring before the biopsies were taken, with increased tissue high-energy phosphate compounds and decreased inorganic phosphate, and a secondary inhibition of glycolytic flux. It should be stressed that GIK infusions did not increase tissue lactate in the infarction tissue; increased tissue lactate has been thought to be an undesirable effect of adding insulin to the wholly ischemic isolated rat heart perfused with glucose.\textsuperscript{34}

In the peri-infarct border zone, GIK infusions were associated with an increased tissue phosphate content but only when compared with the nonligated control group. Even in the absence of changes in other measured effectors of phosphofructokinase, increased glycolytic flux is possible and could account for the increased tissue lactate and lactate/pyruvate ratio in the peri-infarct zone. However, comparing GIK with ligation baboons, tissue glycogen was increased in this zone suggesting increased uptake of glucose and conversion to glycogen. Similar values for inorganic phosphate in ligated, KCl and GIK groups suggest similar rates of glycolytic flux (and anaerobic ATP production) in the absence of other measured effectors of phosphofructokinase. Hence another mode of action of GIK needs to be invoked to explain the increased K\textsuperscript{+}/Na\textsuperscript{+} ratio in the peri-infarct zone (compared with KCl).

Sodi-Pallares and co-workers originally suggested increased transport of K\textsuperscript{+} into ischemic cells as a basis of their "polarizing" therapy,\textsuperscript{35} and GIK effects on tissue K\textsuperscript{+}/Na\textsuperscript{+} ratio would be compatible with the suggestions of Sodi-Pallares. However, GIK caused changes in the central infarct zone where ATP and creatine phosphate doubled (comparing GIK with ligation controls) but without K\textsuperscript{+}/Na\textsuperscript{+} changes. Thus, neither glycolytic flux changes nor "polarization" could explain all the findings with GIK.

GIK decreased circulating FFA concentrations by half, and therefore probably caused a corresponding decrease in the uptake of FFA which is related to the arterial concentration of FFA in both ischemic and nonischemic tissue.\textsuperscript{36} In the isolated rat heart, even physiological concentrations of FFA appeared to promote development of the infarction process as judged by rates of enzyme release, while the addition of glucose and/or insulin decreased enzyme release.\textsuperscript{37} The mechanisms involved are not yet clear but the phenomenon may be relevant to GIK effects. Thus "polarization" and decreased uptake of circulating FFA could possibly have contributed to the effects of GIK on the peri-infarct and infarct zones, respectively.

Changes in Nonischemic Tissue
The findings of Gudbjarnason and co-workers\textsuperscript{38} have suggested that the so-called "normal," nonischemic myocardium (our zone 4) may develop metabolic changes after myocardial infarction, notably decreased contents of ATP and creatine phosphate. Following GIK infusions, lactate and glycogen rose when compared with ligation baboons and creatine phosphate and the ratio K\textsuperscript{+}/Na\textsuperscript{+} rose when compared with KCl-infused baboons. The increased inorganic phosphate following GIK is interpreted as increased uptake of phosphate\textsuperscript{23} because of the absence of changes in high-energy phosphate compounds in this zone. Increased lactate, together with increased glycogen, could result from increased glucose uptake and increased glycolytic flux under the influence of GIK. Contents of hexosemonophosphates approximately doubled in the nonischemic zone, also suggesting increased flow through glycolysis after GIK infusion (tissue contents of other substances regulating phosphofructokinase activity were similar to those in the ligated group). As assessed by our indirect methods, GIK probably increased glycolytic flux to the "nonischemic" zone but probably not to other zones. Unchanged rates of glycolytic flux do not exclude the occurrence of increased glucose uptake and glycogen formation, which could explain increased glycogen in all zones when comparing GIK and ligation groups.

Relation of Biochemical Changes to Tissue Necrosis
Both glycogen depletion and a K\textsuperscript{+}/Na\textsuperscript{+} ratio of less than 1.0 are associated with tissue necrosis in established myocardial infarction.\textsuperscript{39,40} In early experimental myocardial infarction, 6½ hours after coronary artery ligation in the dog, we found a positive correlation between histologic criteria of infarction and the degree of decrease in glycogen or tissue K\textsuperscript{+}/Na\textsuperscript{+}.\textsuperscript{41} Thus, increased tissue glycogen and K\textsuperscript{+}/Na\textsuperscript{+} ratios found in some zones after GIK infusions in the present series would be compatible with an effect of GIK in diminishing tissue necrosis in the baboon, but firm proof of reduction of infarct size by GIK would require further studies over longer experimental periods.

GIK and Epicardial ECG
In our experiments, contrary to findings in the dog,\textsuperscript{6} ST-segment elevation increased with time and the 15 min value could not be used to predict infarct size, nor did GIK alter the rate of rise of the ST segment. However, epicardial tissue in the ischemic area in the
dog is less severely damaged than endocardial tissue, whereas both our microsphere and our biopsy studies show either equal damage to both myocardial layers or lesser damage to endocardial tissue in ischemic baboon tissue. Another difference from the results of Maroko et al., was that ST elevation was greater in our experiments, probably indicating severer degrees of ischemia in the baboon than in the dog.

In the baboon administration of GIK diminished the rate of development of ST-segment depression in peri-infarct zone 3; in the dog the significance of ST depression is not clarified although recent data suggest that ST-segment depression indicates areas of mild ischemia more likely to respond to therapy.

Application to Reduction of Infarct Size in Patients

Reservations in applying the above therapeutic findings on baboons with coronary artery ligation to patients with acute myocardial infarction include the following. First, the infusions were started three minutes after the onset of coronary artery occlusion whereas medical help may not reach a patient for some hours. However, this study was deliberately designed to assess the effects of GIK infusion in the first sixty minutes after infarction because more and more emphasis is placed on reaching the patient as soon as possible. Secondly, sudden ligation of the previously healthy coronary artery in an open-chest animal may well not be a valid model of human myocardial infarction which may occur on the basis of widespread coronary artery disease, or even without obvious coronary artery occlusion. Patients may have pre-existing damage to other parts of the myocardium whereas the baboon myocardium appeared to be entirely healthy before coronary artery ligation. Thirdly, the present experiments (and some showing a beneficial effect of GIK in dogs with coronary artery ligation) involved relatively small infarcts, whereas patients with the largest infarcts may be in greatest need of therapy to reduce infarct size.

Nevertheless, our studies appear to be the first defining an effect of GIK on tissue metabolic parameters in a subhuman primate. It should be noted that our studies do not allow definition of the active principle in GIK, although there were a number of differences between KCl- and GIK-infused baboons. The baboon is anatomically, phylogenetically and behaviorally closer to man than is the dog used in previous animal studies with GIK.

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