EFFECT OF MAGNESIUM IN CARDIOPLEGIC SOLUTION UPON HYPOTHERMIC ISCHEMIC MYOCARDIAL MITOCHONDRIA

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Twelve anesthetized mongrel dogs were subjected to systemic hypothermia and potassium-induced cardioplegia for 60 minutes with or without magnesium-1-aspartate. The effect of magnesium was assessed by indices of mitochondrial oxidative phosphorylation. Cardiac arrest was induced by potassium (20 mEq per liter) (6 dogs) or potassium (20 mEq per liter)-magnesium (8 mM per liter). The heart was reperfused for ten minutes following arrest. Dogs were supported by standard cardiopulmonary bypass with hypothermia at 20°C of myocardial temperature.

Mitochondria were isolated from the endocardium, the epicardium of the left ventricle and the ventricular septum. ADP: O ratio and state 3 respiration were well maintained in both groups following 60 minutes of ischemic arrest and 10 minutes of reperfusion. Magnesium suppressed the non-phosphorylated oxygen consumption of mitochondria, therefore, respiratory control index was significantly enhanced in the group of potassium-magnesium-1-aspartate cardioplegia. These data suggest that magnesium protects functional capacity of mitochondrial phosphorylation in the myocardium from ischemia.

SUBCELLULAR pathophysiology of ischemic myocardium has been extensively studied.^{1,2} Ischemic myocardium may develop explosive cell swelling during coronary artery reperfusion subsequent to defective cell volume regulation.^{3,4} This is partly explained by the alteration of cell membrane permeability in which K-efflux, Mg-efflux and influx of Na, Cl and Ca occur during reperfusion.^{5,6} Movement of these electrolytes across sarcolemmal membrane occurs rapidly in the first few minutes of

reperfusion.⁷ Therefore, the fate of myocardial reversibility is partly determined in the early phase of reperfusion. Cardiac cell survival during the phase of ischemia and reperfusion is dependent on mitochondrial function and the capacity for production of adenosine triphosphate.⁸

The protective mechanism of myocardium from ischemia has not been completely understood yet. However, magnesium was used to prolong the period of viability of anoxic heart^{9,10} or used in the cardioplegic solution.¹¹⁻¹³ Potassium-magnesium-1-aspartate prevents ATP consumption during muscular contraction,¹⁴ and decreases the total ATP-ase activity and the Na-K-ATP-ase activity in the canine heart.¹⁵ Furthermore, important evidence reported by Sordahl and Silver¹⁶ demonstrates

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TABLE I COMPOSITION OF CARDIOPLEGIC SOLUTION

	KCl	Mg-l-aspartate	Glucose	Mannitol	NaHCO ₃	CaCl ₂	Osmolarity
Potassium-Magnesium cardioplegia	20mEq	8mM (1.243g/L)	247mM (44.5g/L)	200mM (36.434g/L)	to adjust pH to 7.4	0.5mM (0.0735g/L)	530 (mOsm/L)
Potassium-cardioplegia	20mEq	~	247mM	200mM	to adjust pH to 7.4	0.5mM	557 (mOsm/L)

Distilled water was used to make up 1 liter solution, Numbers were expressed as concentrations in one liter solution.

that magnesium protects the high energy state and modulates calcium transport and ATP-synthesizing activities of heart mitochondria.

Thus, the effect of magnesium-l-aspartate in cardioplegic solution upon myocardial mitochondria was investigated in the hypothermic canine model with ten minutes of reperfusion following 60 minutes of ischemic cardioplegia. Mitochondrial function was assessed by indices of oxidative phosphorylation.

MATERIALS AND METHODS

Mongrel dogs were used in this investigation. The weight of the 17 dogs ranged between 9.8 and 18.7 Kg. All dogs were anesthetized with thiamylal sodium approximately 30 mg per Kg intravenously to suppress corneal reflexes, and respiration was controlled by a positive pressure ventilator. Arterial and left ventricular pressure and the electrocardiogram were recorded continuously using pressure transducers (Statham P23DB) and a polygraph.

