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# Effect of ATP Synthesis Promoters on Postischemic Myocardial Recovery[1,2]

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**The use of cardioplegia during surgically induced ischemia greatly reduces myocardial metabolic requirements. However, adenosine triphosphate (ATP) depletion may occur, resulting in poor functional recovery after ischemia. This study investigated if augmentation of intracellular ATP could be achieved by delivering known ATP synthesis promoters (adenosine and/or phosphate) during cardioplegic arrest, and whether this could enhance myocardial functional and metabolic recovery following ischemia. Isolated, perfused rabbit hearts were subjected to 120 min of hypothermic (34°C) cardioplegia-induced ischemia. Controls received St. Thomas cardioplegia (CTL); remaining hearts received cardioplegia containing 200 $\mu M$ adenosine (ADO), or 25 $\mu M$ phosphate ($PO_4$), or both ADO and $PO_4$. Following ischemia and reperfusion, recovery of developed pressure (%DP) and postischemic diastolic stiffness was significantly better in adenosine hearts when compared with control or $PO_4$ hearts. To determine if ADO or $PO_4$ minimized depletion of ATP during ischemia or accelerated synthesis of ATP in the postischemic period, nucleotide levels were obtained before, during, and after ischemia. During ischemia, ATP fell equally in all groups, indicating that ADO and $PO_4$ did not alter ischemia-induced depletion of ATP. However, intracellular adenosine was augmented during ischemia in adenosine-treated hearts. Consequently, during reperfusion, ADO and ADO/$PO_4$ hearts had significantly enhanced ATP levels, suggesting that augmenting myocardial adenosine accelerated synthesis of ATP postischemia. The addition of phosphate, a stimulus for ATP synthesis, did not augment postischemic ATP. In fact, the beneficial effect of adenosine may have been decreased when phosphate was added to adenosine. In conclusion, adenosine but not $PO_4$ augments intracellular ATP by allowing better metabolic repletion following ischemia, thereby improving postischemic myocardial functional recovery.** 

## INTRODUCTION

To provide myocardial protection during cardiac surgery, cardioplegia and myocardial hypothermia are routinely utilized. Despite these protective techniques, adenosine triphosphate (ATP) depletion may occur during ischemia [1]. Since intracellular ATP is essential for myocardial contraction and relaxation, ATP depletion may be an important factor contributing to the incomplete recovery of ventricular function following ischemia and reperfusion [2]. In a previous study, we demonstrated that adenosine-supplemented cardioplegia was associated with accelerated repletion of ATP and improved functional recovery after reperfusion [3], consistent with results obtained by other investigators using different experimental models [4–6]. The use of 2-deoxycoformycin, an agent that inhibits adenosine metabolism, was also beneficial [4], supporting the view that adenosine availability during and after global ischemia may be an important determinant of postischemic metabolic and functional recovery. The objective of the present study was to evaluate the potential benefit of another agent that has been reported to stimulate ATP synthesis.

Based upon the demonstration that phosphate stimulates ATP production in the kidney [7], we tested the hypothesis that phosphate is an effective promoter of ATP synthesis in isolated hearts exposed to global ischemia followed by reperfusion. Cardioplegia was administered containing phosphate or phosphate plus adenosine (to ensure substrate availability for ATP synthesis). The effects of these interventions were compared with the results obtained using adenosine in cardioplegia, done concurrently [3], and the results of treatment of a control group of isolated hearts with cardioplegia alone.

## MATERIAL AND METHODS

Studies were performed in 39 isolated, perfused rabbit hearts. Male New Zealand White rabbits (3–4 kg) were anesthetized with intravenous pentobarbital (10 mg/kg), after which cardiectomy was performed through a median sternotomy. The beating heart was immediately immersed in cold (4°C) Krebs–Ringers bicarbonate solution, after

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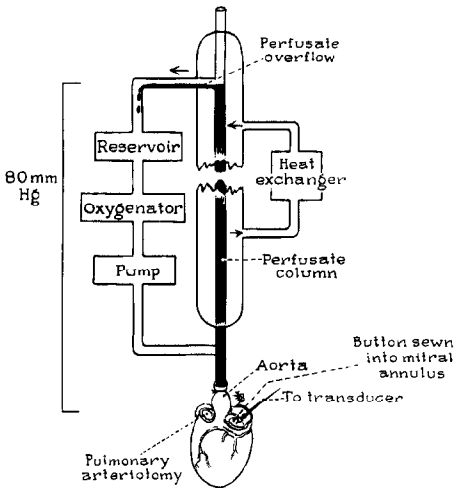


