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This study tests the hypothesis that endogenous adenosine mediates recovery of cardiac function in ischemia/reperfused rabbit hearts. Isolated isovolumic rabbit hearts perfused at constant pressure were subjected to mild ischemia (perfusion pressure 50 cm H_2O) or moderate ischemia (perfusion pressure 30 cm H_2O) for 90 min followed by 60 min of reperfusion. In treated hearts, infusion of 100 μM 8-*p*-sulfophenyl theophylline (SPT) was initiated 20 min before ischemia and maintained throughout the experiment. Adenosine receptor blockade did not affect left ventricular function assessed from pressure-heart rate product (PRP). Lactate release increased to 152 ± 24% of baseline during mild ischemia and 259 ± 26% of baseline during moderate ischemia in untreated hearts. Lactate release was markedly elevated at baseline, ischemia and reperfusion by SPT treatment ($P < 0.05$ compared to untreated). Purine nucleoside release was 4.1 ± 0.7 nmol · min^{-1} · g^{-1} in SPT treated group and 1.8 ± 0.24 nmol · min^{-1} · g^{-1} in untreated group during moderate ischemia ($P < 0.05$). Myocardial efficiency was significantly lower in the SPT treated hearts (240 ± 11 mmHg · g^{-1} · μl^{-1} O_2) compared to untreated hearts (300 ± 22 mmHg · g^{-1} · μl^{-1} O_2) during reperfusion after moderate ischemia. In conclusion, adenosine receptor blockade stimulates glycolysis in normoxic and ischemic myocardium, but does not affect post-ischemic functional recovery.

ADENOSINE RECEPTOR BLOCKADE INCREASES LACTATE AND PURINE RELEASE BUT DOES NOT AFFECT FUNCTIONAL RECOVERY IN ISOLATED RABBIT MYOCARDIUM

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LIST OF ABBREVIATIONS

ADA	adenosine deaminase
ADO	adenosine
AMP	adenosine monophosphate
ADP	adenosine diphosphate
ANOVA	analysis of variance
ATP	adenosine triphosphate
BW	body weight
cAMP	cyclic adenosine monophosphate
CF	coronary flow
CPP	coronary perfusion pressure
Cr	creatine
CrP	creatine phosphate
EHNA	erythro-9-(2-hydroxy-3-nonyl)adenosine
HR	heart rate
HPLC	high performance liquid chromatography
HW	heart wet mass
IMP	inosine 5'-monophosphate
INO	inosine
K_{ATP}	ATP sensitive potassium channels

LACT	lactate
LDH	lactate dehydrogenase
LVDP	left ventricular developed pressure
LVEDP	left ventricular end-diastolic pressure
MVO_2	myocardial oxygen consumption
NAD	nicotinamide adenine dinucleotide
NADH	the reduced form of nicotinamide adenine dinucleotide
pH_i	intracellular pH
PAA	phenylaminoadenosine
P_i	inorganic phosphate
PIA	phenylisopropladenosine
PRP	product of heart rate-pressure
SEM	standard error from mean
SPT	8-*p*-sulfophenyl theophylline
TC	time control
SPT TC	SPT treated time control
$V_{ADO+INO}$	rate of adenosine + inosine release
V_{lac}	rate of lactate release

CHAPTER I

INTRODUCTION

BACKGROUND

Adenosine is an endogenous cardiac nucleoside produced primarily from the degradation of adenosine triphosphate (ATP). Myocardial ischemia causes release of adenosine, and this adenosine activates adenosine receptors, which regulate adenylate cyclase activity through guanosine triphosphate binding proteins.[25] Adenosine receptors are classified as A_1 and A_2 receptors, located predominantly in the myocardium and coronary vasculature, respectively.[42] In the past years, adenosine has been received great attention[27] since its cardioprotective effects are recognized including coronary vasodilation,[28] β-adrenergic antagonism,[29] increasing glucose uptake,[30] and preconditioning.[14]

Myocardium reperfusion has now become an accepted therapy for the treatment of evolving myocardial infarction.[43] Rahimtoola[1,2] defined a phenomenon termed "myocardium hibernation" in which myocardium subjected to sustained moderate ischemia has a reversible decrease in its force of contraction, which fully recovers upon restoration of coronary flow. The mechanism of myocardial hibernation has not been delineated.

