EFFECTS OF GLUCOSE-INSULIN-POTASSIUM SOLUTION ON FREE FATTY ACID METABOLISM IN ISCHEMIC MYOCARDIUM

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The accumulation of intermediates subsequent to impaired oxidation of free fatty acids has been suggested as a cause of cellular damage in ischemic myocardium. Many reviews have supported the theory that glucose-insulin-potassium (GIK) solution has a beneficial effect on the ischemic myocardium. We evaluated the effects of GIK solution on intermediates of free fatty acid metabolism in ischemic myocardium.

The left coronary artery was occluded for 40 minutes in twelve dogs. In six dogs, 10 minutes before coronary artery occlusion, GIK solution (50 per cent of glucose, 50 units/liter of regular insulin, 50 mEq/liter of potassium) was given at the rate of 0.1 ml/kg per minute until the time of excision of the heart.

In the ischemic area, adenosine triphosphate (ATP) level in the GIK group ($3.80 \pm 1.34 \mu\text{mole/g}$) was significantly higher than that in the control group (2.04 ± 0.68 , $p < 0.05$). The free carnitine level was significantly increased was GIK in both ischemic and nonischemic areas ($p < 0.05$). In the control group, the long chain acyl coenzyme A (CoA) level in the ischemic area ($23.0 \pm 7.0 \text{ nmole/g}$) was significantly higher than that in the nonischemic area (17.1 ± 3.5 , $p < 0.05$). On the other hand, GIK prevented the increase in the long chain acyl CoA in the ischemic area (17.8 ± 5.6).

This study suggests that GIK has a protective effect on ischemic myocardium, probably by preventing the accumulation of long chain acyl CoA by improving free fatty acid metabolism.

INTEREST in the glucose-insulin-potassium (GIK) solution started with the work of Sodi-Pallares and his co-worker.¹ They claimed GIK solution to be a polarizing solution for the treatment of ventricular arrhythmias due to myocardial infarction. More recently, Maroko² and other have strongly supported the use of

GIK, and attention has shifted away from the repolarizing action of GIK towards its effects on energy metabolism and its possible effect in restricting infarct size. Treatment with GIK was introduced in the hope of enhancing anaerobic glycolysis. However, glycolysis in the ischemic myocardium is inhibited by the accumulation of H^+ , lactate, and the increased NADH/NAD ratio.³ Therefore, GIK increases the intracellular concentration of glucose, but this effect may not be followed by an acceleration in glycolytic flux.⁴

Other possible mechanisms of GIK include interaction with lipid metabolism, hyperosmolar

Key Words:

Glucose-insulin-potassium solution
Free fatty acid
Carnitine
Long chain acyl CoA
Ischemic myocardium

(Received November 24, 1983; accepted February 16, 1984)

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TABLE 1 EFFECTS OF GIK ON TISSUE LEVELS OF VARIOUS METABOLITES

	Free carnitine (nmole/g)	Long chain acyl CoA (nmole/g)	Long chain acyl carnitine (nmole/g)	Long chain acyl CoA		ATP (μ mole/g)	Free fatty acid (μ Eq/g)
				Free carnitine			
Nonischemic area							
Control (n = 6)	1110 $\pm$ 415	17.1 $\pm$ 3.5	250 $\pm$ 62	1.87 $\pm$ 0.49	5.48 $\pm$ 0.50	8.54 $\pm$ 0.54	
GIK (n = 6)	1418 $\pm$ 370	14.0 $\pm$ 2.4	213 $\pm$ 35	1.06 $\pm$ 0.28*	5.89 $\pm$ 0.63	8.16 $\pm$ 0.45	
Ischemic area							
Control (n = 6)	855 $\pm$ 375##	23.0 $\pm$ 7.0#	279 $\pm$ 117	3.76 $\pm$ 1.89	2.04 $\pm$ 0.68##	8.71 $\pm$ 0.62	
GIK (n = 6)	1104 $\pm$ 320*#	17.8 $\pm$ 5.6†	346 $\pm$ 147	1.63 $\pm$ 0.47†	3.80 $\pm$ 1.34*##	8.30 $\pm$ 0.88	