**FIG. 1.** Perfusion column for isolated heart preparation. Reproduced with permission, from Ref. [4].

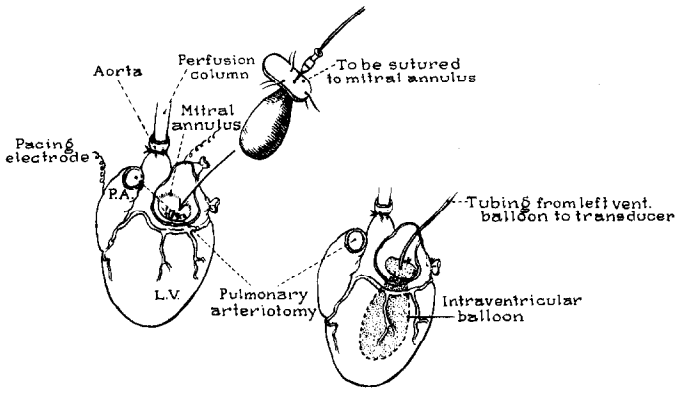


**FIG. 2.** Balloon inserted into LV cavity for recording functional measurements. Reproduced, with permission, from Ref. [4].

which it was suspended from a perfusion column by aortic cannulation within 30 sec of excision. Coronary perfusion was established at 80 mm Hg, and the perfusate used was a modified, oxygenated Krebs–Ringer bicarbonate solution (pH 7.44 to 7.48, 300 to 310 mOsm/liter).

The oxygen tension of the perfusate was maintained between 550 and 650 Torr and the perfusate temperature was kept at 37°C by means of a heat exchanger in the perfusion column (Fig. 1). The perfusion solution was passed through a No. 40 polyester filter to remove particulate matter and was not recirculated.

After coronary perfusion was initiated, the hearts generally regained a sinus rhythm, but in the presence of persistent fibrillation, immediate direct current cardioversion was carried out. While the heart was perfused on the column, a portion of the mitral valve with its chordae was excised and a latex balloon, connected to saline-filled tubing, was introduced into the left ventricle (LV) through the mitral orifice (Fig. 2). The balloon was sewn in place in such a way to allow for passive venting of the LV cavity. The balloon was connected through a catheter to a pressure transducer, and LV pressure was recorded continuously. Output from the pressure transducer was electronically differentiated to enable continuous recording of $dP/dt$. Left ventricular pressure and $dP/dt$ were recorded on a Gould (Model 2600s) pressurized ink chart recorder.

During the preischemic control period, a volume of saline was introduced into the LV balloon to produce an end-diastolic pressure (EDP) of 10 mm Hg. The same volume was used to evaluate ventricular systolic performance during reperfusion. By increasing LV volume in increments, diastolic stiffness was estimated before and after the ischemic interval by measuring the slope of linearized end-diastolic pressure versus end-diastolic volume curves ($d$ EDP/$d$ EDV) for each heart. Coronary flow was measured volumetrically. A thermistor needle was inserted into the midmyocardium to record myocardial temperature.

After a 30-min stabilization period, control measurements of EDP, developed pressure (DP, peak systolic pressure minus end-diastolic pressure), peak positive $dP/dt$, coronary flow, and diastolic stiffness were made in each heart. Hearts were then rendered globally ischemic by interruption of the perfusion column immediately above the aortic cannulation site. The intraventricular balloon was deflated and 60 ml of cardioplegia was administered. The experimental protocol is shown in Fig. 3.

All hearts were maintained at 34°C by means of a circulating water jacket during 120 min of total ischemia. All hearts received 15 ml of cardioplegia every 30 min during ischemia. Control hearts ($n = 15$) received modified St. Thomas cardioplegia, while experimental hearts received modified St. Thomas cardioplegia containing 25 $\mu M$ phosphate ($n = 7$), a combination of 200 $\mu M$ adenosine and 25 $\mu M$ phosphate ($n = 7$), or 200 $\mu M$ adenosine alone ($n = 10$).

