Sustained Effect of Glucose-Insulin-Potassium on Myocardial Performance during Regional Ischemia

ROLE OF FREE FATTY ACID AND OSMOLALITY

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ABSTRACT To evaluate the influence of glucose infusate administered with insulin and potassium on left ventricular function during 4 h of ischemia, as well as mechanism of action, four groups of intact anesthetized dogs were studied. Acute regional ischemia was induced with a balloon tip catheter in the left anterior descending artery and infusates were begun after 20 min of ischemia. A threefold increase of plasma glucose concentration was associated with improved left ventricular function during ischemia, compared to animals receiving isovolumic saline. There was a significant decline of left ventricular end-diastolic pressure associated with elevation of stroke volume and ejection fraction to control levels, as determined by indicator dilution. In a separate subgroup studied by cineangiography, shortening of the ischemic anterior wall, after an initial decline, was increased in response to glucose but there was no evidence of extension of injury. Ischemic tissue exhibited a smaller gain of water as well as Na^+ per gram dry weight as compared to ischemic controls. On precordial electrocardiogram mapping there was a significant decrease in the ΣST (sum of ST elevation) as well as NST (number of ST segment elevations), but the reduction of R wave amplitude was not different from controls. To further evaluate long-term effects, eight controls and six treated animals underwent myocardial ischemia and were sacrificed after 4 mo. Calculated area and weight of scar, as well as degree of wall thinning, were similar in both groups.

The glucose-treated animals had a significant decrease of plasma FFA in contrast to controls which manifested a significant rise. To examine the postulate that the decrease in FFA was important to therapeutic action, a third group was infused with Intralipid (Cutter Laboratories, Inc., Berkeley, Calif.) and heparin, simultaneously with the glucose infusate, to effect an elevation of plasma FFA during ischemia. Changes in myocardial function and electrolyte composition, as well as precordial electrocardiogram mapping, were similar to that of animals receiving glucose alone. Because serum osmolality was increased approximately 40 mosmol during the glucose infusion, the potential role of hyperosmolality was assessed by infusion of 20% mannitol during acute ischemia in a fourth group. After a transient small increase, there was a moderate decline in function by 4 h, suggesting that the response to glucose is not dependent upon extracellular osmolality. Thus, it is concluded that during the initial hours after the onset of myocardial ischemia the glucose infusate improves ventricular performance without evidence of arrhythmia induction or intensification of ischemic injury. Evolution of irreversible necrosis appears to be delayed rather than prevented under the circumstances of this study.

INTRODUCTION

The advent of effective antiarrhythmic therapy has changed the mortality patterns during acute myocardial infarction. Although pump failure has emerged as a major problem, classic treatment for heart failure has not demonstrated notable effects on survival rates (1). The ideal intervention would enhance ventricular function without evoking arrhythmias or extending the

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area of ischemia. Glucose-insulin-potassium (GIK)[1] appears to at least partially fulfill these criteria (2). The incidence of ventricular arrhythmias and fibrillation is reduced in the early period after the onset of experimental ischemia in intact animals with proximal occlusion of the left anterior descending artery (3, 4). In addition, the degree of injury as judged by epicardial electrocardiogram (ECG), myocardial morphology, and enzyme content is diminished (5).

In this investigation we have examined in the intact anesthetized dog the effects of the glucose infusate on myocardial function in the ischemic segment as well as total ventricle over 4 h. The extent and severity of injury were assessed from R wave amplitude as well as ST-segment alterations by precordial ECG mapping. Associated changes in myocardial electrolytes and water were utilized as criteria for the ischemic tissue response to particular interventions.

Because potential therapeutic effects of the glucose infusate have been postulated to depend upon a reduction of plasma and myocardial FFA levels (6, 7), we have used simultaneous infusions of triglyceride with heparin to counter the reduction of plasma FFA produced by infusion of the glucose solution. The alterations of left ventricular function and composition were compared with GIK-treated and control animals undergoing ischemia. In addition, the role of hyperosmolality in the putative therapeutic action of glucose was examined in animals receiving equiosmolar mannitol during the 4-h ischemic period.

METHODS

Healthy, male mongrel dogs with normal hematocrit levels and weighing 19–27 kg were anesthetized with morphine sulfate (3 mg/kg i.m.) and Nembutal (12 mg/kg intravenously; Abbott Laboratories, North Chicago, Ill.), 18 h after eating. Cuffed endotracheal tubes were inserted, and respiration was maintained with a Harvard respiratory pump (Harvard Apparatus Co., Inc., Millis, Mass.). This facilitated the maintenance of arterial oxygen saturation at about 90%. Frequent arterial pH determinations were performed for maintenance of the physiological range. Closed-chest dogs were used for production of coronary artery obstruction because this preparation is relatively stable hemodynamically for observations over a 4-h period. A balloon tip catheter (American Catheter Corp., Rahway, N. J.) was placed in the left anterior descending coronary artery approximately 1.5 cm from its origin, and the balloon was inflated while peripheral coronary pressure was monitored.

To determine the effect of systemic infusion of GIK in normals, GIK was infused alone in nine intact anesthetized dogs over a 1-h period, using the same priming and maintenance dose as in the animals undergoing myocardial ischemia. The animals undergoing ischemia were divided into four groups of similar body weight range. In the high risk period during the initial 20 min of ischemia before starting infusions, ventricular fibrillation occurred in 15% of all animals. Subsequently, no fibrillation occurred in any group; ectopic beats were not quantified because this was not the primary purpose of the study. The lack of fibrillation after the initial 20 min was not unexpected in the GIK group (3, 4) but was unusual for the other groups. This may have been the result of several factors. A modestly reduced incidence was observed previously when isovolumic equiosmolar solutions were substituted for GIK (3). In addition, the previous model involved a thrombus occlusion which appears to affect a greater area of injury than the balloon occlusion of this present study in untreated animals. There is an approximately twofold higher incidence of ventricular fibrillation over a 4-h period in the thrombus model.

Eight animals served as controls (group 1) and received an isovolumic solution of saline. Six animals received 1 liter of 10% glucose, 20 U of insulin, and 40 meq/liter of potassium (group 2). Group 3 was formed to counter the glucose effect in reducing plasma FFA and to simulate the elevations occurring during untreated ischemia. In six animals a solution of 10% Intralipid (Cutter Laboratories, Inc.) was infused in another catheter simultaneously with GIK. A 50-mg bolus of heparin (U. S. Pharmacopeia) was administered intravenously shortly after the onset of the lipid infusion to obtain an early FFA increment (6).

