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Amino Acid Substrate Preloading and Postischemic Myocardial Recovery[1]

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During induced myocardial ischemia for cardiac surgery, myocardial stunning occurs and aerobic metabolism of glucose, fatty acids, and lactate is inhibited as anaerobic pathways predominate. Even following reperfusion, stunned myocardium uses oxygen and substrate inefficiently leading to poor functional recovery as less mechanical work is developed per oxygen utilized. Amino acids potentially can act as cardiac metabolic substrates during and after ischemia, utilizing the transamination of amino acids by the malate-aspartate shuttle to form high energy phosphates via the tricarboxylic acid cycle. We investigated if "preloading" hearts with a physiologic spectrum of amino acids could increase postischemic myocardial recovery. Isolated perfused rabbit hearts were subjected to 120 min of 34°C cardioplegic ischemia. Hearts received cardioplegia alone as controls or were "preloaded" with a 0.05% amino acid perfusion for 30 min prior to cardioplegic ischemia. Following reperfusion, analysis of functional recovery revealed that contractility and cardiac efficiency were improved with amino acids substrate preloading. The mechanism of this may be due to uptake of amino acids prior to ischemia, which are later utilized for internal reparative work during ischemia and external contractile work after ischemia.

INTRODUCTION

During non-ischemic aerobic metabolism amino acids contribute insignificantly (less than 5%) to the generation of energy for the heart, as the myocardium preferentially oxidizes carbohydrates (glucose) and lipids (fatty acids) to form ATP for the contraction (external work) and repair of subcellular structures (internal work) [1]. However, during ischemia, aerobic metabolism ceases and less efficient anaerobic pathways predominate. Anaerobic glycolysis of glucose cannot produce adequate high-energy phosphate compounds to maintain critical cellular function and the result is a breakdown of cellular organelles and poor contractility following reperfusion. Theoretically however, amino acids, through transamination to tricarboxylic acid cycle intermediates and subsequent anaerobic substrate phosphorylation, could provide for energetics during ischemia and protect the ischemic myocardium from damage, thereby improving cardiac function after ischemia. Furthermore, if amino acids could be taken up and were present in adequate quantities at reperfusion, they could serve as a further substrate for energetics upon reflow, during early reperfusion when normal aerobic mechanisms for utilization of glucose and fatty acids continue to be inhibited, despite the reintroduction of oxygen. This present study "preloaded" hearts with amino acids prior to ischemia at a time when the heart has the ability to uptake these compounds and examined functional recovery after an ischemic episode, analogous to that which occurs during clinical cardiac surgery.

MATERIALS AND METHODS

Preparation of isolated hearts. New Zealand White rabbits (male or female, 2–3 kg body weight) were anesthetized with sodium pentobarbital (45 mg/kg) and heparinized (700 units/kg). Heart were rapidly excised and immersed in ice-cold physiologic salt solution (PSS) containing 118.0 m*M* NaCl, 4.0 m*M* KCl, 22.3 m*M* $NaHCO_3$, 11.1 m*M* glucose, 0.66 m*M* K_2HPO_4, 1.23 m*M* $MgCl_2$, and 2.38 m*M* $CaCl_2$. The aorta was cannulated in the Langendorff mode and perfused with PSS which was equilibrated with 95% O_2–5% CO_2 at 37°C and pH 7.4. Perfusion pressure was maintained at 80 mm Hg. The perfusion solution was filtered and was not recirculated. An incision was made in the left atrium and a

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fluid-filled latex balloon was passed through the mitral orifice and placed in the left ventricle. The balloon allowed for passive venting of the left ventricular (LV) cavity. A catheter-mounted micromanometer was positioned in the LV (Model PC250, Millar, Houston, TX) and LV pressure was recorded continuously. The balloon was connected to a Statham P23 XL pressure transducer for continuous measurement of left ventricular pressure (LVP) and output from the pressure transducer was electronically differentiated to enable continuous recording of the first derivative of LVP (dP/dt). End-diastolic volume was inferred from balloon volume. The superior vena cava and inferior vena cava were ligated. The pulmonary artery was cannulated to enable timed collection measurements of coronary flow and the cannula was connected to an oxygen meter (Chemical microsensor, Diamond Electro-Tech, Inc., Ann Arbor, MI) for continuous measurement of the partial pressure of oxygen in the coronary effluent. All hearts were maintained at 37°C by means of a circulating water jacket during the control baseline period.