Studies of short term moderate ischemia, termed "acute hibernation", have noted discordant functional and metabolic responses to myocardial ischemia. Specifically, contractile dysfunction occurs prior to significant decrease in the content of myocardial

ATP.[3,4,5] Schaefer *et al.*[5] developed an isolated, perfused rat heart model of acute hibernation and employed nuclear magnetic resonance techniques to determine ATP and creatine phosphate (CrP) contents and creatine kinase release during ischemia and reperfusion. Coronary flow was reduced from 12.5 to 5.4 ml · min^{-1} for 2 hr, followed by reperfusion. The reduction of flow resulted in a stable 44% reduction in the rate-pressure product. Myocardial CrP content fell significantly by 9% within the first 15 min of ischemia, but recovered to control values by the end of ischemia. Downing and Chen[3,6,7] developed an isolated, nonworking neonatal piglet heart model of hibernation in which coronary flow was lowered to 10% of baseline for 2 hr, and then restored to the pre-ischemic level. The myocardial ATP and CrP contents were identical in both reperfused and time control groups. In another study of acutely hibernating isolated rat hearts, Keller and Cannon[8] found that left ventricular performance fell in proportion to perfusion pressure, but completely recovered when perfusion pressure was restored. Gao *et al.*[9] established a hibernating isolated working guinea pig heart model and found that when coronary flow was lowered 80% for 60 min, heart rate-pressure product and pressure-volume work fell 87% and 75%, respectively, fully recovered when flow was restored. Myocardial ATP phosphorylation potential fell 67% during the first 10 min ischemia, and subsequently recovered to the pre-ischemic value.[9] Offstad *et al.*[24] reported that neither adenosine receptor activation nor K_{ATP} channel opening modulates acute hibernation in red blood cell perfused piglet hearts and crystalloid perfused rabbit hearts. The hearts were subjected to 2 hr low-flow (10%) ischemia and reperfusion. Left systolic pressure recovered to 77% in hearts treated with the adenosine receptor blocker 8-p-sulphophenyl theophylline (SPT), and 74% of pre-ischemic level in hearts treated with

K_{ATP} channel opener aprikalim compared to 78% in control hearts during 60 min of reperfusion.

Adenosine receptors are involved in cardioprotection

Adenosine A_1 receptors located on cardiac myocyte mediate the negative chronotropic, inotropic, dromotropic and anti-adrenergic effects of adenosine, and adenosine A_2 receptors, located predominantly on endothelium and vascular smooth muscle, mediate the positive coronary blood flow effects.[10] Lasley *et al.*[11] used time to onset of ischemic contracture during zero flow global ischemia as an indicator of myocardial ischemic injury in isolated rat hearts. Ischemic contracture was defined as the onset of a continual rise in left ventricular end-diastolic pressure. Hearts were treated with adenosine, the adenosine A_1 receptor agonist, phenylisopropyladenosine (PIA), the adenosine A_2 receptor agonist, phenylaminoadenosine (PAA), and the adenosine receptor blocker, A1433U. The results showed that adenosine and PIA nearly doubled the time to onset of ischemic contracture compared to control hearts, whereas PAA treatment had no such a effect. Treatment with the adenosine receptor blocker A1433U shortened the time to onset of ischemic contracture to 50% of control values. This supported the hypothesis that endogenous adenosine modulates the tolerance of the heart to ischemia and that the effects of adenosine are mediated by adenosine receptor activation. Gao *et al.*[9] reported that adenosine receptor blocker SPT doubled ischemic lactate release, lowered coronary venous purine nucleoside release by 21%, and blunted the subsequent rebound of phosphorylation potential in isolated working guinea pig hearts. These results suggested that activation of adenosine receptors results in recovery

of cytosolic energy level of moderately ischemic, working myocardium. However this energetic recovery is not solely responsible for post-ischemic contractile recovery.[9] Janier *et al.*[23] reported that the salutary effects of adenosine on delayed time of onset of ischemic contracture were completely blocked with 10 μM SPT and that exogenous adenosine attenuated ischemic injury by adenosine receptor-mediated stimulation of anaerobic glycolysis. These findings suggest that a receptor-mediated mechanism plays an important role in the cardioprotective action of adenosine during ischemia and reperfusion.