Although acute hypertriglyceridemia has previously been associated with a modest reduction of coronary blood flow (8), heparin-induced lipolysis was found to normalize flow. Thus, the combination of Intralipid with heparin would not be expected to reduce coronary blood flow. 100 ml of Intralipid contains 10 g of soy bean oil, 1.2 g of phospholipid, and 2.25 g of glycerol. It is assumed that the plasma FFA increment during lipolysis effects a rise of saturated as well as unsaturated fatty acids. The former comprise approximately 50% of this lipid emulsion (6).

In five ischemic animals (group 4) and six normals, an isovolumic solution of 20% mannitol was infused to raise the serum osmolality to the same level noted in group 2 animals. Infusions in all ischemic groups were begun 20 min after the onset of ischemia, determined by ST-segment changes. The animals received a priming dose of 7.8 ml/min for 5 min and 3.6 ml thereafter through a femoral venous catheter, approximating 1 liter of fluid over a 4-h period.

For pressure and indicator dilution studies, an 80-cm 8F National Institutes of Health catheter was passed via the right carotid artery into the left ventricular (LV) chamber; a 50-cm 8F NIH catheter was passed via the left common carotid artery into the aortic root, 1–2 cm above the aortic valve; an 80-cm 8F NIH catheter was inserted from the right femoral artery into the aortic arch; a 120-cm 7F Swan-Ganz flow catheter (Edwards Laboratories, Santa Ana, Calif.) was passed via the jugular vein into the pulmonary artery and a small polyethylene catheter (PE 160) into the femoral vein. For pressure measurement, catheters were connected directly to a strain gauge through a three-way stopcock. Statham pressure transducers (Statham Instruments, Inc., Oxnard, Calif.) for the measurement of LV (P23Gb) and aortic (P23Db) pressures were placed at the midthoracic level and balanced for equisensitivity. Catheters of this dimension have been compared in our laboratory with the Millar catheter tip micromanometer (Millar Instruments, Houston, Tex.) and have given equivalent ventricular systolic and end-diastolic pressures, in accord with a prior study (8, 9). Ventricular end-diastolic pressure was recorded at a sensitivity where 1 mm Hg equaled 5 mm on the tracing at a paper speed of 100 mm/s. The frequency response of the system was linear from 0–30 cycles/s. End-diastolic pres-

[1] *Abbreviations used in this paper:* ECG, electrocardiogram; GIK, glucose-insulin-potassium; LV, left ventricular; MRFS, mean rate of fiber shortening.

sures were measured over at least two respiratory cycles and averaged.

Cardiac output was measured from indication dilution curve samplings from the pulmonary artery after right atrial injection (10). Three successive curves were used to calculate the forward flow. Stroke volume and stroke work were obtained as previously reported. LV ejection fraction was measured by indicator dilution using cold saline rapidly injected into the left ventricle. The thermistor for the ejection fraction was placed within the NIH catheter 1–2 cm above the aortic valve. Three or four such curves were obtained, and end-diastolic volume was calculated as a ratio of mean stroke volume to mean ejection fraction. LV ejection fraction by this technique has shown good reproducibility both in a hydraulic model under nearly ideal conditions of mixing and sampling (11) and in the intact dog (12, 13). The higher values obtained from the angiographic methods at similar levels of heart rate and aortic pressure may be due to a high rate of ejection as a result of the large bolus of contrast material injected in the LV chamber (14). Despite a systematic difference between the two techniques, there is a good correlation of the ejection fraction derived from the indicator dilution and angiographic techniques, with $r = 0.824$ (15).

In terms of end-diastolic volume, values with the angiographic technique have yielded systematically lower values than by the indicator method, but yielded $r = 0.95$ (15). Although an absolute test of accuracy in vivo is not available, two additional studies comparing the indicator dilution technique with independent methods have shown good agreement (16, 17). The dilution curves, pressures, and ECG were recorded on an Electronics for Medicine DR-8 recorder (Electronics for Medicine, White Plains, N. Y.).

Ejection phase indices of contractility were assessed from the ejection fraction as well as from the mean rate of fiber shortening (MRFS). The latter was obtained by dividing the differences between the end-diastolic and end-systolic circumferences by the systolic ejection period. The LV radius at each phase of the cardiac cycle was calculated from the ventricular volume on the assumption that the ventricle was a sphere at the end of the isovolumic period, and circumferential fiber length was calculated as $2\pi r$ (18). Systolic ejection period was obtained from superimposed aortic and LV pressure pulses.

Additional animals in groups 2 and 3 were studied to determine changes in segmental wall motion in the ischemic area. LV cineangiograms were obtained in the left lateral position using 10 ml of Renografin (E. R. Squibb & Sons, Princeton, N. J.) injected into the left ventricle by rapid hand injection. Ventriculograms were obtained at least 30 min before and at 15 min of ischemia, and subsequently at 60, 120, 180, and 210 min of infusion of either saline or GIK. Each ventriculogram was analyzed by two independent observers using quantitative methods to evaluate systolic wall motion as described by Leighton et al. (19). As shown in Fig. 1, the endocardial borders of the ventricle at the onset of ejection (solid lines) and at end-systole (interrupted lines) were traced and superimposed from the projected images of the cine films. A longitudinal axis which corrects for rotation of the apex during systole and eight perpendicular hemiaxes were drawn to divide the ventricle into nine endocardial segments. Because of the large variation of shortening at the basal segments, these were excluded in the estimation of shortening.

To determine the extent of myocardial injury, precordial ECG mapping was obtained from 21 electrodes arranged in three rows as described by Maroko et al. (20). This was obtained during the control state, at 15 min of ischemia before therapy, and at 30-min intervals during infusion of the various solutions. Amplitude of R wave was also determined (21), using electrical calibrations at each interval.