The analog signals were continuously recorded on a pressurized ink chart recorder (Model 2600S, Gould, Inc., Cleveland, OH) and an online computer (AST Premium/386, AST Research Inc., Irvine, CA). During the control period, a standardized volume of saline was introduced into the LV balloon producing an end diastolic pressure of 5.5 ± 3.3 mm Hg. The same volume was used to evaluate and characterize isovolumic cardiac function; developed pressure (DP) was defined as peak systolic pressure minus end-diastolic pressure. The product of heart rate and DP (PRP, mm Hg/min) was calculated to provide an estimate of changes in myocardial work. Myocardial oxygen consumption (MVO_2) was calculated as $MVO_2 = CF \times [(PaO_2 - PvO_2) \times (c/760)]$, where CF is coronary flow (ml/min/g), $(PaO_2 - PvO_2)$ is the difference in the partial pressure of oxygen (PO_2, mm Hg) between perfusate and coronary effluent flow, c is the Bunsen solubility coefficient of O_2 in perfusate at 37°C ($22.7\ \mu l\ O_2 \cdot atm^{-1} \cdot ml$ perfusate). The PO_2 of the perfusate was 663 mm Hg. Coronary flow was measured by performing timed collections of the pulmonary effluent flow with a graduated cylinder. Oxygen extraction (O_2 EXT) was calculated as O_2 EXT = MVO_2/oxygen content in the perfusate. Wet weight of the heart was determined at the conclusion of each experiment after trimming the great vessels and fat and blot drying with eight-layer cotton gauze.

Preparation of amino acids. The solution utilized was a commercially approved and available 10% Travasol Injection which contains 15 amino acids, both essential and nonessential; 730 mg leucine; 600 mg isoleucine; 580 mg lysine (added as the hydrochloride salt); 580 mg valine; 560 mg phenylalanine; 480 mg histidine; 420 mg threonine; 400 mg methionine; 180 mg tryptophan; 2.07 g alanine; 1.15 g arginine; 1.03 g glycine; 680 mg proline; 500 mg serine; 40 mg tyrosine; in each 100 ml (Baxter Healthcare Corp., Deerfield, IL). On the day of each experiment, the amino acid solution with 2.0 m*M* pyruvate was made in 0.05% concentration in PSS.

Experimental protocol. Baseline data were obtained after an equilibration period of approximately 30 min. There were 10 hearts in both the control and amino acid-treated groups. Following collection of baseline data, 60 ml of modified St. Thomas cardioplegia solution was injected into the aorta at 1 ml/sec at the beginning of the 2-hr ischemic period, which was induced by interrupting aortic retrograde flow into the coronaries. All hearts were maintained at 34°C by means of a circulating water jacket during the cardioplegic ischemia period. Fifteen milliliters of cardioplegia solution was injected every 30 min thereafter. At the end of the 2-hr ischemic period, the hearts were reperfused with standard oxygenated PSS solution. Reperfusion was performed with perfusate temperature at 37°C and delivered with pressure at 80 mm Hg. Defibrillation was performed as needed. During the initial 15 min of reperfusion, the intraventricular balloon was kept deflated to allow for recovery. After the initial 15 min of reperfusion, the LV balloon was refilled to the preischemic control volume and isovolumic measurements of cardiac function were obtained. The LV balloon remained inflated for the remainder of reperfusion. Hemodynamic data were recorded every 15 for 45 min to compare with baseline data in order to determine the degree of functional recovery in each heart. The experimental design for this model is well established and has been published previously [2, 3]. All animals received humane care in compliance with the *Principles of Laboratory Animal Care* formulated by the National Society for Medical Research.

Statistical analysis. Values reported are means ± standard deviation. Data were evaluated with repeated measures ANOVA within groups and single factor analysis of variance across groups. When significant *F* values were obtained, Scheffe's test was used to distinguish when time periods or groups differed from one another significantly.