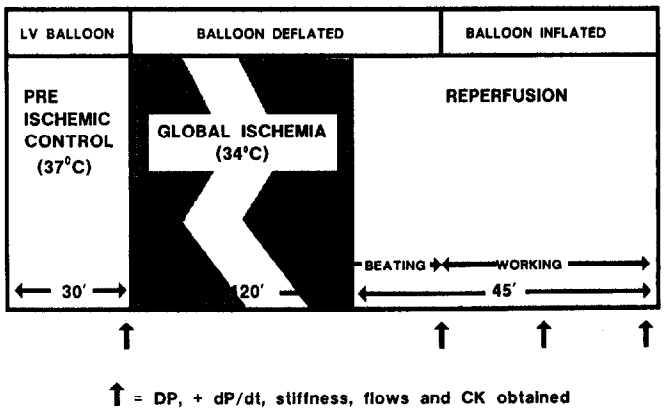


**FIG. 3.** Experimental protocol. DP = developed pressure, $dP/dT$ = change in pressure over time, stiffness = change in slopes of end-diastolic pressure vs end-diastolic volume curves over time, CK = creatine kinase.

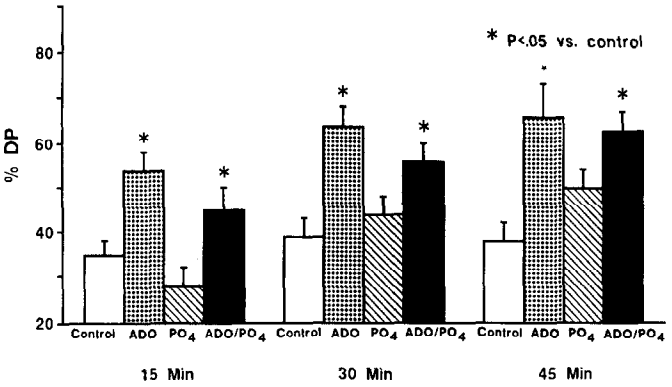


**FIG. 4.** Recovery of developed pressure with time, following reperfusion, comparing control hearts to treated groups. % DP = percentage of developed pressure as compared to preischemic baseline level.

Reperfusion was performed with a perfusate temperature at 37°C and delivered with pressure at 80 mmHg for all hearts in all groups. Defibrillation was performed as needed during the initial 3 min of reperfusion. During the initial 15 min of reperfusion, the intraventricular balloon was kept deflated to simulate the beating, nonworking condition. After the initial 15 min of reperfusion, the LV balloon was refilled to the preischemic control volume and measurements of EDP, DP, $dP/dt$, diastolic stiffness, and coronary flow were made. The balloon remained inflated for the remainder of reperfusion. LV functional measurements were obtained after 15, 30, and 45 min of reperfusion.

After 45 min of reperfusion, all hearts were removed from the perfusion apparatus. The myocardial water content was determined by weighing a fresh specimen of myocardium, allowing this sample to desiccate for 48 hr at 80°C, and finally reweighing the sample. Percentage water was calculated using the formula (1 − dry weight/wet weight) × 100 = % $H_2O$.

Furthermore, a parallel series of experiments were done in an identical fashion for recovery of nucleotide levels. Specimens were taken from the left ventricle by a biopsy gun during the control period, immediately prior to reflow (at end ischemia) and 1 and 15 min during reperfusion, and dropped immediately into liquid nitrogen (−196°C). The specimens were assayed by the high-performance liquid chromatography (HPLC) method, for myocardial cellular ATP, ADP, AMP, adenosine, and phosphocreatine levels. Samples of 20–30 mg were homogenized on a precooled mortar and pestle at the temperature of dry ice. Next, 20-mg samples were suspended in 1 ml of 6% trichloroacetic acid and spun at 5000 rpm for 15 min at 4°C. The precipitate was subsequently dissolved in 0.5 $N$ NaOH and stored at 5°C for later protein analysis. The filtrate was washed with 0.6 $m$ tri-$N$-octylamine prepared as a 22% mixture in Freon. This mixture was vortexed for 2 min and centrifuged for 10 min at 750 $g$. The neutralized extracts were then filtered with a 0.4 m$M$ nitrocellulose filter.