Serial blood samples were taken and plasma analyzed for glucose (22), FFA (23), and triglyceride (24). Serum osmolality was determined by freezing-point depression. At the conclusion of the studies, the thorax was incised and the heart was rapidly arrested with iced Ringer's solution. The ischemic area of left ventricle was excised parallel to and 1 mm lateral to the anterior descending artery, beginning 1 cm below the obstruction site, down to the apex and, then, perpendicular to the anterior descending artery across to the termination of the most inferior diagonal branch or an imaginary extension when this branch terminated short of the apical level. The outer margin was formed at the termination of the main epicardial segment of the other diagonal branches. This formed an approximately triangular-shaped sample with the base at the cardiac apex and the peak just below the obstruction site. In previous studies we have observed that injection of Evans Blue dye distal to the obstruction site at diastolic pressure levels stains this area, except where

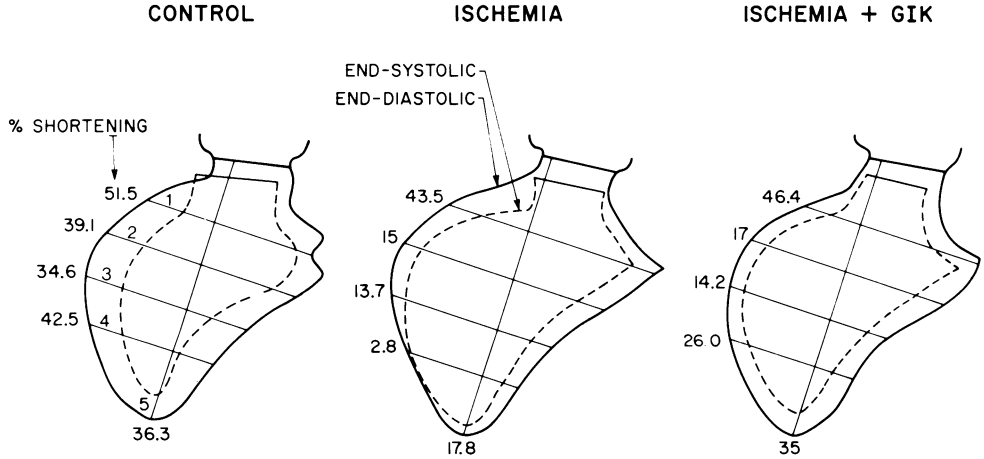

FIGURE 1 Left ventriculograms before and during ischemia in a single experiment. The decreased wall motion of segments 2–5 during ischemia is improved after 4 h of treatment with GIK.

there is aberrant vessel distribution (25). Such animals were excluded from this study. A similar size segment approximating 12 g was taken from the nonischemic posterior wall.

In view of the potential heterogeneity of the myocardial metabolic response (26), the ventricle was divided into inner and outer layers; the tip of the papillary muscle was excluded and the epicardial adipose tissue removed. To analyze for sodium and potassium concentrations, samples were homogenized and extracted for 48–72 h in distilled water, a sufficient time for complete extraction. Potassium and sodium were determined in duplicate on an Auto-Analyzer system (Technicon Tarrytown, N. Y.) with flame attachment. Water content was determined by drying samples in an oven at 100°C to constant weight. FFA (23) and triglyceride (24) were analyzed in extracts of the homogenates using heptane-isopropyl alcohol for the former and chloroform-methanol for triglyceride. A group of normal, intact anesthetized dogs without ischemia underwent similar tissue studies for comparison with the experimental groups.

To further evaluate the extent of protection produced by glucose infusion in terms of long-term influence on the manifestations of myocardial damage, eight controls and six treated animals underwent myocardial ischemia in a manner similar to the previously described animals, but under sterile conditions. The animals from both groups were sacrificed approximately 4 mo later and the heart removed. The length and width of the endocardial and epicardial surface involved with scar was measured to calculate area. Because the latter usually approximated an ellipse, the area formula was applied where area = $\pi\ a\ b$, which represent the major and minor hemiaxes. Three longitudinal incisions at 1-cm intervals and parallel to the anterior descending artery were made from the apex to a level near the base, 0.5 cm below the site of occlusion. The transmural thickness of the wall in this area was measured at 15 points in 1-cm intervals and averaged for each animal. The group means were compared for significance. Several points on the posterior wall of the left ventricle were measured and averaged to determine the wall thickness in the nonischemic area. Grossly visible scar was carefully dissected from the anterior wall and patches of muscle associated with scar were trimmed. Although a mixture of cardiac muscle cells and scar were undoubtedly present on a microscopic scale, there is no basis for believing this occurred in a systematically different manner between the two groups. The weight of scar was compared with the weight of the remainder of the left ventricle and septum to assess the percentage of left myocardium involved by scar.

Statistical analyses were performed using conventional methods for small samples. Differences between groups were evaluated by Student's unpaired *t* test; for comparison of various stages in animals of the same group, a paired *t* test was used.

RESULTS

Infusion of the GIK solution in normal animals raised serum glucose approximately threefold and serum osmolality by 40 mosmol/liter. This was associated with no significant change of heart rate, aortic pressure, or end-diastolic pressure and volume (Table I). The systolic indices of ventricular function were all significantly enhanced, including stroke volume, ejection fraction, and mean rate of fiber shortening. In view of the fact that ventricular function was enhanced without increments of preload or afterload, the response of animals undergoing acute regional myocardial ischemia was assessed (group 2) and compared to animals receiving an isovolumic infusion of normal saline (group 1). In both groups of animals the preinfusion period of ischemia up to 20 min was associated with similar hemodynamic changes (Tables II and III, Fig. 2). There was a trend toward elevation of end-diastolic pressure and volume, as well as reduced stroke volume at this time but this was not significant in either group. However, in subsequent hours these parameters were significantly more abnormal in group 1 compared with the glucose-infused group, without a significant difference in heart rate responses. Aortic pressure in group 1 was 130±8 in the control state and 126±10 mm Hg during the hemodynamic measurements indicated in the experimental period. Group 2 pressures declined nonsignificantly from 142±15 to 134±12 mm Hg. Both LV ejection fraction and MRFS were significantly enhanced in the GIK-treated group, returning to control levels by 60 min and were sustained for the subsequent 3 h of observation. These systolic parameters and end-diastolic pressures, when compared to the ischemic preinfusion values, were

TABLE I
Hemodynamic Changes in Normal Animals Receiving GIK

	Heart rate	Mean aortic pressure	End-diastolic pressure	End-diastolic volume	Stroke volume	Ejection fraction	Mean rate of fiber shortening
	beats/min	*mm Hg*	*mm Hg*	*ml/kg*	*ml/kg*	*%*	*cm/s*
C*	150	119	4.1	2.7	0.6	23.4	7.7
	±9	±4	±1.5	±0.1	±0.05	±3.2	±0.9
E‡	148	127	5	3.1	0.81	29.1	9.9
	±9	±4	±1.8	±0.13	±0.07	±3.0	±0.8
P§	NS	NS	NS	NS	<0.005	<0.05	<0.05

* Control with mean and standard error.
‡ Infusion (60 min) of GIK.
§ Level of significance for change from control to end of infusion using paired *t* test.