Values are expressed per gram wet tissue weight and represented as mean $\pm$ S.D.. The significances of the changes are represented as follows:
 nonischemic area vs ischemic area (paired t test) ## = $p < 0.01$, # = $p < 0.05$; the control group vs the GIK group (non-paired t test) * = $p < 0.05$,
 † = $p < 0.1$

action and a restoration of intracellular potassium level.^{7,21} It is noted that GIK acts on adipose tissue to inhibit lipolysis and reduce circulating free fatty acids and their uptake by the myocardium.⁵⁻⁷ During myocardial ischemia, beta-oxidation of free fatty acids is inhibited by the limited supply of oxygen and long chain acyl coenzyme A (CoA) and long chain acyl carnitine increase in the cytosol. The accumulation of intermediates subsequent to impaired oxidation of free fatty acids has been suggested as a cause of the cellular damage in ischemic myocardium⁸⁻¹⁰ and reducing these metabolites may be desirable.¹¹⁻¹⁴ Therefore, it is interesting to ascertain whether GIK has any effect on these metabolic changes in ischemic myocardium. The purpose of this study is to observe the changes in the tissue levels of free fatty acid, carnitine derivatives, long chain acyl CoA and adenosine triphosphate (ATP) following myocardial ischemia and to evaluate the effect of GIK solution on these metabolites.

MATERIALS AND METHODS

Twelve mongrel dogs of either sex, weighing 10 to 12 kg, were anesthetized by a single intravenous injection of sodium pentobarbital (30 mg/kg). The trachea was intubated and respired with room air using a Harvard respirator pump. After attachment of limb leads for electrocardiographic recording, thoracotomy was performed in the left fifth intercostal space. The pericardium was opened and the heart was suspended in a pericardial cradle. The left anterior descending coronary artery was dissected free from surrounding tissue below the first diagonal branch and occluded with an intracranial arterial clamp for 40 minutes. Dogs which demonstrated cyanosis on the anteroapical wall of the heart were used in this study. In six dogs, 10 minutes before occlusion of the coronary artery, an infusion of GIK solution (50 per cent of glucose, 50 units/liter of regular insulin, 50 mEq/liter of potassium) into the left external cervical vein was given at the rate of 0.1 ml/kg per minute until the time of excision of the heart. The six dogs in the control group received an equal volume of saline.

After forty minutes occlusion the beating hearts were removed from the dogs and transmural myocardial samples representing the ischemic area (supplied by the occluded left descending coronary artery) and the nonischemic

Fig.1. Postulated Effects of GIK Solution on Ischemic Myocardial Metabolism.

Circulating glucose is increased (1). Glucose uptake across the cell membrane is increased (2). Cardiac glycogen rises (3). Glycolytic flux is increased (4). Circulating free fatty acids are decreased by inhibition of lipolysis (5). Free fatty acids uptake across the cell membrane is decreased (6). Removal of acyl CoA (7). Acyl CoA inhibits the mitochondrial translocase system which would account for the decreased myocardial content of ATP (dotted line). Acyl CoA oxidation is increased by maintaining at a high level of free carnitine (8).

area (supplied by the circumflex coronary artery) were rapidly excised. Tissue samples were immediately frozen with a Wollenberger clamp maintained at the temperature of liquid nitrogen for the determination of the metabolites. Analytic details are described elsewhere.¹⁴ Free carnitine was determined enzymatically using carnitine acetyl transferase by the method of Marquis and Fritz.¹⁵ Long chain acyl carnitine was assayed as free carnitine after alkaline hydrolysis by the method of Pearson et al.¹⁶ Long chain acyl CoA was assayed as free CoA after alkaline hydrolysis by the method of Veloso and Veech.¹⁷ ATP was determined by enzyme assay using hexokinase and glucose-6-phosphate dehydrogenase according to the method of Lamprecht and Trautshold.¹⁸ Free fatty acid was determined by the method of Itaya and Ui.¹⁹ Values were expressed for wet tissue weight as mean $\pm$ S.D.. Statistical analysis was performed by paired or non-paired Student's t test and a probability of less than 0.05 was used to indicate a significant difference.