AMP, ADP, ATP, and adenosine were separated on a C-18 column by isocratic, reverse-phase, ion pairing chromatography. Purines were quantitated by measurement of peak heights compared with the peak heights of corresponding standards run under identical conditions with each group of tissue extracts, and values are expressed as micromoles of nucleotide per milligram of protein. Statistical analysis was performed with analysis of variance and Student's two-tailed $t$ test, where appropriate. A $P$ value less than 0.05 was considered significant. All results are expressed as means ± SEM. All animals received human care in compliance with the *Principles of Laboratory Animal Care* formulated by the National Society for Medical Research.

## RESULTS

### *Left Ventricular Function*

There were no significant differences in the prearrest DP or $dP/dt$ values between control hearts and those receiving adenosine, $PO_4$, or ADO/$PO_4$ for any group. Preischemic developed pressure for all hearts was 108 ± 4 mm Hg. Left ventricular systolic and diastolic functional measurements of DP and $+dP/dt$ were made after 15, 30, and 45 min of reperfusion and were expressed as percentages of return of function as compared to the prearrest isovolumic control values. Left ventricular EDP was also measured in 15-min intervals following reperfusion and was expressed as an increase in EDP over the preischemic control EDP value of 10 mm Hg. Diastolic stiffness, measured by the slope of linearized end-diastolic pressure vs end-diastolic volume curves, was assessed for each heart at 15-min intervals following reperfusion and is expressed as raw data.

After 120 min of hypothermic ischemia and 45 min of reperfusion control hearts recovered to 42 ± 4% of the preischemic DP and 44 ± 4% of preischemic $dP/dt$. There was significantly better recovery of LV function in the adenosine-augmented hearts (Fig. 4) with 66 ± 7% recovery of DP and 64 ± 3% recovery of preischemic $dP/dt$ in the hearts treated with 200 $\mu M$ exogenous adenosine ($P < 0.05$ vs control hearts). Furthermore, the ADO/$PO_4$ hearts had significantly better recovery of function than the control hearts. ADO/$PO_4$ hearts recovered to 63 ± 4% of preischemic DP and 65 ± 6% of preischemic $dP/dt$. However, $PO_4$ hearts recovered only 50 ± 4% of preischemic DP and 49 ± 2% of preischemic $dP/dt$ (nonsignificant vs control).

Left ventricular EDP, while only an estimate of diastolic function in this model, was significantly lower at all times during reperfusion in the ADO- and ADO/$PO_4$-augmented hearts compared with control. After 45 min of reperfusion, control hearts demonstrated elevated left ventricular end-diastolic pressures, with a 42 ± 6 mm Hg rise in LVEDP, whereas the 200 $\mu M$ adenosine hearts had a 17 ± 4 mm Hg elevation in LVEDP. Furthermore,

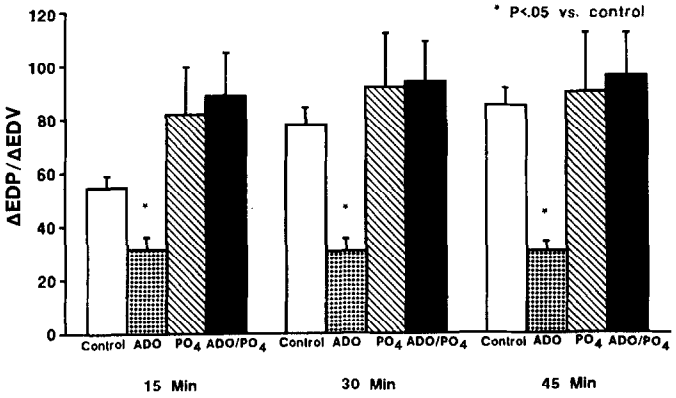


**FIG. 5.** Change with time in slopes of end-diastolic pressure vs end-diastolic volume curves, comparing control hearts to treated groups. sEDP/sEDV = change in end-diastolic pressure/change in end-diastolic volume.

while the ADO/$PO_4$ hearts showed a 24 ± 5 mm Hg rise in LVEDP, the $PO_4$ hearts demonstrated a 39 ± 10 mm Hg rise in LVEDP.