TABLE II
Hemodynamics during 4 h of Ischemia

	Animals	Heart rate			LV End-diastolic pressure			LV End-diastolic volume		
		C	I	E*	C	I	E	C	I	E
		beats/min			*mm Hg*			*ml/kg*		
I	Saline									
	1	170	146	150	5	10	19	3.6	6.7	4.1
	2	140	130	116	8	8	13	4.7	5.3	3.7
	3	140	110	90	5	10	29	2.6	4.6	4.5
	4	115	144	130	6	8	16	3.4	3.8	2.5
	5	110	140	130	4	8	20	5.4	4.5	4.4
	6	133	100	100	8	12	16	3.0	3.4	6.3
	7	202	170	160	7	22	26	3.2	3.7	6.3
	8	126	140	140	7	10	26	5.0	4.6	3.4
	Mean	142	135	128	6.3	11.0	20.6	3.9	4.6	4.4
	SE	12	7	9	0.5	1.6	2.0	0.4	0.4	0.5
II	GIK Only									
	1	160	140	110	7	9	12	2.4	3.3	6.2
	2	210	155	129	7	9	14	4.6	4.5	5.0
	3	120	100	90	2	5	8	5.1	5.3	4.0
	4	170	160	120	4	14	10	5.1	6.2	3.9
	5	155	130	110	5	10	13	5.2	6.5	7.1
	6	110	130	113	8	16	10	4.9	5.6	4.9
	Mean	154	136	112	5.5	10.5	11.2*‡	4.6	5.2	5.1
	SE	15	9	15	0.9	1.6	1.0	0.4	0.5	0.5
	P vs. I	NS	NS	NS	NS	NS	<0.01	NS	NS	<0.05
III	GIK + Intralipid									
	1	110	96	96	7	15	7	3.0	3.3	3.6
	2	110	100	140	5	8	7	3.7	2.8	3.0
	3	110	170	135	7	10	9	5.2	7.3	4.3
	4	90	120	90	11	16	14	3.8	4.4	3.9
	5	140	100	90	6	14	6	3.3	6.6	4.0
	6	170	170	160	2	10	6	2.6	3.1	3.0
	Mean	122	126	119	6.3	12.2	8.2‡	3.6	4.6	3.7
	SE	12	14	12	1.1	1.2	1.2	0.37	0.4	0.25
	P vs. I	NS	NS	NS	NS	NS	<0.01	NS	NS	NS
	P vs. II	NS	NS	NS	NS	NS	NS	NS	NS	<0.05

* C = preischemic control; I = 20 min of ischemia while in sinus rhythm; E = maximal change during ischemia at the end of infusion.
‡ $P < 0.05$ to <0.001 for change between states I and E vs. group I.

significantly improved in contrast to the same comparison in group 1.

To determine whether the enhanced function of the left ventricle reflected a change in function of the ischemic segment, two groups of animals were studied by cineangiography before and during the onset of ischemia and after intervention with saline or the glucose solution. Both groups had similar heart rate and aortic pressure levels. As seen in Figs. 1 and 3, there was a substantial decline in shortening of the anterior wall of the left ventricle that persisted through 4 h in the saline-treated group. This was compared with the glucose-treated animals by summing the percent shortening in the five segments of the anterior ischemic wall. The decline of shortening showed initial improvement by 60 min in the group receiving glucose which was significant by 4 h of treatment.

The potential role of reduced levels of plasma FFA in the hemodynamic response to GIK was examined in group 3 during the simultaneous infusion of GIK with Intralipid and heparin in a volume of 220 ml over a 4-h period. Plasma triglyceride rose from 0.37±0.1 to 3.36±1.0 meq/liter. Plasma glucose in group 3 was raised from 4.7±0.3 to 13.2±2.5 mmol/liter, similar to the increment in group 2 in which glucose was elevated from 4.3±0.5 to 11.9±1.3 mM/liter by 4 h. The hemodynamic response in group 3 animals during ischemia was similar to the response in those receiving GIK alone (Fig. 2). Despite a fivefold increase in both plasma FFA and the fatty

TABLE III
Hemodynamics during 4 h of Ischemia

	Animals	Stroke volume			LV Ejection fraction			Mean rate of fiber shortening		
		C	I	E*	C	I	E	C	I	E
		ml/kg			*%*			*cm/s*		
I	Saline									
	1	1.04	0.96	0.55	29.1	14.2	13.4	12.8	7.1	5.6
	2	0.85	0.89	0.63	18.3	16.7	17.1	6.7	6.0	4.6
	3	0.96	0.90	0.83	36.9	19.5	18.4	11.8	5.7	3.8
	4	0.71	0.57	0.43	20.8	15.0	17.5	9.1	6.3	4.6
	5	1.04	0.82	0.47	19.4	18.4	11.2	8.4	7.3	3.8
	6	0.55	0.89	0.66	18.2	26.4	10.5	4.5	10.9	4.0
	7	0.57	0.52	0.31	17.7	13.9	5.0	8.6	6.5	2.2
	8	0.89	0.77	0.52	17.5	16.9	15.3	8.8	7.2	6.6
	Mean	0.83	0.79	0.55	22.0	17.6	13.6	8.8	7.1	4.4
	SE	0.07	0.06	0.06	2.0	1.0	1.6	0.9	1.6	0.5
II	GIK Only									
	1	0.41	0.42	1.06	17.1	12.7	17.2	7.5	5.5	5.6
	2	0.74	0.64	1.03	16.3	14.3	12.8	7.1	6.2	5.8
	3	0.96	1.17	0.89	18.9	21.9	22.0	6.0	7.1	5.8
	4	1.06	1.04	1.12	20.8	16.9	29.1	7.6	5.3	7.1
	5	0.89	0.96	1.02	17.1	14.7	14.3	8.1	6.5	5.6
	6	0.75	0.59	0.61	15.2	10.4	12.4	5.9	4.1	4.3
	Mean	0.80	0.80	0.89‡	17.6	15.2	18.0‡	7.0	5.8	5.7‡
	SE	0.09	0.12	0.09	0.8	1.6	2.7	0.04	0.4	0.4
	P vs. I	NS	NS	<0.02	NS	NS	NS	NS	NS	NS
III	GIK + Intralipid									
	1	0.73	0.52	0.69	24.5	15.7	19.2	6.6	4.1	5.0
	2	1.1	0.73	0.59	29.1	26.4	16.5	7.0	5.6	4.8
	3	1.3	1.1	0.9	24.5	14.5	21.9	7.5	6.5	8.0
	4	0.9	0.7	0.8	22.1	15.8	20.8	7.5	5.8	6.3
	5	0.7	1.4	1.5	20.6	20.6	35.2	5.4	5.9	8.1
	6	0.51	0.45	0.6	19.7	14.5	19.7	6.8	4.6	6.2
	Mean	0.87	0.79	0.85‡	23.4	17.9	22.2‡	6.8	5.6	6.4‡
	SE	0.12	0.1	0.14	1.3	1.8	2.5	0.3	0.5	0.6
	P vs. I	NS	NS	NS	NS	NS	<0.05	NS	NS	<0.05
	P vs. II	NS	NS	NS	NS	NS	NS	NS	NS	NS