Adenosine is a mediator of preconditioning

Preconditioning the myocardium with a sublethal period of ischemia renders it highly resistant to infarction from a subsequent ischemic insult. This phenomenon has been found in dogs,[44] pigs,[45] and rabbits.[14] The protection conferred by ischemic preconditioning has been subsequently mimicked by pretreatment with adenosine, or an adenosine agonist.[14] In contrast to other species, adenosine does not appear to mediate preconditioning in rat heart.[12] Liu *et al.*[13,14] examined the effect on infarct size of pretreatment with the adenosine receptor blocker SPT in both control and preconditioned *in situ* rabbit hearts. Hearts underwent 30 min of regional ischemia plus 3 hr of reperfusion, and infarct size was measured with tetrazoluim staining. Infarct size averaged 39% of the zone at risk in controls but only 8% in preconditioned hearts. Preconditioned and nonpreconditioned hearts receiving SPT had infarcts not different in size from the controls. Liu *et al.*[13,14] also found that a 5-min intracoronary infusion of adenosine was as effective as 5 min of ischemia in protecting perfused isolated rabbit hearts against infarction from a subsequent 45-min ischemic insult.

The ability of adenosine receptor blocker to block preconditioning confirmed that the activation of adenosine receptors is involved in the protective effect.

Adenosine enhances preservation of myocardial ATP

ATP is the final source of energy for the contractile process. Mentzer's group[15,16] found that augmentation of myocardial adenosine during ischemia and reperfusion would facilitate the repletion of myocardial adenine nucleotide content and could thereby enhance the recovery of post-ischemic ventricular contractile function. Isolated rat hearts were perfused at a constant coronary flow and paced at 280 beats · min^{-1}. Hearts subjected to 10 min of global ischemia and 60 min of reperfusion in the presence of 100 μM adenosine had a greater recovery of post-ischemic left ventricular developed pressure than untreated hearts. Adenosine treatment was also associated with higher tissue ATP content after ischemia. Adenosine may act by preserving ATP, since ATP concentration and synthesis increase after ischemia when isolated hearts are treated with exogenous adenosine.[17,18,19] However, a number of studies have provided evidence that during ischemia and reperfusion, cardiac function is not strongly correlated with myocardial ATP content.[46-48] The cytosolic ATP phosphorylation potential has been shown to be strongly correlated with cardiac function.[32,52] This potential is defined as [ATP]/[ADP][P_i], where P_i is inorganic phosphate. It is important to note that the ATP phosphorylation potential is not dependent on ATP content, as the potential can be high even at low ATP levels if ADP and P_i levels is low. Does adenosine treatment increase ATP phosphorylation potential? Bünger *et al.*[49-50] tested the effects of 100 μM adenosine treatment on adenylate contents and the phosphorylation state of creatine

phosphate in normoxic and post-ischemic isolated guinea pig hearts. In normoxic hearts adenosine treatment had no effect on the phosphorylation state of creatine phosphate. In contrast, the exogenous adenosine markedly increased the phosphorylation state of creatine phosphate in post-ischemic hearts.[49] These observations suggest that the cytosolic ATP phosphorylation potential was increased by adenosine during reperfusion.

Effects of adenosine receptor activation on glycolysis

Wyatt *et al.*[30] demonstrated that exogenous adenosine stimulates glycolysis in the isolated perfused normoxic hearts and that this stimulation is mediated by adenosine A_1 receptors. Lasley *et al.*[31] observed that isolated perfused rat hearts treated with adenosine plus the adenosine deaminase inhibitor EHNA during low flow ischemia exhibited increased lactate release and a delayed onset of contracture, whereas adenosine receptor blockade markedly reduced lactate release and accelerated the onset of contracture. Moreover, when glucose was removed from the perfusate, adenosine plus EHNA treatment had no effect on lactate release or time to onset of ischemic contracture. This means that glucose is an important requirement for post-ischemic recovery of contractile function,[32] and adenosine attenuates myocardial ischemic contracture may be related to glucose metabolism. Mainwaring and his colleagues[34] found that adenosine increased glucose uptake in constant flow perfused normoxic rat hearts. Law *et al.*[35] suggested a possible mechanism by which adenosine increases glucose uptake. Adenosine may improve glucose transport by its insulin-like characteristics.[35]