RESULTS

There were no significant differences among the two groups in terms of heart wet weight (6.02 ± 1.21 g in control group and 6.18 ± 0.98 g in amino acid group, respectively), postischemic myocardial water content (86.2 ± 1.9% in control group and 86.1 ± 1.2% in amino acid group), or end diastolic volume (0.97 ± 0.16 ml in control group and 0.97 ± 0.36 ml in amino acid group). During baseline recording, developed pressure was 100–140 mm Hg for all of the hearts. There were no significant hemodynamic differences among the groups during baseline conditions with the exception of positive and

TABLE 1

Hemodynamics (Means ± SD)

	Baseline	REP 15 Min	REP 30 Min	REP 45 Min
PSP (mm Hg)				
CONT	119.6 ± 8.4	84.1 ± 11.9**	84.7 ± 8.5**	80.8 ± 10.8**
AM	119.5 ± 10.4	90.4 ± 11.4**	91.4 ± 12.2**	92.2 ± 12.7*,**
EDP (mm Hg)				
CONT	6.7 ± 3.1	48.4 ± 16.4**	35.5 ± 14.7**	30.0 ± 13.3**
AM	4.4 ± 3.6	32.4 ± 17.7*,**	23.7 ± 15.4**	20.5 ± 13.8
DP (mm Hg)				
CONT	112.9 ± 9.2	35.7 ± 8.4**	49.2 ± 11.0**	50.8 ± 10.8**
AM	115.1 ± 11.6	58.0 ± 26.1*,**	67.7 ± 23.8*,**	71.7 ± 23.4*,**
dP/dt_{max} (mm Hg/sec)				
CONT	1812 ± 268	560 ± 151**	788 ± 182**	853 ± 224**
AM	1608 ± 152*	772 ± 301**	1009 ± 301**	1035 ± 309**
$-dP/dt_{max}$ (mm Hg/sec)				
CONT	1306 ± 145	422 ± 104**	564 ± 111**	589 ± 110**
AM	1102 ± 194*	534 ± 252**	632 ± 219**	676 ± 217**
HR (beats/min)				
CONT	185.6 ± 26.9	168.6 ± 26.9	176.5 ± 29.3	176.0 ± 31.4
AM	195.7 ± 21.5	175.1 ± 14.3	181.8 ± 18.1	183.8 ± 19.6
PRP (10^3 mm Hg/min)				
CONT	21.00 ± 3.85	5.98 ± 1.55**	8.66 ± 2.27**	8.84 ± 1.98**
AM	22.65 ± 4.16	10.22 ± 4.60*,**	12.41 ± 4.55*,**	13.30 ± 4.61*,**
CF (ml/min/g tissue)				
CONT	9.11 ± 2.55	6.57 ± 1.25**	6.16 ± 1.53**	5.83 ± 1.12**
AM	8.08 ± 1.09	6.78 ± 0.86**	6.56 ± 1.03**	6.73 ± 0.92**
MVO_2 (μl/min/g tissue)				
CONT	106.6 ± 29.8	60.4 ± 20.3**	65.0 ± 17.5**	67.8 ± 13.3**
AM	111.9 ± 16.5	60.3 ± 21.6**	69.3 ± 26.9**	74.1 ± 28.0**
O_2 EXP (%)				
CONT	59.9 ± 11.4	46.7 ± 15.6	54.0 ± 12.5	58.9 ± 7.8
AM	70.1 ± 9.4	44.8 ± 15.6**	52.6 ± 18.0	55.2 ± 19.7

Note. Hemodynamic indexes as determined in isolated reperfused hearts at 15, 30, and 45 min of reperfusion as described under Materials and Methods. Ten hearts were used per group. PSP, peak systolic pressure; EDP, end diastolic pressure; DP, developed pressure; dP/dt_{max}, maximum first derivative of left ventricular pressure; HR, heart rate; PRP, product of HR and DP; CF, coronary flow; MVO_2, myocardial oxygen consumption; O_2 EXT, oxygen extraction; CONT, control group; AM, amino acid-treated group.

* $P < 0.05$ compared to controls.

** $P < 0.05$ compared to baseline.

negative dP/dt_{max} values. There was no hemodynamic deterioration or change during the 30 min of preinfusion of amino acid.

