EFFECT OF GLUTAMATE-ASPARTATE REPERFUSION ON POSTISCHEMIC NEONATAL MYOCARDIUM

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Objective: We postulated that L-glutamate- and L-aspartate-enriched perfusate would improve functional recovery of postischemic neonatal rabbit hearts. **Methods:** Isolated working neonatal rabbit hearts were perfused with Krebs-Henseleit buffer and then subjected to 1 hour of hypothermic cardioplegic arrest with St. Thomas' Hospital solution. Hearts were then reperfused with L-glutamate- and L-aspartate-enriched (20 mmol/L) Krebs-Henseleit buffer (AA-enriched Krebs-Henseleit buffer). Hearts reperfused with Krebs-Henseleit buffer alone acted as controls (experiment A). Another group of hearts underwent a similar protocol but were reperfused with the AA-enriched Krebs-Henseleit buffer with correction of the sodium content (experiment B). **Results:** Hearts reperfused with AA-enriched Krebs-Henseleit buffer showed a significant decrease in aortic flow at both 15 ($p = 0.04$) and 30 ($p = 0.025$) minutes compared with controls. Arrhythmias were frequent. Sodium content of the AA-enriched Krebs-Henseleit buffer was 174 ± 0.5 mmol/L. In experiment B, hearts reperfused with the AA-enriched Krebs-Henseleit buffer with correction of the sodium content exhibited no difference in aortic flow and cardiac output at either 15 or 30 minutes ($p = 0.95$ and 0.5 and 0.48 and 0.78 , respectively) compared with controls. No arrhythmias were observed. The sodium content of the AA-enriched Krebs-Henseleit buffer was 146 ± 0.7 mmol/L. **Conclusions:** A beneficial effect on functional recovery of neonatal hearts reperfused with AA-enriched Krebs-Henseleit buffer was not demonstrated. (J Thorac Cardiovasc Surg 1997;113:462-6)

Optimization of postischemic neonatal myocardial function remains an elusive goal. Amino acid supplementation of cardioplegic solutions has been advocated to improve protection of both adult¹⁻³ and immature myocardium⁴ after ischemia. However, adult results are mixed⁵ and experimental data

is lacking in neonatal hearts. Studies in the neonatal rabbit heart have been limited to models of hypoxia,⁶ which is a metabolically distinct condition from ischemia.⁷ No studies have examined the effects on functional recovery of postischemic neonatal working hearts reperfused with solutions enriched with amino acid substrates. Intuitively this should lead to sustained improvement in functional recovery on the basis of earlier studies. We investigated the effect of an amino acid-enriched perfusate on functional recovery of postischemic neonatal hearts in a clinically relevant model of neonatal myocardial ischemia.

Materials and methods

Isolated working heart model. Neonatal 8- to 12-day-old rabbits were kept with their does until use. After premedication with intramuscular ketamine hydrochloride (50 mg/kg) followed by intraperitoneal somnotol (40 mg/kg) and heparin (50 units/kg), a tracheostomy was performed and the animal was connected to a Harvard rodent ventilator. Ventilation prevented hearts from becoming ischemic before excision. After a median sternotomy incision, the hearts were rapidly excised and mounted

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Table I. Preischemic control values in experiment A

Group	n	AoF (ml/min)	CoF (ml/min)	CO (ml/min)	SP (mm Hg)	HR (beats/min)
G+A (20 mmol/L)	8	28.2 ± 2.0	13.2 ± 1.2	41.5 ± 3.1	95.4 ± 3.6	331 ± 15
K-H	8	24.1 ± 1.7	12.3 ± 1.2	36.3 ± 2.8	87.3 ± 4.2	319 ± 16
p Value		0.139	0.6	0.24	0.17	0.6

Data are mean ± standard error of the mean.

AoF, Aortic flow; CoF, coronary flow; CO, cardiac output; SP, systolic pressure; HR, heart rate; G+A, L-glutamate (20 mmol/L) + L-aspartate (20 mmol/L) and Krebs-Henseleit solution; K-H, Krebs Henseleit solution.