RESULTS

The effects of GIK solution on tissue levels of various metabolites were shown in Table I. The ATP level in the ischemic area decreased markedly both in the control and the GIK groups. However, the ATP level in the GIK group remained at a higher level than that in the control group ($3.80 \pm 1.34 \mu\text{mole/g}$ vs $2.04 \pm 0.68 \mu\text{mole/g}$, $p < 0.05$). Free fatty acid showed no significant differences between the ischemic and the nonischemic areas both in the control and the GIK groups. Free carnitine levels in the ischemic areas were significantly decreased compared with those in the nonischemic areas both in the control ($p < 0.01$) and the GIK groups ($p < 0.05$). Furthermore in the ischemic area, the free carnitine level in the GIK group was at a higher level than that in the control group ($1104 \pm 320 \text{ nmole/g}$ vs $855 \pm 375 \text{ nmole/g}$, $p < 0.05$). Long chain acyl carnitine showed no significant differences between the ischemic and the nonischemic areas both in the control

and the GIK groups. In the control group, the long chain acyl CoA level in the ischemic area increased significantly compared with that in the nonischemic area (23.0 ± 7.0 nmole/g vs 17.1 ± 3.5 nmole/g, $p < 0.05$). On the other hand, in the GIK group, there was no statistically significant difference between the ischemic and the nonischemic areas (17.8 ± 5.6 nmole/g vs 14.0 ± 2.4 nmole/g). The long chain acyl CoA level in the GIK group was at a lower level than that in the control group.

In the ischemic area, the ratio of long chain acyl CoA to free carnitine, which was an index of relative deficiency of carnitine, was markedly elevated in the control group (1.87 ± 0.49 to 3.76 ± 1.89), whereas it was small in the GIK group (1.06 ± 0.28 to 1.63 ± 0.47).

DISCUSSION

GIK solution was first popularized by Sodi-Pallares and his co-workers¹ as a polarizing agent that could restore K^+ loss from the ischemic area and hence improve mitochondrial function. More recently, Maroko et al² have shown that GIK solution can inhibit myocardial necrosis in dogs and there are many reports about the beneficial effects of GIK solution on ischemic myocardium. We found in this study that GIK increased the ATP level in the ischemic area. This finding was in agreement with the experiences of other investigators.^{6,20} Opie and Owen²⁰ reported that GIK solution improved high energy phosphate compounds in the infarcting myocardium. GIK solution was introduced in the hope of enhancing anaerobic glycolysis (Fig. 1) but further increase in glycolysis is limited by the accumulation of H^+ and lactate and by the increased NADH/NAD ratio.⁴ Other possible beneficial effects of GIK solution include an increase in contractility due to hyperosmolar action, a reduction in circulating free fatty acids and their uptake by the myocardium, and a restoration of intracellular potassium level^{7,21} (Fig. 1). Mantle et al²⁷ have shown that the left ventricular function improves during GIK infusion, suggesting improved mechanical performance of the infarcted left ventricle. But its definitive mechanism has not yet been described in detail. In this study, GIK solution increased the ATP level and the free carnitine level and improved the accumulation of long chain acyl CoA in the ischemic myocardium. Moreover, the long chain acyl CoA to free