Hemodynamic results are summarized in Tables 1 and 2. The amino acid hearts were characterized by higher postischemic developed pressures as summarized in Fig. 1. Average results of percentage recovery of peak positive dP/dt are summarized in Fig. 2. After 15, 30, and 45 min of reperfusion, developed pressure recovered to 32 ± 8, 44 ± 11, and 45 ± 11% of baseline, respectively, in the control group. In the amino acid group, recovery was significantly better averaging 50 ± 20, 58 ± 17, and 62 ± 17% of baseline, respectively. The dP/dt_{max} recovered to 31 ± 9, 44 ± 11, and 48 ± 14% of the baseline value, respectively, in controls. The counterpart values in the amino acid group were significantly higher, averaging 48 ± 17, 63 ± 18, and 64 ± 17% of baseline, respectively.

End-diastolic pressure was significantly lower in the amino acid group compared to the control group after reperfusion (Table 1). The recovery of end diastolic LV pressure is also summarized in Fig. 3. The pressure-rate products were significantly higher in the amino acid group than those in control group at all time during reperfusion (Table 1). The recovery of coronary flow was significantly higher in the amino acid group only at 45 min of reperfusion compared to the control group (84 ± 14% of baseline in the amino acid group vs 66 ± 14% of baseline in controls, Fig. 4). O_2 EXT was higher in control group at 45 min reperfusion time point, perhaps induced by the lower CF. Myocardial oxygen consumption

TABLE 2

Postischemic Recovery (Percentage of Baseline, Means ± SD)

	REP (15 min)	REP (30 min)	REP (45 min)
DP			
CONT	31.8 ± 8.2	43.8 ± 10.7	45.3 ± 11.1
AM	49.7 ± 20.0*	58.2 ± 17.6*	61.8 ± 17.2*
dP/dt_{max}			
CONT	31.4 ± 9.2	44.2 ± 11.6	48.0 ± 14.6
AM	47.5 ± 17.7*	62.7 ± 18.9*	63.8 ± 17.1*
$-dP/dt_{max}$			
CONT	32.4 ± 7.7	43.4 ± 8.3	45.4 ± 8.9
AM	48.8 ± 23.2*	57.9 ± 19.9*	61.8 ± 19.7*
HR			
CONT	90.8 ± 5.1	95.2 ± 8.4	94.8 ± 9.5
AM	90.2 ± 9.7	93.4 ± 9.3	94.4 ± 9.6
PRP			
CONT	28.9 ± 7.3	41.7 ± 10.5	42.7 ± 9.8
AM	43.9 ± 16.9*	53.7 ± 15.4	57.7 ± 15.6*
CF			
CONT	74.9 ± 14.5	70.5 ± 18.1	66.8 ± 14.4
AM	85.0 ± 14.2	81.9 ± 13.0	84.5 ± 14.9*
MVO_2			
CONT	58.3 ± 20.3	63.7 ± 19.9	66.3 ± 14.2
AM	53.0 ± 15.9	60.8 ± 19.4	65.6 ± 20.7
O_2 EXT			
CONT	81.6 ± 33.9	94.3 ± 32.7	102.2 ± 26.5
AM	62.9 ± 17.2	73.6 ± 18.8	77.4 ± 21.6*

Note. The hemodynamic indexes were determined in isolated reperfused hearts at 15, 30, and 45 min of reperfusion as described under Materials and Methods. Ten hearts were used in each group. DP, developed pressure; dP/dt_{max}, maximum derivative of left ventricular pressure; HR, heart rate; PRP, product of HR and DP; CF, coronary flow; MVO_2, myocardial oxygen consumption; O_2 EXT, oxygen extraction; CONT, control group; AM, amino acid-treated group.

* $P < 0.05$ compared to control group.

was not significantly different between the two groups during reperfusion.