Table II. Percent recovery of function at 15 minutes in working mode

Group	AoF (ml/min)	CoF (ml/min)	CO (ml/min)	SP (mm Hg)	HR (beats/min)
G+A (20 mmol/L)	45 ± 7	71 ± 8	53 ± 7	80 ± 7	90 ± 5
K-H	70 ± 9	90 ± 9	76 ± 8	87 ± 4	98 ± 5
p Value	0.04	0.17	0.045	0.34	0.42

on the aortic cannula of a water-jacketed Langendorff perfusion apparatus. All animals received humane care in compliance with the guidelines of the Canadian Council on Animal Care. Hearts were then perfused at a hydrostatic pressure of 55 cm H₂O with Krebs-Henseleit solution maintained at 39° ± 1° C. Solution composition (in millimoles per liter) was as follows: NaCl 118.0, KCl 4.7, NaHCO₃ 25.0, MgSO₄ 1.2, KH₂PO₄ 1.2, glucose 11.1, and CaCl₂ 2.0. The perfusion solution was bubbled with a mixture of 95% O₂ and 5% CO₂ and continuously filtered with 5 µm microfilters (Gelman Sciences Inc., Ann Arbor, Mich.). During a 10-minute stabilization period, excess mediastinal tissue was removed and the left atrium was cannulated with simultaneous ligation of the pulmonary veins for conversion to the working mode as described by Neely⁸ and modified by Bove and Stammers.⁹ In this preparation the oxygenated Krebs-Henseleit fluid enters the left atrium from a reservoir located 14 cm above the heart. The perfusate is ejected into a compliance chamber and then against a hydrostatic pressure of 55 cm H₂O. After 15 minutes in the working mode, aortic flow was measured with timed collections from the aortic ejection line. Coronary flow was measured by timed collections underneath the heart. Aortic effluent was recirculated and filtered continuously, whereas coronary effluent was discarded. Systolic and diastolic aortic pressures, as well as heart rate, were measured continuously with a pressure transducer connected to a strip chart recorder. Myocardial temperature was measured with a needle thermistor placed in the right ventricle. Hearts exhibiting coronary-to-aortic flow ratios greater than 60% were discarded because this ratio level could indicate ischemic injury.¹⁰ The heart was arrested with St. Thomas' Hospital cardioplegic solution containing (in millimoles per liter) NaCl 110.0, KCl 16.0, MgCl₂ 15.0, CaCl₂ 1.2, and NaHCO₃ 10.0, pH 7.8, delivered for 3 minutes at a temperature of 10° C from a height of 55 cm by way of a sidearm of the aortic ejection line. To simulate clinical conditions, we placed the heart in a cold water bath at 10° to 15° C for 1 hour and then retrogradely reperfused it for

10 minutes followed by 30 minutes in the working mode. Postischemic aortic and coronary flows, as well as systolic and diastolic pressures, were measured at 15 and 30 minutes and were expressed as percentage of control.

Experimental groups. Hearts were reperfused with Krebs-Henseleit solution alone or with the addition of L-glutamate and L-aspartate (20 mmol/L; Ajinomoto Co., Los Angeles, Calif.)(experiment A) or with Krebs-Henseleit solution alone or with the addition of L-glutamate and L-aspartate (20 mmol/L) but with correction of sodium content (experiment B). The sodium content of Krebs-Henseleit solution was lowered by decreasing the amount of sodium chloride used to give a final sodium content that was normal for the solution (144 ± 0.78 mmol/L).

Statistical analysis. Data are reported as mean ± standard error of the mean. Comparison between groups was made with the Student's *t*-test with the level of significance at *p* ≤ 0.05.

Results

Preischemic control values are shown for experiment A in Table I. No significant difference was found in hemodynamic values between the control and experimental groups. In experiment A, at the end of ischemia, the temperature of the hearts was 12.7° ± 0.7° C and 11.5° ± 0.7° C in group G+A and K-H (*p* = 0.29). During reperfusion in experiment A, significantly decreased aortic flows and cardiac output were seen at 15 minutes; these values were independent of the heart rate (Table II). Aortic flows were also decreased at 30 minutes of reperfusion, and, although cardiac output was less, this result was not statistically significant (Table III). The sodium content of the L-glutamate and L-aspartate (20 mmol/L) + Krebs-Henseleit solution without correction of sodium was 174 ± 0.5 mmol/L, with osmolarity 365 mOsm/kg H₂O and pH 7.64

Table III. *Percent recovery of function at 30 minutes in working mode*

Group	AoF (ml/min)	CoF (ml/min)	CO (ml/min)	SP (mm Hg)	HR (beats/min)
G+A (20 mmol/L)	28 ± 7	67 ± 10	40 ± 7	71 ± 8	84 ± 11
K-H	56 ± 9	88 ± 12	63 ± 9	80 ± 4	93 ± 8
<i>p</i> Value	0.025	0.20	0.06	0.30	0.52

Abbreviations are given in Table I.