However, Finegan *et al.*[36] reported that glycolysis was inhibited by adenosine pre-treatment in isolated working rat hearts. During reperfusion, glycolysis was also inhibited by adenosine treatment, either concurrently or pre-ischemic treatment. Additionally, Dale *et al.*[37] reported inhibition of glycolysis by the adenosine A_1-receptor agonist PIA. In addition, adenosine deaminase enhances the effect of insulin on glucose uptake.[37] There is evidence that adenosine exerts a biphasic effect on lactate production in isolated guinea pig hearts. Adenosine elevated lactate production in normoxic hearts, but it did not in the hypoxic hearts.[53] In our laboratory, Gao *et al.*[9] recently found that isolated guinea pig hearts treated with SPT doubled ischemic lactate release compared to untreated hearts during ischemia.

SPECIFIC AIMS

The above review suggests that adenosine is a mediator of mechanisms which are involved in cardioprotection, myocardial preconditioning, preservation of myocardial ATP, and mediation of glycolysis. These protective effects of adenosine on myocardial ischemia and reperfusion injury are mediated through activation of adenosine receptors. The release of endogenous adenosine during myocardial ischemia may serve as an important protective mechanism to maintain energy reserves by reducing myocardial oxygen consumption through its negative inotropic and chronotropic and antiadrenergic effects.[38,39] During reperfusion, endogenous adenosine may be effective as cardioprotective agent to prevent reperfusion injury by inhibition of neutrophil activation and free radical formation and decreasing intracellular calcium.[40] Infusion of adenosine in the reperfusion period has been reported to significantly enhance myocardial salvage beyond that due to reperfusion alone.[41] This

cardioprotection was associated with preservation of the structure and metabolic function of the reperfused myocardium, probably through activation of adenosine receptors. Adenosine receptor activation has been particularly effective in attenuating ischemic contracture and increasing ATP content in severely ischemic rabbit hearts.[23] Presently, no studies have determined if adenosine receptor activation is cardioprotective in rabbit hearts subjected to moderate, reversible ischemia, *i.e.* acute myocardial hibernation. I proposed that adenosine receptor activation decreases contractile function and slows metabolism of ischemic myocardium, and thus preserves energy reserves. The following hypotheses were tested in rabbit hearts subjected to moderate, reversible ischemia:

1) *Adenosine receptor blockade blocks the cardioprotective effects of adenosine on mechanical and metabolic functions.*
2) *Adenosine receptor blockade elevates glycolysis and lactate release.*
3) *Adenosine receptor blockade increases purine nucleoside release through an enhanced feedback stimulation of adenosine formation.*

SIGNIFICANCE

This study answers the following questions: 1) Does endogenous adenosine interact with its receptors to enhance the functional recovery of isolated rabbit hearts during reperfusion? To determine if an adenosine-receptor mechanism mediates the functional recovery of ischemia/reperfused hearts will greatly contribute to the development of therapeutic approaches to treat patients with chronic heart disease. 2) Does adenosine

receptor blockade increase glycolysis and lactate release during myocardium ischemia? Since glycolysis is the only significant source of ATP during myocardial ischemia, it is important to delineate the effects of adenosine on glycolysis and lactate release. 3) Is purine nucleoside release affected by adenosine receptor blockade? To determine if purine nucleoside release from ischemic myocardium is affected by adenosine receptor blockade will lead to further understanding of how myocardium achieves contractile functional and energetic adaption to ischemia.