DISCUSSION

The beneficial effect of amino acid preloading upon diastolic recovery and the higher recovery of DP and dP/dt_{max} indicate that amino acid preloading, possibly by metabolic intervention, improved ischemic tolerance and/or minimized the effects of reperfusion in this model. This beneficial effect of amino acid preloading was not felt to be due to changes in coronary vasodilation, as coronary flow during the 30-min preloading period was similar in both groups (controls, 9.11 ± 2.55 vs amino acid preload, 8.08 ± 1.09, ml/min/g tissue), nor was this a primary effect upon alteration in myocardial

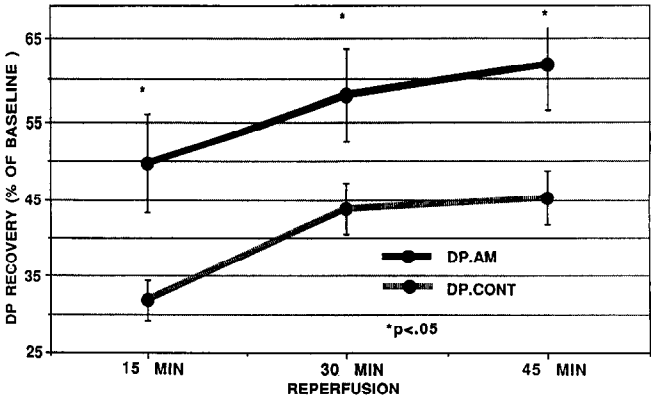

FIG. 1. Average data (means ± SE) on the left ventricular pressure during baseline 15, 30, and 45 min after reperfusion. Developed pressure was significantly higher than control (CONT) with amino acid preloading (AM).

oxygen consumption which remained unchanged between groups during the entire time course.

The metabolic pathways for anaerobic energy production via amino acid substrate level phosphorylation during ischemia include transamination of glutamate with pyruvate to alanine and α-ketoglutarate with subsequent conversion to succinate and transamination of aspartate to oxaloacetate, which is converted subsequently to malate, fumarate, and then to succinate. Other amino acids can be utilized by alternate incorporation in the tricarboxcylic acid cycle including glycine, lysine, and methionine. Still others may provide for energetics by interconversion (arginine, phenylalanine, leucine, isoleucine, valine, and tyrosine) before inclusion into a metabolic pathway. These pathways ultimately lead to formation of high-energy compounds in the mitochondria [4].

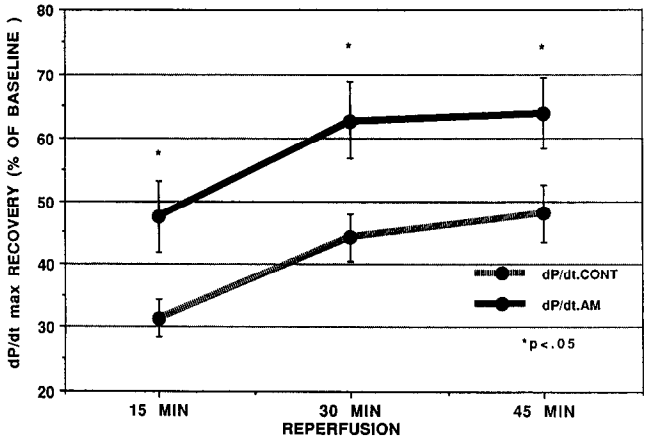

FIG. 2. Average data (means ± SE) on the percentage recovery of peak positive dP/dt at 15, 30, and 45 min after reperfusion. Data are presented as percentages of baseline values. Similar to the findings with developed pressure, amino acid preloading (AM) resulted in a significant improvement of this parameter of systolic cardiac performance compared with control (CONT).

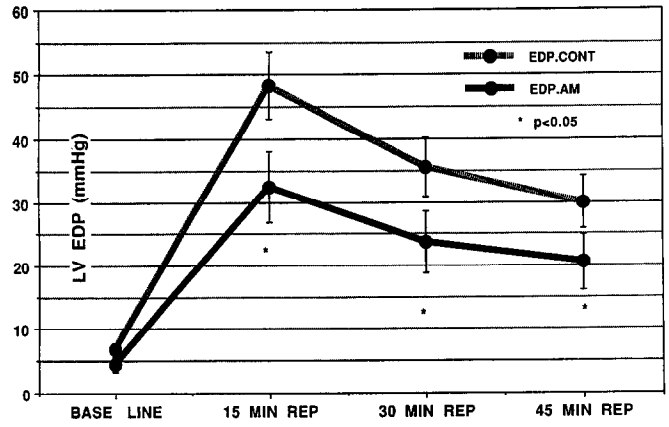

FIG. 3. Average data (means ± SE) on the percentage recovery of left ventricular end diastolic pressure at 15, 30, and 45 min after reperfusion. Data are presented as mm Hg. Amino acid preloading (AM) resulted in a significant improvement of this parameter of diastolic cardiac performance compared with control (CONT).