* C = preischemic control; I = 20 min of ischemia while in sinus rhythm; E = maximal change during ischemia at the end of infusion.

‡ $P < 0.05$–<0.001 for change between states I and E vs. group I.

acid/albumin ratio (Table IV), group 3 animals exhibited a significantly lower end-diastolic pressure and end-diastolic volume than the control group infused with saline (Fig. 2). In addition, from 1 to 4 h there was a sustained restoration of ejection fraction and mean rate of fiber shortening to control levels. Thus, it would appear that diminished plasma FFA levels are not essential to the therapeutic responses observed with the glucose solution during ischemia. In the animals from these groups, the cold-arrested myocardium was analyzed for FFA and triglycerides. The concentrations of these lipids were not found to differ significantly in the ischemic area from those of the saline-treated group when compared to normals (Table V).

In the animals of group 4 undergoing ischemia, the serum osmolality was raised to a similar extent as the glucose-infused animals by infusions of mannitol, from 304±3 to 348±4 mosmol/liter. There was a sustained elevation of end-diastolic volume and transient rise of end-diastolic pressure which subsequently reverted to control levels (Fig. 4). The most pronounced effect, however, was represented in the parameters of systolic function. Although function was somewhat improved by 60 min, after 4 h the animals infused with mannitol had a substantially diminished ejection fraction and MRFS similar to the saline-infused group, despite a higher end-diastolic volume in the former. Thus, it would appear that the hemodynamic response to the glucose infusate was not dependent on enhanced osmolality of extracellular fluid.

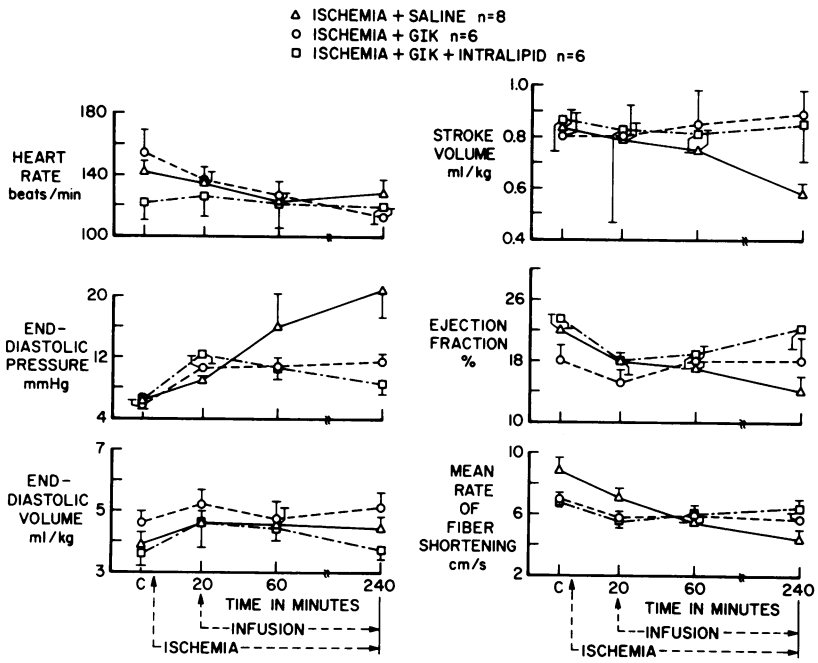

FIGURE 2 Hemodynamic response to ischemia. In the untreated animals the LV end-diastolic pressure rose, the systolic ejection phase parameters including stroke volume, ejection fraction and mean rate of fiber shortening declined over 4 h of ischemia. In the group treated with GIK alone combined with simultaneous Intralipid solution, the LV end-diastolic pressure and the ejection phase indices returned toward control levels despite sustained ischemia.

To assess whether this response to mannitol was observable in normal muscle, seven normal animals were infused with the same quantity of mannitol for a 4-h period (Fig. 4). In these animals, stroke volume, stroke work, ejection fraction, and MRFS increased significantly without a change in heart rate, preload, or afterload. This, however, was followed by a slight increase in end-diastolic volume associated with a significant reduction of ejection fraction and fiber shortening as well as stroke volume. It would, therefore, appear that the failure of mannitol to induce a sustained increase in performance of the ischemic ventricle may well be related to an effect on the nonischemic ventricle during ischemia.

Because the effects of GIK solution might be secondary to limitation of the area of injury, ECG mapping was obtained from the 21 precordial electrodes. The ΣST (sum of ST elevation) as well as the number of sites showing ST elevation of at least 0.2 mV, were reduced after 4 h of ischemia after GIK infusions (Table VI). A similar reduction was observed in the group receiving GIK combined with Intralipid. In none of these animals was there a significant rise of arterial plasma K^+ concentrations to explain the reduced ST segment (27). The group infused with mannitol did not demonstrate a significant change compared to the saline-infused animals. It is noteworthy that the diminished amplitude of the R wave observed in the precordial leads in the control group was not significantly effected in any of the three treatment groups (Table VI).