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CHAPTER II

INTRODUCTION

Adenosine is an endogenous purine nucleoside that possesses numerous physiological properties, including potentially beneficial effects for salvaging ischemic and reperfused myocardium,[1] coronary vasodilation,[2,3] β-adrenergic antagonism,[4] increasing glucose uptake,[6,7] replenishment of high-energy ATP,[5] and mediating myocardial preconditioning.[11] Administration of adenosine and adenosine receptor agonist improves post-ischemic functional and metabolic recovery in isolated hearts.[9] These effects are primarily due to enhanced ATP repletion through uptake and incorporation of adenosine into the adenine nucleotide pool and due to adenosine receptor-mediated processes. Lasley *et al.*[10] reported that exogenous adenosine reduces the time to onset of ischemic contracture by activation of adenosine A_1 receptors. Downey *et al.*[11] found that adenosine receptor blockade blocks the protective effect of ischemic preconditioning and adenosine appears to mediate preconditioning via its receptor-activated mechanism. Janier *et al.*[12] reported that the salutary effect of adenosine on time to onset of ischemic contracture was completely blocked with 10 μM nonspecific adenosine receptor blocker SPT, and that exogenous adenosine attenuated ischemic injury by receptor-mediated stimulation of anaerobic glycolysis. These findings suggest that an adenosine receptor-mediated mechanism plays an important role in cardioprotective effects of adenosine. In the present study, effects of the adenosine receptor blocker SPT on contractile function, lactate release and purine nucleoside release during mild

or moderate ischemia and during reperfusion were compared to those in untreated hearts. The purpose of the present study was to determine whether endogenous adenosine interacts with its receptors to improve contractile function and to affect glycolysis and purine nucleoside release during ischemia and reperfusion in isolated rabbit hearts.

MATERIAL AND METHODS

Isolated heart preparation

The animal experiments were approved by the institutional animal care and use committee and conformed to the *Guide for the Care and Use of Laboratory Animals* (NIH publication 85-23, revised 1995). New Zealand white rabbits of either sex, weighing from 1 to 3 kg, were intravenously anesthetized with sodium pentobarbital (30 mg/kg). Rabbits were heparinized (1000 units, intravenously) for anticoagulation 30 min before surgery. The heart was rapidly excised and immediately placed in ice cold Krebs-Henseleit buffer to produce cardiac arrest. Following cannulation of the aorta on a Langendorff apparatus, hearts were perfused at a constant perfusion pressure with Krebs-Henseleit buffer consisting of (in mmol · liter^{-1}): NaCl 118.5, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 24.8, $CaCl_2$ 2.5, and glucose 10. The Krebs-Henseleit buffer was gassed with 95% O_2 - 5% CO_2, which resulted in a PO_2 of 600 mmHg and pH of 7.4 - 7.5. The pulmonary artery was cannulated for anaerobic collection of coronary effluent. The venae cavae were ligated. A filter (pore size 3 μm) was incorporated into the perfusion system to filter microaggregates from the perfusate. After the aorta was cannulated, the perfusion pressure was gradually increased to

100 cm H_2O. The heart was enclosed in a small, stoppered, water-jacketed chamber. The temperature of the perfusate and the water-jacketed chamber surrounding the heart was maintained at 37°C. A fluid-filled latex balloon, connected via PE 240 tubing to a pressure transducer (Gould Statham P23 ID), was inserted into the left ventricle via the left atrium and inflated to an end-diastolic pressure of 0 to 5 mmHg to record left ventricular pressure and its electrically differentiated derivative, dP/dt. Once the volume of the balloon was set, it was not changed for the remainder of the experiment. The coronary perfusion pressure was maintained constant at 100 cm H_2O by an overflow system. A drawing of the perfusion system is presented in Fig. 1.

Perfusion pressure was monitored through a short, saline-filled PE tubing with one end of the tubing inserted into the perfusion line just above the aorta and the other end of the tubing attached to a pressure transducer (Gould Statham P23 ID). All hearts were allowed to equilibrate for 30 min before beginning the experimental protocol, and the study was continued only if left ventricular developed pressure exceed 80 mmHg after 20 min equilibration. Heart rate, left ventricular developed pressure, dP/dt and perfusion pressure were recorded continuously on a Grass 79D chart recorder. The vasodilator and bradycardia effects of 100 µl bolus injections of 2 mM adenosine before and after SPT were compared in a preliminary experiment. Addition of 100 µM SPT, unspecific adenosine receptor blocker completely blocked the bradycardia effect and partially inhibited the vasodilation induced by adenosine.