The transaminase reactions which catalyze the conversion of pyruvate to alanine and α-ketoglutarate to glutamate are major metabolic branch points joining amino acid and carbohydrate metabolism. Pyruvate, as provided in this study is the probable source of the three carbon fragment from which alanine is produced in cardiac muscle and glutamate is the source of the amino nitrogen for the transanimation reaction. During anaerobic metabolism, citrate is formed from glutamate via 2-oxoglutarate and isocitrate, utilizing pyruvate as an amino acceptor. Because citrate inhibits the glycolytic enzyme phosphofructokinase which is the regulatory enzyme for turning "on" aerobic myocardial glucose utilization, anaerobic metabolism with its obligate production of citrate inhibits aerobic glucose utilization even after the reintroduction of oxygen. The quantity of myocardial citrate release during postischemic recovery has been positively correlated with a return of aerobic function. These findings suggest that myocardial ischemia may have a profound effect on myocardial metabolism which lasts many times longer than the ischemic interval itself. Indeed many but not all myocardial metabolic studies have demonstrated that the stunned postischemic heart is inefficient in its utilization of delivered oxygen [5]. This oxygen utilization inefficiency disables the heart from repleting high energy phosphates or meeting the internal reparative work required for the myocardium and the end result is poor postischemic performance [6].

Clinical studies have documented that myocardial metabolism is abnormal during the first hours of reperfusion after a period of myocardial ischemia. Despite the reintroduction of oxygen, there is little or no uptake of carbohydrate or lipid substrates up to 4 hr after coronary artery bypass operations and consequently resumption of aerobic metabolism is suppressed [7]. Although elevated serum levels of glucose, lactate, pyruvate, and β-OH-butyrate were noted, no uptake of these substrates was observed. Interestingly however, amino acids were the only exogenous substrates taken up by the heart. The pattern of uptake of amino acids suggested an adaptation of postischemic myocardial metabolism, as almost all amino acids were extracted from serum with glutamate and branch chain amino acids—leucine isoleucine and valine being the quantitatively most important. These findings suggest that amino acids may serve a very important function in immediate postischemic metabolism.

Confirming this adaptive change despite a "return" to the aerobic state, in a second study [8] the metabolism of cardiac and skeletal muscle following cardiac surgery was examined. While there was a net loss of amino acids from peripheral skeletal muscle, indicating continued aerobic metabolism, there was positive uptake of amino acids in the postischemic myocardium. The amino acids most significantly taken up in the myocardium were glutamate, aspartate, leucine, isoleucine, and tyrosine. The uptake of amino acids by human ischemic myocardium has been documented in patients who are made transiently ischemic by atrial pacing [9]. In these patients with known coronary artery disease, rapid atrial pacing resulted in increased myocardial glutamate extraction and alanine and glutamine turnover, indicating that glutamate extraction is closely connected with the energy supply status of the ischemic myocardium. These alterations of myocardial amino acid metabolism during and following ischemia had been confirmed in other studies [10, 11], which demonstrated that patients with coronary artery disease subjected to ischemic stress all demonstrated increase myocardial uptake of glutamate and release of citrate indicating actual utilization of amino acids through the tricarboxylic acid cycle.

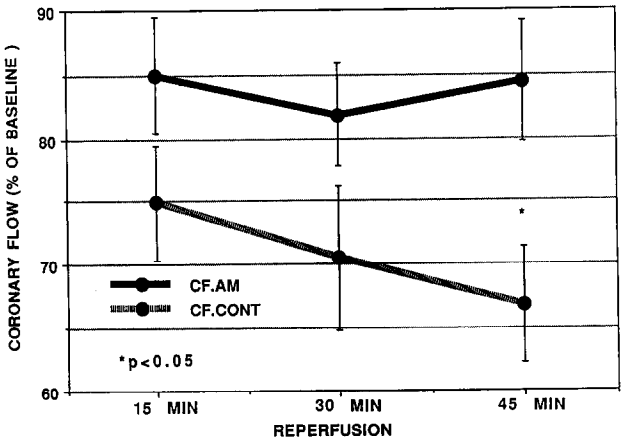

FIG. 4. Average data (means ± SE) on the percentage recovery of coronary flow (CF) at 15, 30, and 45 min after reperfusion. Data are presented as percentages of baseline values. Amino acid treatment (AM) resulted in significantly higher CF compared with control (CONT) at 45 min of reperfusion.