carnitine ratio, an index of relative deficiency of carnitine in tissue was improved by GIK solution. According to GIK interaction with the free fatty acid metabolism, Opie et al⁵ suggest that GIK solution may promote glycolysis and provide an increased supply of alpha-glycerophosphate for reesterification of intracellular free fatty acids, thus removing the postulated free fatty acids or acyl CoA accumulation in ischemic myocardium. During myocardial ischemia, beta-oxidation of free fatty acid is inhibited by the limited supply of oxygen and the resulting buildup of long chain acyl CoA and long chain acyl carnitine in the cytosol. These metabolites exert several deleterious effects: a) Long chain acyl CoA inhibits further formation of CoA esters from free fatty acid entering the cell, hence leading to an accumulation of free fatty acids. A high level of free fatty acid can impair sarcolemma integrity with their detergent action and can also precipitate arrhythmias.⁹ b) Long chain acyl CoA inhibits the activity of adenine nucleotide translocase, an important enzyme located in the inner mitochondrial membrane which transfers ATP synthesized in the mitochondria to the cytosol.⁸ Thus the lack of energy caused by ischemia is accentuated by the failure of newly synthesized ATP to reach the site of its utilization (Fig. 1). In fact, an excess of free fatty acid causes a significant decline in left ventricular work¹⁰ and ventricular arrhythmias²² in the ischemic heart.

Fatty acyl derivatives can not penetrate the inner mitochondrial membrane, hence carnitine is essential for fatty acyl derivatives to be transported to the site of oxidation in the mitochondria.^{23,24} A reduction of the tissue carnitine level has been demonstrated in the ischemic myocardium²⁵ and is considered to aggravate the ischemic damage, presumably by accelerating the accumulation of fatty acyl derivatives. It has already been mentioned that carnitine restores the translocation of adenine nucleotides inhibited by long chain acyl CoA.⁸ From this point of view, the protective effects of exogenous carnitine on metabolic derangements have been reported by several investigators.^{11-14,26} This study showed that GIK solution increased the ATP level, maintained a high level of free carnitine and improved the accumulation of long chain acyl CoA in the ischemic myocardium. Thus, GIK solution acts as an agent to alter free fatty acid metabolism and this action may also contribute to the beneficial effect of GIK solution on myocardial

ischemia.

This suggests that GIK solution has a protective effect on ischemic myocardium, probably by preventing the accumulation of long chain acyl CoA by improving free fatty acid metabolism.