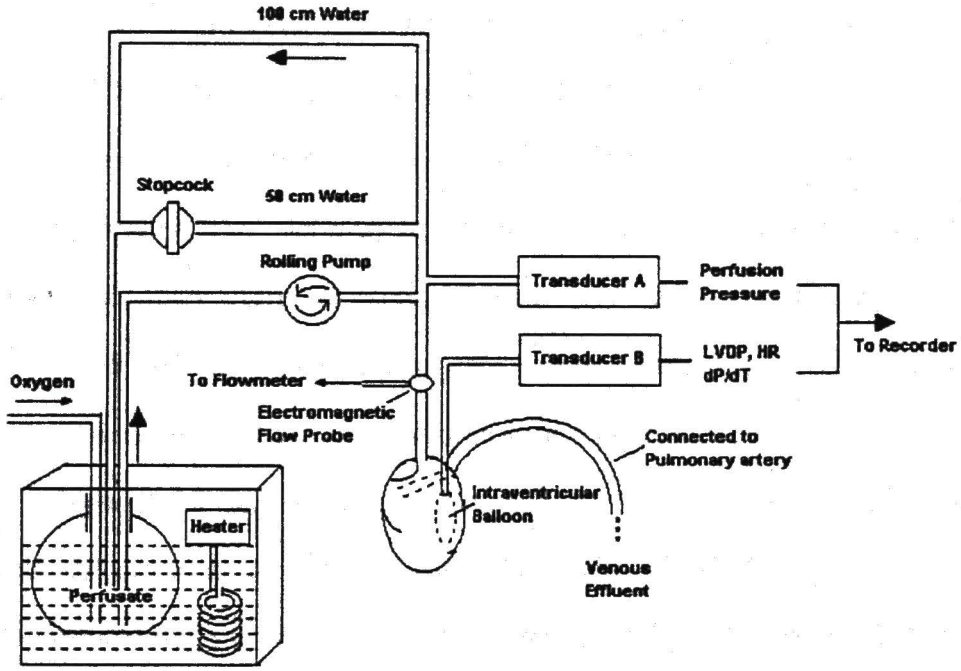

Figure 1. Langendorff system for constant pressure perfusion of isolated heart. During hypoperfusion the heart was perfused at either 50 cm water or 30 cm water. The height of the column of perfusate above the heart is adjustable to either 50 cm or 30 cm.

Hemodynamic parameters

Pressure-rate product (PRP): Left ventricular performance was expressed by the product of left ventricular developed pressure (LVDP) and heart rate (HR). LVDP was defined as peak systolic pressure minus end-diastolic pressure.

Coronary flow: Coronary flow rate was measured by timed collections of effluent from the pulmonary artery and expressed as ml · min^{-1} · g^{-1} wet heart mass.

dP/dt: The values of maximum positive dP/dt (dP/dt_{max}) and maximum negative dP/dt (dP/dt_{min}) were analyzed as indices of contractility and relaxation.

Myocardial oxygen consumption (MVO_2): Krebs-Henseleit buffer and coronary venous effluent samples were collected anaerobically. PO_2, PCO_2 and pH of these samples were measured with a Corning 175 Automatic pH/Blood Gas System. MVO_2 was computed from coronary flow times the arteriovenous difference of oxygen content and expressed as μl O_2 · min^{-1} · g^{-1} wet heart mass. MVO_2 was calculated from the following formula:[52]

$$MVO_2\ (\mu l\ O_2 \cdot min^{-1} \cdot g^{-1}) = CF\ (ml \cdot min^{-1} \cdot g^{-1}) \times (P_aO_2\ (mmHg) - P_vO_2\ (mmHg)) \times (c/760\ (mmHg \cdot atm^{-1}))$$

where CF is coronary flow (ml · min^{-1} · g^{-1}); P_aO_2 and P_vO_2 are the partial pressure of O_2 (mmHg) in the coronary perfusate and coronary effluent, respectively; c is the Bunsen solubility coefficient of oxygen dissolved in Krebs-Henseleit buffer at 37°C (23 μl O_2 · atm^{-1} · ml $perfusate^{-1}$). Heart wet mass (HW) was converted from body weight (BW) based on a linear regression between heart wet mass and body weight for rabbits determined previously in this laboratory: HW (g) = 0.0015 × BW (g) + 1.36, (n = 10; R^2 = 0.9).