Table IV. *Preischemic control values in experiment B*

Group	<i>n</i>	AoF (ml/min)	CoF (ml/min)	CO (ml/min)	SP (mm Hg)	HR (beats/min)
G+A (20 mmol/L)*	9	25.2 ± 1.8	12.8 ± 0.9	38.1 ± 2.5	99.6 ± 4.6	330 ± 9
K-H	7	25.4 ± 2.7	12.3 ± 1.1	37.8 ± 3.6	96.7 ± 5.3	299 ± 16
<i>p</i> Value	0.9	0.95	0.73	0.94	0.69	0.1

Abbreviations are given in Table I.

*Sodium corrected

when bubbled with 95% O₂ + 5% CO₂ at 39° C. The slightly alkaline pH is probably a reflection of the ability of the Krebs-Henseleit perfusate to buffer the acidosis produced by the amino acid additives. Arrhythmias were frequent and were seen in seven of eight hearts during reperfusion.

Preischemic values for experiment B are shown in Table IV with no significant differences in hemodynamic parameters noted between groups. At the end of ischemia the temperature of the hearts was 12.4° ± 0.5° C and 12.5° ± 0.4° C in group G+A and K-H (*p* = 0.83). In experiment B hearts were reperfused with the amino acid-enriched solution as in experiment A; however, the sodium content was corrected to 146 ± 0.7 mmol/L, the osmolarity was 291 mOsm/kg H₂O, and the pH was 7.45. No difference was found in either aortic flow or cardiac output at 15 or 30 minutes of reperfusion (Tables V and VI). Coronary flow was significantly increased in the control group at 15 minutes of reperfusion and may have resulted from an atrial leak. No arrhythmias were observed on reperfusion.

Discussion

Experimental studies have shown that glutamate- and aspartate-enriched perfusate improved functional and metabolic recovery of adult myocardium after hypoxia.^{11,12} Hochachka and others^{13,14} showed that anaerobic glycolysis was the primary pathway for energy production in diving vertebrates and that the anaerobic catabolism of the amino acids glutamate and aspartate yielded succinate, a component of the Krebs cycle. Julia and associates¹⁵ showed in puppies that more glutamate was used

and more succinate was produced after normothermic global ischemia than in a control group. They suggested that this may be one mechanism that improves the tolerance of the immature heart to ischemia. Pisarenko and his group¹⁶ have shown that when glutamic acid, 20 mmol/L, was added to a cold blood cardioplegic solution in patients undergoing repair of Tetralogy of Fallot, tissue levels of glutamate and adenosine triphosphate were significantly higher than in a control group of patients undergoing the same repair; this correlated with a significant difference in cardiac index 2 hours after the operation. In a similar fashion, aspartate^{17,18} has been shown to improve functional recovery of adult rat myocardium when added to cardioplegic solutions.

We postulated that normothermic amino acid-enriched reperfusion of postischemic isolated neonatal working hearts would enhance functional recovery on the basis of the previous studies. An ischemic period of 1 hour at temperatures between 10° and 15° C was chosen because of its clinical relevance to neonatal cardiac surgery. However we have shown that the addition of L-glutamate, and L-aspartate, 20 mmol/L, to Krebs-Henseleit solution during reperfusion of postischemic neonatal rabbit hearts is detrimental to functional recovery. Many hearts were arrhythmic in contrast to control hearts which were in sinus rhythm at reperfusion. This result may be explained by the high sodium content and osmolarity of the Krebs-Henseleit solution once the monosodium salts of the amino acids have been added to it. After correction of the sodium content and osmolarity, we found no significant difference in functional recovery between control and amino

Table V. Percent recovery of function at 15 minutes in working mode

Group	AoF (ml/min)	CoF (ml/min)	CO (ml/min)	SP (mm Hg)	HR (beats/min)
G+A (20 mmol/L)*	72 ± 11	87 ± 8	77 ± 9	82 ± 5	83 ± 7
K-H	73 ± 7	119 ± 8	86 ± 6	87 ± 4	97 ± 2
p Value	0.95	0.02	0.48	0.40	0.13

*Sodium corrected.