The slope of linearized end-diastolic pressure vs end-diastolic volume curves was determined for each heart in the prearrest period as well as during reperfusion. Diastolic stiffness in the adenosine-augmented hearts was significantly lower at all times during reperfusion compared to untreated control hearts (Fig. 5). After 45 min of reperfusion the control hearts were characterized by a diastolic stiffness slope value of 82 ± 10 (a 8.2 ± 1.0 mm Hg rise in end-diastolic pressure for each 0.1-ml increase in end-diastolic volume), compared with a diastolic stiffness value of 41 ± 6 in the 200 $\mu M$ adenosine hearts ($P < 0.05$). The $PO_4$ and ADO/$PO_4$ groups were not significantly different from one another in terms of diastolic stiffness, with diastolic stiffness values of 91 ± 19 and 93 ± 16, respectively ($P < 0.05$ compared with control).

Coronary flow during reperfusion demonstrated no differences between any group at any time during reperfusion. Prearrest values were also similar in all of the groups. The myocardial water content following 45 min of reperfusion was not statistically different between any of these groups.

*Nucleotide Levels*

Adenosine, AMP, ADP, and ATP were measured in the control, adenosine, phosphate, and ADO/$PO_4$ groups ($n = 4$ each) to investigate the mechanism of the favorable action on recovery of ventricular function. All results are expressed as micromolar nucleotide per milligram of protein, measured by Lowry assay, and are listed in Table 1. The change in adenosine levels during ischemia and reperfusion is demonstrated in Fig. 6 and the change in ATP levels during that same time period is demonstrated in Fig. 7. Baseline readings of adenosine and ATP were equivalent in all groups measured, (control, adenosine, phosphate, and ADO/$PO_4$).

**TABLE 1**

**Nucleotide Levels During Ischemia and Reperfusion**

| | Level ($\mu M$/mg protein) | | | |
|---|---|---|---|---|
| | ATP | ADP | AMP | ADO |
| Control | | | | |
| Baseline | 2.3 ± 0.01 | 4.0 ± 0.15 | 1.2 ± 0.04 | 2.2 ± 0.04 |
| End ischemia | 0.8 ± 0.01 | 0.8 ± 0.8 | 0.8 ± 0.04 | 2.0 ± 0.12 |
| 1 min postreflow | 1.8 ± 0.05 | 1.2 ± 0.17 | 1.9 ± 0.07 | 1.9 ± 0.05 |
| 15 min postreflow | 1.3 ± 0.19 | 2.1 ± 0.08 | 1.6 ± 0.09 | 1.5 ± 0.07 |
| Adenosine | | | | |
| Baseline | 2.6 ± 0.11 | 6.8 ± 0.06 | 1.5 ± 0.02 | 2.4 ± 0.06 |
| End ischemia | 0.8 ± 0.04 | 0.7 ± 0.15 | 0.7 ± 0.02 | 5.6 ± 0.17 |
| 1 min postreflow | 5.9 ± 0.05 | 8.0 ± 0.12 | 8.0 ± 0.06 | 5.9 ± 0.09 |
| 15 min postreflow | 6.9 ± 0.07 | 8.9 ± 0.14 | 10.0 ± 0.11 | 4.0 ± 0.11 |
| Phosphate | | | | |
| Baseline | 2.4 ± 0.09 | 5.2 ± 0.09 | 1.4 ± 0.04 | 1.9 ± 0.09 |
| End ischemia | 0.6 ± 0.15 | 0.8 ± 0.06 | 0.9 ± 0.06 | 2.1 ± 0.07 |
| 1 min postreflow | 1.9 ± 0.06 | 1.4 ± 0.14 | 1.8 ± 0.08 | 2.0 ± 0.06 |
| 15 min postreflow | 1.5 ± 0.12 | 2.0 ± 0.07 | 2.1 ± 0.10 | 1.8 ± 0.07 |
| Adenosine and phosphate | | | | |
| Baseline | 2.3 ± 0.11 | 7.4 ± 0.06 | 1.9 ± 0.02 | 2.0 ± 0.13 |
| End ischemia | 0.8 ± 0.05 | 1.0 ± 0.15 | 1.0 ± 0.06 | 3.1 ± 0.13 |
| 1 min postreflow | 3.3 ± 0.15 | 4.0 ± 0.12 | 6.1 ± 0.06 | 3.7 ± 0.10 |
| 15 min postreflow | 3.8 ± 0.23 | 6.8 ± 0.14 | 8.0 ± 0.11 | 4.1 ± 0.10 |