Left thoracotomy was performed, and cardiopulmonary bypass (CPB) was instituted using a pediatric-sized bubble oxygenator (Bentley). The left ventricle was vented through the left atrium during total perfusion and cross-clamping of the aorta. Prime solution contained 1,000 ml of Ringer's lactate, 500 ml of low-molecular-weight dextran in saline, and 50 mEq of sodium bicarbonate. No blood was added to the prime solution. Flow rate of perfusion during CPB was approximately 100 ml per Kg per minute, however, it was adjusted to maintain the mean arterial pressure between 70 and 100 mmHg. The pH of arterial blood was 7.41 ± 0.01 during cross-clamping of the aorta and perfusate with blood mixed during reperfusion had a pH of 7.42 ± 0.02 and an osmolarity of 318 mosm per liter.

Systemic hypothermia with cold prime solution (10°C) was employed using a heat exchanger. The aorta was cross-clamped when tem-

perature reached 28°C . The duration to obtain 28°C of myocardial temperature ranged between 3 and 5 minutes. Myocardial temperature was monitored in the mid-layer of ventricular septum using needle thermister (Yellow Springs Instrument, Inc.). Care was taken to eliminate spontaneous warming of myocardium by removing the light source from the exposed thoracotomy. Moreover, the apical portion of left ventricle was selected for myocardial biopsy since this portion of ventricle was covered with the chest wall and presumed to be equivalent to the septal temperature. Immediately after cross-clamping of the aorta, cold cardioplegic solution ($4-10^{\circ}\text{C}$) was infused with the approximate perfusion pressure of 30 mmHg by gravity through the ascending aorta using an 18 G needle until the heart stopped electrical activity. Infusion of cold cardioplegic solution enabled myocardial cooling down to 20°C in 10 to 15 minutes after cross-clamping of the aorta. Myocardial temperature was maintained between 18 and 20°C during 60 minutes of cross-clamping of the aorta.

No rewarming was attempted in this investigation after the aorta was unclamped in this experiment. Reperfusion was observed for ten minutes under a perfusion pressure between 70 and 100 mmHg and approximate flow rate of 100 ml/Kg/min.

Experiments were grouped as follows:

Group 1 (6 dogs)—potassium-magnesium-l-aspartate induced cardioplegia for 60 minutes at 20°C .

Group 2 (6 dogs)—potassium induced cardioplegia for 60 minutes at 20°C .

Group 3 (5 dogs)—thoracotomy without CPB, control studies of mitochondrial respiration.

Composition of cardioplegic solutions are shown in Table I. At the end of the experiments dogs were sacrificed and hearts were removed immediately, then rinsed with cold saline ($0-4^{\circ}\text{C}$). Approximately 7 grams each of endocar-

TABLE II SUMMARY OF MYOCARDIAL MITOCHONDRIAL FUNCTION
(Substrate: glutamate)

	Tissue	$K^+ \cdot Mg^{2+} \cdot l\text{-aspartate}$ $n = 6$	K^+ $n = 6$	control $n = 5$
ADP : o ratio	Endo	3.18 ± 0.02	3.18 ± 0.04	3.17 ± 0.06
	Epi	3.17 ± 0.02	3.15 ± 0.02	3.21 ± 0.03
	Sep	3.15 ± 0.03	3.20 ± 0.04	3.21 ± 0.02
State 3 respiration ($n\text{Atom} \cdot 0 \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$)	Endo	150.3 ± 12.9	177.0 ± 8.9	162.6 ± 19.8
	Epi	152.5 ± 18.9	172.5 ± 13.7	147.6 ± 15.0
	Sep	179.2 ± 18.7	190.5 ± 16.1	161.6 ± 13.9
State 4 respiration ($n\text{Atom} \cdot 0 \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$)	Endo	10.0 ± 0.8 ($p < .001$)	14.4 ± 1.1	11.6 ± 0.8
	Epi	9.4 ± 0.5 ($p < .002$)	13.2 ± 1.0	10.1 ± 0.5
	Sep	10.9 ± 0.8 ($p < .01$)	14.1 ± 1.1	11.5 ± 0.6
RCI	Endo	15.0 ± 0.7 ($p < .01$)	12.6 ± 0.9	13.9 ± 1.2
	Epi	16.2 ± 1.6 ($p < .03$)	13.2 ± 0.9	14.6 ± 1.2
	Sep	16.5 ± 1.2 ($p < .02$)	13.7 ± 0.8	14.1 ± 1.2

RCI=Respiratory Control Index, Endo=Endocardium, Epi=Edpicardium, Sep=Ventricular Septum
P value indicates significance to K^+ cardioplegic group.