The influence of these interventions on myocardial cation composition in the ischemic area was assessed

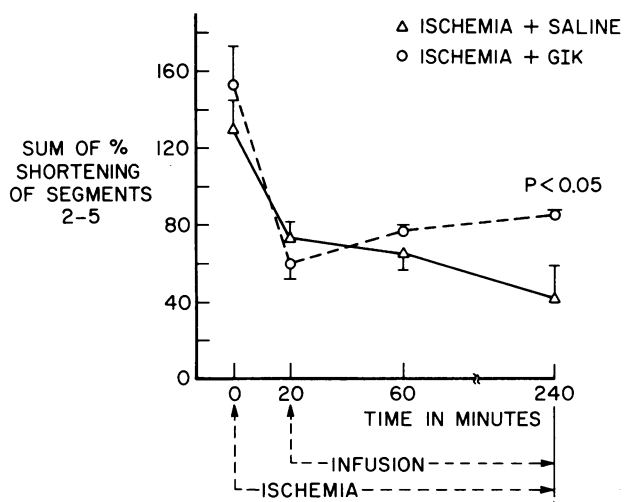

FIGURE 3 The mean and standard error of the changes in wall motion of the anterior wall during ischemia (n = 7) and after treatment with GIK (n = 6). Ischemic segments in each animal were averaged. There was marked reduction in the percent shortening beginning soon after the onset of ischemia, with improvement during GIK infusion, that was significant using a t test for unequal variances.

TABLE IV
Plasma Substrates and Osmolality

Group	Glucose		Osmolality		FFA		FFA/Albumin molar ratio		Triglycerides	
	C	E*	C	E	C	E	C	E	C	E
	mM/liter		*mosmol/liter*		*μeq/liter*				*meq/liter*	
1 Ischemia + saline (n = 5)	5.3 ±0.4	5.1 ±0.1	294 ±4	300 ±5	245 ±43	888‡ ±205		§	0.38‖ ±0.02	0.39‖ ±0.03
2 Ischemia + GIK (n = 6)	4.3 ±0.5	11.9‡ ±1.3	292 ±3	335‡ ±4	377 ±52	143‡ ±24	1.09 ±0.11	0.54‡ ±0.1	0.49 ±0.11	0.39 ±0.08
P vs. 1¶	NS	<0.003	NS	<0.001	NS	<0.001			NS	NS
3 Ischemia + GIK + Intralipid (n = 6)	4.7 ±0.3	13.2‡ ±2.5	302 ±5	348‡ ±9	386 ±63	1,436‡ ±178	0.95 ±0.17	4.68‡ ±0.55	0.37 ±0.1	3.36‡ ±1.0
P vs. 1	NS	<0.01	NS	<0.003	NS	NS			NS	<0.03
P vs. 2	NS	NS	NS	NS	NS	<0.001	NS	<0.0001	NS	<0.001

* C = preischemic control; E = at the end of infusion.
‡ P vs. preinfusion control (using paired t test) <0.05–0.001.
§ Albumin not measured in group 1.
‖ Values obtained in additional group of animals studied under same conditions as group 1.
¶ Level of significance between groups (using unpaired t test).

in the inner and outer half of the affected segment. Cation and water composition of the nonischemic area did not differ from the normal group in the four experimental groups. Plasma K^+ was normal in all four groups before infusion and did not change significantly in the subsequent 4 h. Group 1 had a substantial reduction of K^+ in both the inner and outer half of ischemic myocardium, associated with a gain of sodium and water compared with normal animals (Table VII). The GIK group had a significantly lower sodium content per gram dry weight after 4 h of ischemia as well as a modest reduction in water content. This response was also seen in the GIK group receiving Intralipid simultaneously. Although the latter two groups had a higher K^+ content than group 1, this was not significant when expressed per unit of dry weight. By contrast, concentrations of K^+ expressed per gram of wet weight were significantly higher in both layers of myocardium compared with group 1, in part a result of greater dilution by water in the untreated group. Because biologic activity of the cation is presumably dependent on concentration in water, the wet weight expression would appear to be more relevant. The K^+ differences are illustrated by the concentrations in the inner half of myocardium. Group 1 was 33.2±2.5 μeq/g wet wt com-

TABLE V
*LV Lipid Concentrations**

Group	FFA	Triglycerides
Normals (n = 7)	5.9±0.3	2.3±0.2
1 Ischemia + saline (n = 8)	5.8±0.4	2.1±0.4
2 Ischemia + GIK (n = 6)	5.5±1.0	2.4±0.6
P vs. 1	NS	NS
3 Ischemia + GIK + Intralipid (n = 6)	5.7±0.3	2.72±0.6
P vs. 1	NS	NS
P vs. 2	NS	NS

* Values in microequivalents per gram of left ventricle (taken from midlayers of ischemic area).

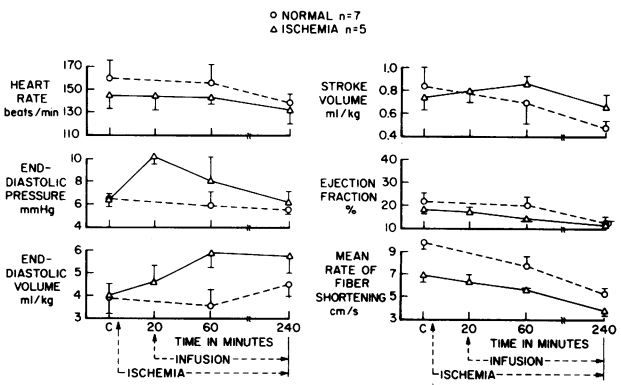

FIGURE 4 Hemodynamic response to mannitol infusion in normal dogs and animals with 4 h of left anterior descending artery occlusion. The LV systolic indices including stroke volume, ejection fraction, and mean rate of fiber shortening ultimately decreased in both groups during mannitol infusion. The end-diastolic pressure declined and end-diastolic volume rose in the two groups with or without ischemia.