During ischemia, ATP levels fell equally in all groups, with end-ischemic ATP level decreasing to approximately 0.8 $\mu M$/mg. However, myocardial adenosine was augmented in some treated groups during ischemia. Control hearts demonstrated adenosine levels of 2.0 ± 0.12 $\mu M$/mg at end ischemia as compared to 5.6 ± 0.17 and 3.1 ± 0.03 $\mu M$/mg in the adenosine and ADO/$PO_4$ groups, respectively (both $P < 0.05$ vs control). As expected, hearts treated with $PO_4$ alone demonstrated no augmentation of adenosine during ischemia (2.1 ± 0.07 $\mu M$/mg, nonsignificant vs control).

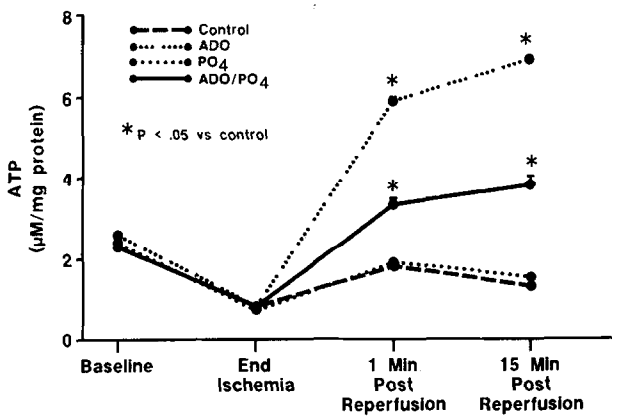


**FIG. 6.** Change in adenosine level measured during ischemia and reperfusion, expressed as $\mu M$ adenosine/mg of protein. ADO = adenosine treated hearts, $PO_4$ = phosphate-treated hearts, ADO/$PO_4$ = adenosine- and phosphate-treated hearts.

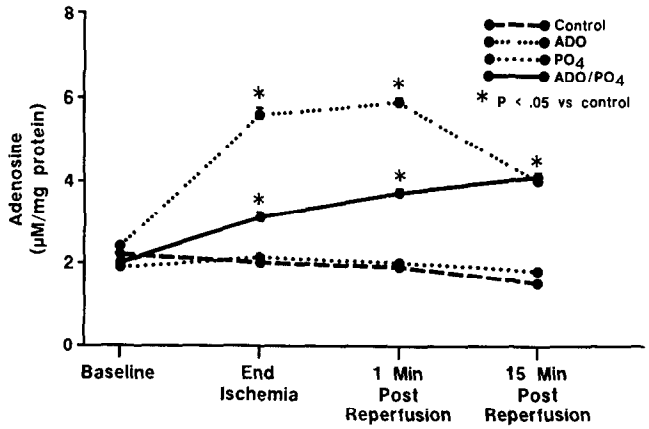


FIG. 7. Change in ATP level measured during ischemia and reperfusion, expressed as $\mu M$ ATP/mg of protein. ADO = ATP-treated hearts, $PO_4$ = phosphate-treated hearts, $ADO/PO_4$ = adenosine- and phosphate-treated hearts.

During reperfusion, both at 1 min and at 15 min following reflow, control hearts were characterized by depressed adenosine levels ($1.9 \pm 0.5$ and $1.5 \pm 0.07$ $\mu M$/mg). As a result, ATP levels remained depressed in the control group and were only $1.8 \pm 0.05$ and $1.3 \pm 0.19$ $\mu M$/mg at 1 and 15 min following reperfusion. However, this was not the case in the ADO or $ADO/PO_4$ groups. The adenosine-treated hearts demonstrated the best augmentation of adenosine nucleotide levels ($5.9 \pm 0.09$ and $4.0 \pm 0.11$ $\mu M$/mg at 1 and 15 min following reflow). Consequently, the adenosine-treated hearts also had substantially higher levels of ATP at 1 and 15 min following reflow, $5.9 \pm 0.05$ and $6.9 \pm 0.07$ $\mu M$/mg, respectively ($P < 0.05$ vs control). Although the $ADO/PO_4$-treated group did not achieve the high levels of ATP augmentation observed in the adenosine-treated group, it was characterized by significant augmentation of nucleotide levels during reperfusion compared with the control group. $ADO/PO_4$-treated hearts demonstrated adenosine levels of $3.7 \pm 1.0$ and $4.1 \pm 10$ $\mu M$/mg at 1 and 15 min following reperfusion and ATP levels of $3.3 \pm 0.15$ and $3.8 \pm 0.23$ $\mu M$/mg at the same times, respectively. Disappointingly, $PO_4$ hearts did not show any augmentation of adenosine nucleotide levels ($1.9 \pm 0.06$ $\mu M$/mg and $1.5 \pm 0.06$ $\mu M$/mg at 1 and 15 min following reflow). Furthermore, phosphate-treated hearts did not demonstrate any augmentation or neosynthesis of ATP during reflow with ATP levels remaining quite depressed ($2.0 \pm 0.06$ and $1.8 \pm 0.08$ $\mu M$/mg, respectively, at 1 and 15 min following reflow).