Table VI. Percent recovery of function at 30 minutes in working mode

Group	AoF (ml/min)	CoF (ml/min)	CO (ml/min)	SP (mm Hg)	HR (beats/min)
G+A (20 mmol/L)*	58 ± 10	87 ± 10	67 ± 9	77 ± 4	84 ± 6
K-H	47 ± 11	114 ± 15	71 ± 6	79 ± 4	97 ± 3
p Value	0.50	0.12	0.78	0.79	0.15

Abbreviations are given in Table I.

*Sodium corrected.

acid-enriched reperfusion groups. The approximately 70% recovery of aortic flow and cardiac output during reperfusion in the control group may be attributable to the calcium content of the St. Thomas' Hospital cardioplegic solution used (1.2 mmol/L).^{19,20}

The disparity of our results with others may have several explanations. Previously,^{6,12} a model of hypoxia, a metabolically distinct condition from ischemia, was used. Hochachka and Storey¹⁴ have shown that porpoises and other divers have elevated levels of aspartate and alanine amino transferases to aid in the transition from anaerobic to aerobic metabolism. In developing sheep and guinea pig hearts, however, decreased activities of these amino acid transferases have been shown.²¹⁻²³ Galinanes and associates²⁴ have shown the mildly improved postischemic function of perfused adult rat hearts arrested with L-aspartate-enriched (20 mmol/L) cardioplegic solution to be related to the sodium content of the cardioplegic solution and not necessarily to the amino acid component. They concluded that the ionic composition of amino acid-enriched cardioplegic solutions requires consideration before attributing the cardioprotective properties to the additives. Although Weldner and associates⁴ attributed improved functional recovery of 3- to 4-week-old rabbit hearts to enrichment of a standard blood cardioplegic solution with L-glutamate, 20 mmol/L, they did not compare this with another group where the blood cardioplegic solution sodium content was increased by 20 mmol/L. The decreased function of hearts exposed to monosodium glutamate and aspartate immediately after ischemia may also be explained by increased

sodium-hydrogen and sodium-calcium exchange resulting in worsening postischemic reperfusion injury.²⁵ Nakanishi²⁶ has shown the negative inotropic effect of a solution containing Na⁺, 200 mmol/L, on maximal rate of tension rise of neonatal rabbit septa.

Potential weaknesses of this study are that it was performed with the use of crystalloid perfusates and the absence of biochemical data. Qui and Hearse²⁷ have shown that retrograde blood perfused isolated rabbit hearts were more stable, had less coronary flow, were more responsive to cardioplegic protection, and exhibited a significantly improved recovery of left ventricular pressure after ischemia as compared with crystalloid-perfused preparations. It may be postulated that these amino acids will not be effective when added to a crystalloid cardioplegic solution at temperatures between 10° and 15° C. The results presented in this article cannot be generalized to adult hearts.

Both glutamate and aspartate are excitatory neurotransmitters and are neurotoxic at sufficiently high concentrations.²⁸ Newborn mice exposed to systemic glutamate have experienced retinal injury, and, on reaching maturity, have been noted to have neurodegenerative lesions.²⁸ These excitatory neurotransmitters accumulate under conditions of hypoxia and ischemia, resulting in increased cellular permeability to sodium- and calcium-mediated cell death.²⁹ Deep hypothermia and total circulatory arrest are techniques frequently used in neonatal cardiac surgery. We postulate that the use of these amino acids during repair of neonatal congenital heart disease could contribute to a poor neurologic

outcome in addition to the potential detrimental effect on the heart.

In summary we have shown lack of a beneficial effect on functional recovery of postischemic neonatal rabbit hearts reperfused with Krebs-Henseleit solution enriched with L-glutamate and L-aspartate with or without correction of sodium. We advise caution in the use of these amino acids as cardioplegic additives in neonatal cardiac surgery because of their potential neurotoxicity, especially in the setting of circulatory arrest.

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