Myocardial efficiency: It was estimated as the ratio of work per unit oxygen consumed and calculated as product of left ventricular developed pressure times heart rate divided by MVO_2.[12]

$$\text{Efficiency (mmHg} \cdot g^{-1} \cdot \mu l^{-1}\ O_2) = \text{PRP (mmHg} \cdot \text{min}^{-1}) / MVO_2\ (\mu l\ O_2 \cdot \text{min}^{-1} \cdot g^{-1})$$

Myocardial metabolites

Adenosine and inosine release: The venous effluent samples for measuring adenosine and inosine concentrations were heated in a 100^{0}C water bath for 5 min immediately after collection to inactivate adenosine deaminase and then stored in a minus 4^{0}C freezer until analysis by high performance liquid chromatography (HPLC; Shimadzu LC-600 system equipped with a SPD-6A spectrophotometric *uv - vis* detector, computerized chromatography data system and SIL-9A sample auto-injector). 50 µl samples were injected onto an ODS-Hypersil reverse-phase 5 µm C-18 column and absorbance of the eluate was monitored at 254 nm by a spectrophotometric *uv - vis* detector. HPLC solvents were 100 mM $NH_4H_2PO_4$ in 1% methanol at pH 5.3 (solvent A), and 25% methanol in pH 5.58 (solvent B). A nonlinear bisolvent gradient are employed at a flow rate of 1.3 ml/min: 0 - 4 min: 100% A and 0% B; 4 - 9 min: 70% to 60% A and 30% to 40% B; 9 - 15 min: 0% A and 100% B. Peaks were identified by comparing retention times to known standards, and quantified by peak area. Rates of adenosine and inosine release were calculated as the respective coronary effluent concentrations times coronary flow.

Lactate release: The venous effluent samples for measuring lactate release were stored in a minus 4°C freezer immediately after collection, and the samples were analyzed by standard enzymatic spectrophotometric methods (Perkin Elmer Lambda *uv/vis* spectrophotometer, measuring wavelength: 337 nm).[51] Lactate release was calculated as coronary effluent concentration times coronary flow. 400 µl venous samples were added to 750 µl glycine-hydrazine buffer with 50 µl NAD. Lactate and NAD was converted to pyruvate and NADH by lactate dehydrogenase (LDH, 10 µl). Absorbance of NADH is directly proportional to the concentration of substrate. Thus, lactate concentration in venous effluent was calculated by comparing absorbance from NADH with those of known standards. We found that SPT also absorbs at 337 nm. To correct for SPT absorbance in venous samples from the SPT treated hearts, each of the samples was divided into two sets. One set of samples was measured with LDH added to the cuvette and the other set of samples was measured without LDH added to the cuvette as a control. The lactate concentration in a sample from the SPT treated hearts was calculated by subtracting the concentration measured with LDH from the concentration measured without LDH.

Myocardial metabolite contents: At the end of the experiments, the hearts were freeze clamped with Wollenberger tongs prechilled in liquid nitrogen and then stored in a minus 90°C freezer until analysis. The following standardized extraction procedure was performed. Stop-frozen myocardium was carefully powdered in a mortar filled with liquid nitrogen. Approximately 0.2 g tissue powder was desiccated in a oven (100°C) overnight for determination of dry mass. 0.3 - 0.4 g pulverized myocardium was added to 2.5 ml ice-cold

0.3 N perchloric acid ($HCLO_4$) immediately followed by 1 min stirring for homogenization. Then the supernatant was neutralized to pH of 5.5 - 6.5 with small aliquots of refrigerated (4^0C) 1 M and 0.1 M KOH after centrifugation (10,000 × g) at 4^0C for 10 min. After standing 30 min in ice, potassium perchlorate was removed by another centrifugation at the same condition, and aliquots of the extracts were immediately assayed by standard enzymatic spectrophotometric methods in a Perkin Elmer Lambda-2 dual wavelength *uv - vis* spectrophotometer (measuring wavelength: 337 nm; reference wavelength: 417 nm; $\epsilon = 5.65$ $M^{-1} \cdot cm^{-1}$).[13] Myocardial ATP, creatine phosphate, creatine, and lactate contents were calculated from the respective concentrations time their extract factors and expressed as μmol $\cdot g^{-1}$ dry mass.