TABLE III SUMMARY OF MYOCARDIAL MITOCHONDRIAL FUNCTION
(Substrate: succinate + rotenone)

	Tissue	$K^+ \cdot Mg^{2+} \cdot l\text{-aspartate}$ $n = 6$	K^+ $n = 6$	Control $n = 5$
ADP : 0 ratio	Endo	1.87 ± 0.01	1.94 ± 0.02	1.88 ± 0.05
	Epi	1.87 ± 0.03	1.95 ± 0.02	1.89 ± 0.04
	Sep	1.91 ± 0.04	1.97 ± 0.02	1.90 ± 0.05
State 3 respiration ($n\text{Atom} \cdot 0 \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$)	Endo	141.7 ± 9.1	170.0 ± 11.0	144.8 ± 9.1
	Epi	140.0 ± 13.1	160.2 ± 12.3	136.8 ± 11.0
	Sep	153.8 ± 9.9	167.0 ± 12.9	148.6 ± 10.2
State 4 respiration ($n\text{Atom} \cdot 0 \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$)	Endo	35.3 ± 1.5 ($p < .01$)	40.3 ± 2.6	38.4 ± 1.7
	Epi	31.9 ± 1.2 ($p < .01$)	36.8 ± 1.8	35.7 ± 2.1
	Sep	35.2 ± 1.0 ($p < .01$)	38.1 ± 2.4	36.6 ± 2.4
RCI	Endo	4.0 ± 0.2	4.2 ± 0.1	3.8 ± 0.2
	Epi	4.4 ± 0.3	4.3 ± 0.2	3.8 ± 0.2
	Sep	4.3 ± 0.2	4.4 ± 0.1	4.1 ± 0.3

RCI=Respiratory Control Index, Endo=Endocardium, Epi=Epicardium, Sep=Ventricular Septum
P value indicates significance to K^+ cardioplegic group

dium, epicardium and ventricular septum were excised from the left ventricle. These tissues were processed for isolation of mitochondria as outlined below.

MITOCHONDRIAL STUDIES

Excised myocardial tissues were immediately processed as previously described by publica-

tion.¹⁷

Indices of oxidative phosphorylation as described by Chance and Williams¹⁸ were obtained by methods modified from Sordahl et al.¹⁹ The oxygen consumption curves were recorded from a vibrating platinum electrode in a 1.5 ml solution of 0.15 M sucrose, 10 mM PO_4 , 0.10 M Tris, and 10 mM glutamate substrate or 10 mM succi-

nate with 2.5 μg rotenone at pH 7.4 and a temperature of 29°C. Mitochondrial protein (1.0 mg), determined by the Lowry method²⁰ was added to the cuvette. Approximately 250 nmoles of adenosine diphosphate (ADP) were added in each of three successive determinations of oxygen consumption with glutamate substrate in each of three mitochondrial suspensions. Two successive determinations were made with succinate-rotenone substrate. An average of four runs on each suspension were studied for six determinations with glutamate and four with succinate for the mitochondria from each of the three tissue suspensions in each experiment.

The indices were defined: ADP : O ratio = nanomoles of ADP phosphorylated per nanomole of oxygen consumed, state 3 respiration = the rate of oxygen consumption during ADP phosphorylation, state 4 respiration = the rate of oxygen consumption after ADP phosphorylation is completed, and respiratory control index (RCI) = the ratio of state 3 oxygen consumption to state 4 oxygen consumption. A value for each of these indices were obtained in each experiment for the endocardial and epicardial layers from the left ventricle and the ventricular septum.

RESULT

Mitochondrial function following cardioplegia compared to normal myocardium are summarized in Table II and III.

ADP : O ratio remained unchanged following 60 minutes of cardioplegia either by potassium-magnesium or potassium cardioplegia compared to normal.

State 3 respiration was well protected from 60 minutes of hypothermic cardioplegia both in the group of potassium and potassium-magnesium cardioplegia when either glutamate or succinate with rotenone was substrated and their functional capacity ranged in that of normal myocardium.

State 4 respiration was enhanced significantly following potassium cardioplegia when either glutamate or succinate with rotenone was substrated. Hence, respiratory control index (RCI) was lowered in potassium-induced cardioplegia. On the other hand, RCI in potassium-magnesium-induced cardioplegia group became the highest value in these experimental groups.