TABLE VI
Precordial EKG Mapping

Group	ΣST*		NST‡		R̄§		
	B‖	E¶	B	E	C**	B	E
	mv						
1 Ischemia + saline (n = 8)	4.3 ±1.0	5.2 ±0.3	9.6 ±1.0	13.0 ±1.2	1.7 ±0.13	1.4 ±0.13	0.89 ±0.09
2 Ischemia + GIK (n = 6)	7.3 ±1.7	3.9 ±1.2	9.1 ±1.0	3.9 ±1.2	1.95 ±0.13	2.0 ±0.17	1.19 ±0.12
P vs. 1‡‡	<0.001		<0.002		NS	NS	
3 Ischemia + GIK + Intralipid (n = 6)	4.0 ±1.4	1.7 ±1.2	11.0 ±2.2	6.0 ±3.2	1.19 ±0.34	1.18 ±0.29	0.66 ±0.16
P vs. 1	<0.005		<0.05		NS	NS	
P vs. 2	NS		NS		NS	NS	
4 Ischemia + mannitol (n = 5)	4.0 ±0.8	5.8 ±1.5	10.2 ±0.9	10.5 ±1.0	1.40 ±0.14	1.20 ±0.07	0.96 ±0.15
P vs. 1	NS		NS		NS	NS	
P vs. 2	<0.0001		<0.003		NS	NS	
P vs. 3	<0.001		<0.05		NS	NS	

* Sum of ST elevation in millivolts.
‡ Number of sites with ST-segment elevation.
§ Mean R wave in millivolts in leads with ST-segment elevation.
‖ Before starting infusion of various solutions after 20 min of steady ischemia while in sinus rhythm.
¶ At the end of infusion.
** Preischemic Control.
‡‡ Level of significance between groups for changes from 20 to 240 min of ischemia.

TABLE VII
*Myocardial Cations at 4 h of Ischemia**

Group	Na^+		K^+		Water	
	Outer	Inner	Outer	Inner	Outer	Inner
					%	
Normals (n = 9)	160 ±10	164 ±5	348 ±16	313 ±5	78.8 ±0.86	77.8 ±0.27
1 Ischemia + saline (n = 8)	446 ±47	506 ±51	230 ±26	213 ±18	83.6 ±0.75	83.3 ±0.4
2 Ischemia + GIK (n = 6)	227 ±22.0	222 ±26	284 ±14	256 ±24	80.1 ±1.4	79.3 ±1.1
P vs. 1	<0.001	<0.0005	NS	NS	<0.05	<0.01
3 Ischemia + GIK + Intralipid (n = 6)	330 ±26	318 ±21	265 ±8	259 ±18	80.9 ±1.0	82.1 ±0.7
P vs. 1	<0.04	<0.005	NS	NS	<0.05	NS
P vs. 2	<0.01	<0.01	NS	NS	NS	<0.05
4 Ischemia + mannitol (n = 5)	228 ±18	246 ±26	241 ±23	254 ±31	78.2 ±1.3	81.3 ±0.7
P vs. 1	<0.001	<0.001	NS	NS	<0.01	<0.02
P vs. 2	NS	NS	NS	NS	NS	NS
P vs. 3	<0.01	<0.05	NS	NS	NS	NS

* Cations are expressed as microequivalents per gram dry weight of ischemic tissue.

pared with group 2 with 53.0±5.2 ($P < 0.01$) and group 3 at 48.1±1.9 ($P < 0.001$).

A qualitatively similar response to that of groups 2 and 3 was observed in the mannitol-infused animals so that cation alterations, cell swelling, and leak of K^+ appear to be modified by a sustained increase in extracellular osmolality.

In the separate groups of animals followed 4 mo after occlusion of the anterior descending artery, there was no evidence of infection, anemia, or weight loss. The calculated scar area, as well as its major and minor hemiaxes in endocardium, were similar in controls and GIK-treated animals (Table VIII). The area extending to the epicardium and the percentage of left ventricle involved by scar were similar in both groups. Furthermore, the degree of thinning of the anterior wall did not differ in the treated and control animals.

DISCUSSION

This study has demonstrated that a sustained infusion of GIK effecting a threefold increase of plasma glucose concentrations can enhance LV performance during myocardial ischemia. This response was sustained throughout the 4 h of observation in animals with relatively large areas of segmental ischemia, contrasted with the response of animals receiving an equal volume of saline in which LV dysfunction was progressive.

Inasmuch as the GIK infusion in normal animals elicited an increase of LV function, it was anticipated that the enhanced function in the ischemic ventricle might be solely related to a response of the nonischemic myocardium. However, anterior wall motion was improved, although not normalized by the intervention, and this persisted for 4 h. Despite the enhanced function of ischemic muscle, there was no evidence of extension of injury as judged by precordial ECG mapping. In addition, the ischemic tissue was less swollen, with a smaller gain of sodium and water and higher potassium concentrations. Thus, the effects of the glucose intervention on mechanical function did not appear to occur at the expense of tissue integrity. Despite the fact that the inner wall is considered to be more ischemic than the outer wall (26), the effects of the glucose intervention appeared to be relatively similar in terms of electrolyte composition in these locations. Consequently, one could not, on this basis, conclude that only the less ischemic zones demonstrated a therapeutic effect in terms of cation alterations. This contrasts with a prior study of several zones in the ischemic area which indicated that potassium/sodium ratios were affected in the epicardial or marginal muscle (28). The absolute concentrations of ions were not provided in the latter study presumably because of greater variability in tissue samples of smaller size.

TABLE VIII
Myocardial Scar in Control and Glucose-Treated Ischemic Groups

Group	Endocardium	Epicardium	Wall thickness		LV scar*
			Anterior	Posterior	
	area cm²	*area cm²*	*cm*		*%*
Control ($n = 8$)	10.8 ±1.6	0.65 ±0.36	0.51 ±0.04	1.15 ±0.04	5.13 ±0.49
GIK ($n = 6$)	10.3 ±1.3	1.4 ±0.91	0.48 ±0.06	1.15 ±0.12	4.95 ±0.81

* Excised scar compared to weight of grossly normal left ventricle and septum.

Enhanced function of the ventricle in normals as well as during ischemia may well be related to an increase in contractility because preload and afterload were not significantly altered. This has been more clearly demonstrated in an isolated heart preparation (4). Although the mechanism is unknown, it is of interest that plasma levels of epinephrine have been found to increase up to 55% after the ingestion of glucose (29), and cardiac sympathetic activity is enhanced after carbohydrate feeding in fasted animals (30). Whether small increments in sympathetic activity without significant effects on heart rate and aortic pressure may be responsible for the observed myocardial effect remains to be demonstrated.