## DISCUSSION

Many studies have shown that myocardial ATP and adenosine levels decrease during ischemia [1–5]. Since high-energy phosphate compounds are essential for myocardial contraction and relaxation, as well as maintenance of cellular integrity, any reduction in high-energy phosphate compounds may have severe effects on myocardial cells. Reduction in myocardial ATP content has been correlated with the incomplete recovery of ventricular function observed following ischemia [1].

In a previous study from this laboratory [4], a beneficial effect of exogenously administered adenosine upon recovery of ventricular function was demonstrated in an isolated rabbit heart model of 2 hr of hypothermic (32°C) multidose cardioplegia-induced ischemia. In that study, control hearts which received only modified St. Thomas cardioplegia recovered $47 \pm 3\%$ of their initial developed pressure following 45 min of postischemic reperfusion, whereas hearts receiving cardioplegia supplemented with 100, 200, or 400 $\mu M$ adenosine had significantly increased recovery of function ($63 \pm 4$, $78 \pm 3$, and $70 \pm 4\%$ of initial developed pressure, respectively). Nucleotide levels were not measured in that study.

In a follow-up study from our laboratory [3] we investigated if augmentation of myocardial adenosine during global ischemia improves functional recovery by decreasing depletion of ATP during ischemia or by enhancing repletion of ATP after reperfusion. Isolated adult rabbit hearts were again subjected to 120 min of mildly hypothermic cardioplegia-induced ischemia (34°C). Myocardial adenosine levels were augmented during ischemia by providing exogenous adenosine in cardioplegia or by inhibiting adenosine degradation with 2-deoxycoformycin, a noncompetitive inhibitor of adenosine deaminase. Results from this study demonstrated that adenosine augmentation with adenosine, deoxycoformycin, or both significantly enhanced recovery of ventricular function following ischemia as compared to standard cardioplegia-treated controls. Furthermore, there was better diastolic function in adenosine-augmented groups.

In this follow-up study [3], nucleotide levels were measured by HPLC. During ischemia adenosine levels were significantly elevated in the adenosine-augmented groups, while ATP decreased equally in all four groups, indicating that augmenting myocardial adenosine had no effect on depletion of ATP during ischemia. After reperfusion, ATP levels were depressed in the control group but increased in the other groups above baseline values, suggesting that improvement in functional recovery was due to accelerated repletion of adenine nucleotide stores in the adenosine-augmented groups. Other studies have also shown a favorable effect of ATP precursors on the recovery of ventricular function following ischemia in many different models [5, 6].

In contrast to these previous studies that attempted to enhance high-energy phosphate levels following ischemia by increasing the availability of ATP precursors, several other studies have been undertaken to directly stimulate ATP synthesis during and following ischemia. The first study [7] utilized a canine renal model, in which stimulation of ATP synthesis in hypothermically perfused kidneys was shown to be enhanced when the combination of high-dose adenosine (10 $\mu M$) and phosphate (25 $\mu M$) was

utilized. The use of either of these agents alone was less effective in stimulating the synthesis of ATP.