There was no significant difference in mitochondrial respiratory function among the layers of the left ventricle.

DISCUSSION

Magnesium-1-aspartate in cardioplegic solution did add the protective effect upon mitochondrial state 3 respiration following 60 minutes of cross-clamping of the aorta compared to cardioplegia without magnesium at 20°C of myocardial temperature. However, state 4 respiration of mitochondria isolated from the myocardium following 60 minutes of cardioplegia was significantly reduced by magnesium. Therefore, respiratory control index was increased in the myocardium following magnesium-potassium cardioplegia.

Magnesium has been utilized for protecting myocardium from ischemic insult. Webb et al.¹⁰ reported that magnesium sulfate increased the period of viability of anoxic rat hearts in normothermia. Nakae et al.⁹ showed magnesium sulfate provided a several-fold prolongation of viability of cardiac muscle and it also extended the tolerance of normothermic canine heart to ischemia to nearly 3 hours. In 1959, Sealy et al.¹³ used cardioplegic solution for surgical treatment of ventricular septal defect and tetralogy of Fallot, in which magnesium sulfate (2.4) was used with potassium citrate and neostigmine under moderate hypothermia. Recently, magnesium has been advocated to be mixed in cardioplegic solution for the protection of myocardium from ischemia. Hearse and Braimbridge^{11,21} have suggested the importance of magnesium in cardioplegic solution to maintain better recovery in aortic flow and pressure from ischemic insult. German cardiac surgeons¹² have extensively investigated cardioplegia induced by procaine-magnesium-1-aspartate and they have accumulated evidences to prove the protective effect of their cardioplegic solution combined with moderate hypothermia.

Nakahara et al.¹⁴ showed that potassium-magnesium-1-aspartate is closely related to electrolyte transport and prevents ATP consumption during muscular contraction. Furthermore, Fedelesova et al.¹⁵ demonstrated that potassium-magnesium-aspartate decreased the total ATPase activity and the Na-K-ATPase activity of canine heart. Sordahl and Silver¹⁶ reported the two important evidences; the first, in the presence of magnesium, mitochondria apparently retained the capability to phosphorylate ADP after calcium uptake, the second, an effect of magnesium on calcium deposition in the intramitochondrial space was shown, in which two types of calcium

crystals, a spheroidal — amorphous and a destructive needle — like crystalloid mass were demonstrated. Their evidence suggests that magnesium protects the high energy state and modulates calcium transport and ATP-synthetizing activities of heart mitochondria. Furthermore, modulation of calcium uptake by magnesium may play an essential role in protecting myocardial mitochondria during ischemia and during early reperfusion of ischemic myocardium. During reperfusion, as Shen et al⁶ reported, calcium accumulation in the intramitochondrial space is critical to determine the myocardial functional recovery from ischemia. In this investigation, ischemic myocardium induced either by potassium-magnesium-l-aspartate or potassium alone maintained normal capacity of oxidative phosphorylation. This result is consistent with that of Sordahl and Silver¹⁶. And the non-phosphorylated oxygen consumption was elevated in mitochondria isolated from myocardium induced by potassium cardioplegia, whereas, remained in the normal range in mitochondria isolated from myocardium induced by potassium-magnesium-l-aspartate. Non-phosphorylated oxygen consumption (state 4 respiration) has been known to be affected by electrolyte movement across membranes, particularly, calcium enhances calcium-related non-phosphorylated oxygen consumption.²² In the previous investigation dealing with reperfused ischemic myocardium, an increase in non-phosphorylated oxygen consumption related to calcium movement has been demonstrated.¹⁷ Thus, the effect of magnesium-l-aspartate inhibiting an increase in non-phosphorylated oxygen consumption is critically important to preserve the integrity of mitochondrial function.

Magnesium has been known to be negative chronotropic effect and this effect was more pronounced at a low calcium concentration and was not abolished by a lowering of temperature.^{23,24} In this investigation, complete study of magnesium upon hemodynamics was excluded. However, Hearse et al²¹ advocated the importance of magnesium (12–16 mM) to improve hemodynamic recovery from ischemic arrest. Furthermore, magnesium sulfate (4–5 mEq L) in hyperalimentation has been widely recommended without changes in hemodynamics.²⁵

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