REFERENCES

1. SODI-PALLARES D, TESTELLI MR, FISHLEDER FL: Effects of an intravenous infusion of a glucose-potassium-insulin solution on the electrocardiographic signs of myocardial infarction. A preliminary clinical report. *Am J Cardiol* 9: 166, 1962
2. MAROKO PR, LIBBY P, SOBEL BE, BLOOR CM, SYBERS HD, SHELL WE, COVELL JW, BRAUNWALD E: Effects of glucose-insulin-potassium infusion on myocardial infarction following experimental coronary artery occlusion. *Circulation* 45: 1160, 1972
3. ROVETTO MJ, LAMBERTON WF, NEELY JR: Mechanisms of glycolytic inhibition in ischemic rat hearts. *Circ Res* 37: 742, 1975
4. LIEDTKE AJ, NELLIS SH, NEELY JR, HUGHES HC: Effects of treatment with pyruvate and tromethamine in experimental ischemia. *Circ Res* 39: 378, 1976
5. OPIE LH: Metabolism of free fatty acids, glucose and catecholamines in acute myocardial infarction. Relation to myocardial ischemia and infarct size. *Am J Cardiol* 36: 938, 1975
6. OPIE LH, OWEN P: Effect of glucose-insulin-potassium infusions on arteriovenous differences of glucose and of free fatty acids and on tissue metabolic changes in dogs with developing myocardial infarction. *Am J Cardiol* 38: 310, 1976
7. OPIE LH: Effects of regional ischemia on metabolism of glucose and fatty acids. Relative rates of aerobic and anaerobic energy production during myocardial infarction and comparison with effects of anoxia. *Circ Res* 38 (Suppl I): I-52, 1976
8. SHUG AL, SHRAGO E, BITTAR N, FOLTS JD, KOKE JR: Acyl-CoA inhibition of adenine nucleotide translocation in ischemic myocardium. *Am J Physiol* 228: 689, 1975
9. KJEKSHUS JK, MJOS OD: Effect of free fatty acids on myocardial function and metabolism in the ischemic dog heart. *J Clin Invest* 51: 1767, 1972
10. LIEDTKE AJ, NELLIS S, NEELY JR: Effects of excess free fatty acids on mechanical and metabolic myocardium in swine. *Circ Res* 43: 652, 1978
11. SUZUKI Y, KAMIKAWA T, YAMAZAKI N: Protective effects of L-carnitine on ischemic heart. In *Carnitine: Biosynthesis, Metabolism, and Function*, ed by FRENKEL RA, MACGARRY JD, Academic Press, New York, 1980, p 341
12. FOLTS JD, SHUG AL, KOKE JR, BITTAR N: Protection on the ischemic dog myocardium with carnitine. *Am J Cardiol* 41: 1209, 1978
13. LIEDTKE AJ, NELLIS SH: Effects of carnitine in ischemic and fatty acid supplemented swine hearts. *J Clin Invest* 64: 440, 1979
14. SUZUKI Y, KAMIKAWA T, KOBAYASHI A, MASUMURA Y, YAMAZAKI N: Effects of L-carnitine on tissue levels of acyl carnitine, acyl CoA and high energy phosphate in ischemic dog hearts. *Jpn Circ J* 45: 687, 1981
15. MARQUIS NR, FRITZ IB: Enzymological determination of free carnitine concentrations in rat tissues. *J Lipid Res* 5: 184, 1964
16. PEARSON DJ, CHASE JFA, TUBBS PK: The assay of (1)-carnitine and its o-acyl derivatives. *Methods in Enzymology* 14: 612, 1969
17. VELOSO D, VEECH RL: Stoichiometric hydrolysis of long chain acyl-CoA and measurement of the CoA formed with an enzymatic cycling method. *Anal Biochem* 62: 449, 1974
18. LAMPRECHT W, TRAUTSHOLD I: Adenosine-5-triphosphate determination with hexokinase and glucose-6-phosphate dehydrogenase. *Methods of Enzymatic Analysis* 4: 2101, 1975
19. ITAYA K, UI M: Colorimetric determination of free fatty acids in biological fluids. *J Lipid Res* 6: 16, 1965
20. OPIE LH, BRUYNEEL K, OWEN P: Effects of glucose, potassium and insulin infusion on tissue metabolic changes within first hour of myocardial infarction in the baboon. *Circulation* 52: 49, 1975
21. BOLLI R: Protection of ischemic myocardium in experimental animals and in man: A review. *Cardiovasc Res Cent Bull* 21: 1, 1982
22. SUZUKI Y, KAMIKAWA T, YAMAZAKI N: Effects of L-carnitine on ventricular arrhythmias in dogs with acute myocardial ischemia and a supplement of excess free fatty acids. *Jpn Circ J* 45: 552, 1981
23. FRITZ LB, KAPLAN E, YUE KNT: Specificity of carnitine action on fatty acid oxidation by heart muscle. *Am J Physiol* 202: 117, 1962
24. PANDE SV: A mitochondrial carnitine acyl-carnitine translocase system. *Proc Nat Acad Sci USA* 72: 883, 1975
25. SHUG AL, THOMSEN JH, FOLTS JD, BITTAR N, KLEIN KI, KOKE JR, HUTH RJ: Changes in tissue levels of carnitine and other metabolites during myocardial ischemia and anoxia. *Arch Biochem Biophys* 187: 25, 1978
26. KOBAYASHI A, SUZUKI Y, KAMIKAWA T, HAYASHI H, MASUMURA Y, NISHIHARA K, ABE M, YAMAZAKI N: Effects of L-carnitine on ventricular arrhythmias after coronary reperfusion. *Jpn Circ J* 47: 536, 1983
27. MANTLE JA, RUSSEL RO Jr, MCDANIEL HG, ROGERS WJ, RACKLEY CE: Improved left ventricular mechanical performance during glucose-insulin-potassium infusion in patients with acute myocardial infarction. *Clin Res* 23: 513a, 1975