Phosphate has been shown to suppress catabolism of adenine nucleotides [8] by inhibiting the action of 5'-nucleotidase, as well as decreasing the intraconversion of the various purine nucleosides [9]. Both of these actions would lead to a net increase in precursor availability. Interestingly, in our experiments phosphate did not show a synergistic effect on the favorable action of adenosine-induced synthesis of ATP. Furthermore, in our study, high-dose phosphate, while not demonstrating a significant decrease in myocardial compliance (when added to adenosine or added alone), tended to show a detrimental effect in terms of myocardial diastolic stiffness (as compared to adenosine alone or control hearts). This tendency could be due to calcium phosphate binding, at the actin–myosin coupling level, leading to poor diastolic relaxation [10]. In another study, free inorganic phosphate was shown to have no protective effect on the ischemic myocardium; in fact, inorganic phosphate was found to have a negative inotropic effect on the myocardium if present in high doses throughout ischemia [11].

Other investigators have attempted to stimulate myocardial adenine nucleotide biosynthesis directly by using pentoses, pentitols, and 5-aminoimidazole-4-carboxamide riboside (AICAR) with variable results [12, 13]. Finally, direct exogenous administration of the two major intracellular high-energy phosphate compounds, ATP and creatine phosphate, has been utilized in an attempt to benefit postischemic functional recovery in the myocardium [14, 15]. However, their utilization is hindered by the inability of these compounds, which are both very large and highly charged, to cross the cell membrane [16].

Adenine nucleotides are resynthesized following ischemia by two major pathways [17]. The first pathway is by *de novo* synthesis of the nucleotide pool. However, this pathway is extremely slow, with only 0.4% of the total nucleotide pool being resynthesized per hour. It would take up to 1 week for complete restoration of ATP via the new synthesis pathway, if ATP levels were reduced to one-half of their preischemic level [18]. The second pathway involves rephosphorylation of adenosine by the enzyme adenosine kinase to AMP, where it would be available for regeneration to ATP. This salvage pathway involving rephosphorylation of adenosine is quite efficient and rapid. Intracellular phosphate, even if present at only low levels, is available in sufficient quantity for this pathway. However, the salvage pathway does depend on the availability of intracellular adenosine as a precursor for the kinetics of the pathway to move to ATP resynthesis. Therefore, if, following ischemia, there is a washout of adenosine from the interstitial and extracellular space, then these nucleotide precursors are not available for the salvage pathway. The prolonged period required for the resynthesis of ATP is due not to an inability of the mitochondria to rephosphorylate precursors but, perhaps, to an inadequate availability of adenosine for the salvage pathway itself, because of the washout of adenosine [4–6].

Studies in intact suspended cardiomyocytes from guinea pig hearts [19] have demonstrated that under ischemic conditions, there is a slow release of high-energy phosphorylated compounds from myocytes. However, with the addition of coronary endothelial cells, there is a rapid appearance in the medium suspension of nucleotide precursor compounds such as adenosine and adenosine's breakdown products, inosine and hypoxanthine. The conclusion from these data is that once high-energy phosphate compounds from myocytes are degraded *in vivo*, they are rapidly broken down to adenosine and inosine and removed from the nucleotide pool into the vascular space, and upon reperfusion are lost to the heart.

In animal studies, the loss of adenine nucleotides from the heart via the coronary sinus has been noted to be an excellent marker for irreversible heart failure [20], and most recently, a significant correlation has been shown between the loss of adenosine from the coronary sinus of patients and significant coronary stenoses [21].

In the present study, systolic and diastolic functional recovery and ATP resynthesizing capacity after an ischemic episode were best when adenosine was administered exogenously in cardioplegia. The mechanism of adenosine's favorable action in this model is not definitively established, but may be due to the increased availability of intracellular adenosine at reperfusion, which facilitates resynthesis of ATP and better functional recovery. Very high doses of phosphate (25 $\mu M$), however, did not enhance ATP synthesis and were not beneficial to recovery of ventricular function. The combination of adenosine and phosphate resulted in no better recovery than use of adenosine alone, and had the tendency to suggest the possibility that phosphate negatively influenced the salutary effects exerted by adenosine. Additional investigation will be necessary to verify this possibility and pursue its mechanism. In conclusion, this study demonstrates that adenosine administered during the time of ischemia can favorably enhance ATP synthesis and, therefore, functional recovery following ischemia. Although, this is a crystalloid perfused model, and not completely applicable to clinical use, adenosine may be useful in cardiac surgery, as protection against myocardial injury during ischemia.

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