By contrast with this and prior studies of regional ischemia (3, 4), a previous report of global ischemia indicated that enhanced glucose and insulin concentrations resulted in greater reduction of ventricular performance and survival time in the intact working swine heart (31). This may represent a real difference in the response to glucose infusate. However, in the latter model coronary perfusion was maintained with a closed circulatory system and an initial perfusate potassium concentration that was normal. In the course of the experiment, the addition of insulin may have resulted in hypokalemia in the recirculated perfusate and accounted for some of the observed effects. Furthermore, the transfused erythrocytes used in the perfusate could have undergone some degree of aggregation in response to a fivefold increase of glucose concentration, with associated hyperosmolality resulting in enhanced ischemia.

The glucose-treated animals had a qualitatively different plasma FFA response than the controls during ischemia. The latter exhibited a rise in FFA over a period of 4 h of ischemia, whereas the glucose group had a reduction in plasma FFA. The resultant decrease in myocardial FFA uptake in response to the glucose solution (28) has been postulated to be a basis for the therapeutic action on the ischemic heart (32). However, when the FFA reduction was counteracted by the si-

multaneous infusion of Intralipid and heparin with a resultant substantial elevation of plasma FFA during ischemia, the changes of myocardial function and composition as well as precordial ECG mapping were similar to that of the animals receiving glucose alone. This supports the view that enhanced ventricular performance after glucose is not dependent upon reduced FFA levels. In this regard, the isolated rat heart treated with glucose after coronary artery ligation effected a reduced loss of the enzyme lactic dehydrogenase even in the absence of FFA in the perfusate (33).

Although the uptake of FFA by the myocardium was not measured in the study, FFA oxidation is known to decline with ischemia (34). The finding of normal tissue levels of FFA in accord with a prior study (35) suggests that a substantial reduction of fatty acid uptake occurred despite plasma FFA increments. Although interstitial concentrations of FFA complexed to albumin are presumed to be relatively low on the basis of the lower interstitial protein content and although plasma space accounts for about 6% of tissue volume, one would have anticipated some increase of tissue fatty acid with the prevailing plasma lipid levels. The possibility exists that fatty acid was converted to water soluble acyl CoA compounds which were not measured by the technique employed. Thus, although the final distribution and fate of the infused lipid in terms of myocardium is not explained, elevated fatty acids in plasma do not appear to modify the hemodynamic, ECG, and myocardial electrolyte responses to the glucose infusate during ischemia.

The therapeutic response after GIK is apparently not based on increased blood flow to the ischemic site (3). Enhanced glycolytic flux in ischemic tissue has been postulated as one consequence of GIK infusion (28). In a prior report, enhanced glucose uptake was observed after GIK but not after equiosmolar amounts of nonglucose containing hypertonic solutions (3).

Although that study did not include examination of tissue electrolytes or precordial ECG mapping, leakage of K^+ from the ischemic myocardium, as judged by sampling the great cardiac vein, was diminished by both glucose- and nonglucose-containing solutions (3). This observation is qualitatively consistent with the tissue K^+ alterations after mannitol. Although studies of stroke and end-diastolic volumes were not performed (3), gross indices of ventricular function as measured by LV pressures and first derivative did not differ significantly from the animals receiving GIK. In contrast, the mannitol group, after a modestly improved function early in the course of ischemia as previously reported (36), exhibited progressive deterioration of LV function through 4 h of ischemia as well as persistent ST-segment abnormalities. A similar ineffectiveness has been reported during 2 h of hypoxia (37). It would appear probable that effects on the nonischemic myocardium were operative because normal animals infused with mannitol for a similar period of time also demonstrated depressed ventricular function after initial improvement. Consistent with this view is the observation that release of lysosomal acid phosphatase from normal myocardium occurred during a 4-h infusion of mannitol (38).

The significance of this therapeutic intervention may be considered from several viewpoints. Enhanced LV function and reduced arrhythmia incidence (3, 4) are not usually characteristic of a single therapeutic modality during ischemia. The discordancy between the ventricular functional response and one of the electrical parameters of injury, R wave amplitude, has some precedence. Intervention with nitroprusside (39) or isoproterenol (40) during ischemia has been associated with improved ventricular function despite unchanged or increased ST-segment elevation.

The failure to observe a difference in R wave amplitude in the treated group despite significantly improved repolarization abnormalities may be the result of several factors. Diminished R wave amplitude in the precordial ECG during acute infarction is thought to represent the loss of electrically active myocardium (41) as verified with intramural electrodes (42). Studies of tissue composition with GIK infusion maintained up to the time of tissue sampling at 7 or 24 h after the onset of ischemia (43, 44) have indicated that ischemic tissue by chemical analyses and morphologic examination underwent less severe injury than in nontreated animals. It is conceivable that some of the cells with the degree of histologic preservation observed at these time intervals may still fail to propagate electrical impulses or that conduction delay induced by ischemia may be unaltered and account for the reduced amplitude of R wave that was, in the treatment group, similar to the controls. None of the available studies answers this question as to whether the improved chemical or histologic parameters after glucose represent a delay in the ultimate development of irreversible damage or permanent protection to these injured cells. The former view is supported in a study of sympathetic beta blockade (45), at least in regard to the central ischemic area.

Our observations on the amount of scar and reduction of wall thickness in animals studied 4 mo after infarction would support this interpretation. Therapy of acute ischemia with GIK did not result in less myocardial thinning or a reduction in the amount of scar. A previous attempt to evaluate the influence of insulin administered once per day without glucose or potassium for 10 wk after ligation of multiple coronary artery branches showed no significant difference in thickness of scar measured at the center in animals without malnutrition (46). Although aneurysms were reported in a higher incidence in the controls, such were not present in our untreated or treated animals at 4 mo postinfarc-

tion. The question remains unanswered whether the amount of ultimate scarring would be reduced with a more sustained intervention with glucose. Data are at hand in terms of the healing of cardiomyopathic process treated with the glucose infusate over a period of weeks (47). The resultant increase in the amount of fibrosis would suggest caution in the use of this modality for prolonged periods after the acute stage of ischemia.

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