Many studies [12, 13], have demonstrated an increased tolerance to ischemia of the immature heart. The neonatal heart has been shown to contain a higher level of myocardial glutamate than adult hearts. It has been postulated that the altered amino acid metabolism noted during and following ischemia in conjunction with the higher endogenous supply of amino acids in the immature heart may be responsible for this increased neonatal ischemic tolerance noted. Neonatal hearts [12], subject to metabolic blockade of amino acid transanimation with aminooxyacetic acid (AOA), which inhibits glutamate and aspartate utilization during ischemia, had severely impaired recovery of function as opposed to a control group not treated with AOA. This observation has lead to the use of glutamate [14] to enrich energy-depleted adult hearts during ischemia. Glutamate improved recovery of postischemic oxygen consumption and ventricular performance.

To examine the potential mechanisms of this beneficial effect of amino acids on myocardial energy metabolism and return of cardiac function many studies have been undertaken. In isolated working rat hearts made ischemic for 60 min and reperfused for 30 minutes, ischemia reduced ATP and all high energy phosphate compounds 50% or greater. In control hearts, postischemic function recovered only to 60%. However, in hearts perfused with glutamate, cardiac function returned to 90% of baseline, but in this study high-energy phosphate recovery was no better than in the controls. However, there was increased production of succinate indicating utilization of the TCA cycle and altered amino acid metabolism in the glutamate-treated hearts [15]. In contrast to that study [16], others have shown the addition of glutamate to a cardioplegic solution not only increased postischemic recovery of function but also was associated with a lesser decline in the content of high-energy phosphate compounds during ischemia and lead to significantly better restoration of high-energy phosphate content and ATP during reperfusion. This positive effect of amino acid addition upon myocardial metabolism, postischemic function, and high-energy phosphate content has been confirmed by others [17] utilizing glutamate, fumarate, malate, and oxaloacetate to perfuse isolated rat hearts.

This protective effect of amino acids has been confirmed in other models including the isolated rabbit heart where the effect of glutamate on isolated perfused newborn and adult rabbit hearts was shown to result in increased ATP production during reperfusion and improvement of contractile function in both neonate and adult rabbit hearts [18]. Furthermore, with glutamate administration an association between increased production of α-ketoglutarate and succinate denoting utilization of the TCA cycle for myocardial energetics and postischemic cardiac contractile performance was shown [19].

The effect of amino acids on enhancing mechanical recovery of ischemic myocardium was reconfirmed in perfused interventricular rabbit septa, which demonstrated that arginine, glutamate, ornithine, and aspartate significantly maintained developed tension during ischemia and enhanced postischemic recovery twofold over that noted in control septa not receiving amino acids. AOA again inhibited this protective effect supporting the hypothesis that amino acids act through anaerobic intermediary transamination metabolic reactions [4]. In hypoxic ventricular papillary muscle strips the conversion of aspartate and glutamate to succinate for eventual metabolic use was stimulated 20-fold by glutamate, as shown by radiolabeled glutamate oxidation [20], confirmed by increased production of succinate in response to cardiac hypoxia [21] and *de novo* synthesis of alanine [22]. Finally, in isolated ischemic rat myocytes [1], the same amino acids as used in the present study significantly increased oxygen consumption indicating myocardial substrate metabolism is influenced by available amino acids.

As amino acid oxidation accounts for only about 5% of oxygen utilization and metabolism that occurs in the normal aerobic heart, the contribution of amino acids to myocardial energetics has been neglected. However, during ischemia and the critical period of early reperfusion when aerobic mechanisms are still inhibited, available amino acid stores are critical in the repair of subcellular structures (internal work) and in the production of ATP for contraction (external work). While this is only a preliminary study, in this model, amino acid preloading improved postischemic function, perhaps by providing substrate during these periods and enhancing high-energy compound production. This approach may be of a clinical benefit and warrants further study to identify mechanisms and usefulness.

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