Experiment protocol

The experiment protocol is shown in Fig. 2. In preliminary experiments, four hearts were normally perfused (perfusion pressure 100 cm H_2O) for 3 hr as the time control group. Another four hearts were perfused in the same way as the time control hearts but treated with 100 μM SPT. These time control and SPT time control groups tested the stability of the preparation. Data were collected at 30 min intervals for the 3 hr time control experiments. Two ischemic models were studied: Mild and moderate ischemia, each for 90 min (see below). All hearts were subjected to 60 min of reperfusion following ischemia. At conclusion of the protocol, the experiments were terminated by freeze clamping the hearts.

Mild ischemia: This ischemic model was produced by reducing coronary perfusion pressure from 100 cm H_2O to 50 cm H_2O for 90 min. The hearts subjected to mild ischemia

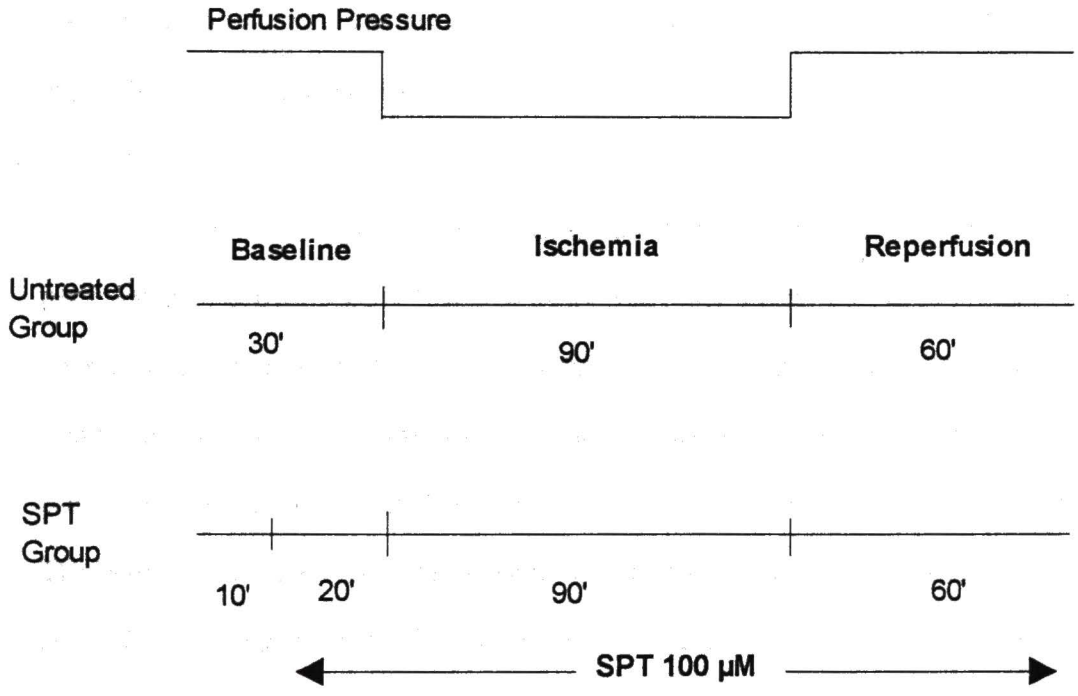

Figure 2. Experiment protocol: The baseline period began after the isolated heart equilibrated as demonstrated by stabilized LVDP, HR and CF for 30 min. SPT was infused from 20 min before ischemia until the end of experiment in the SPT treated group. 1) Mildly ischemic model: Perfusion pressure was 50 cm H_2O during ischemia; 2) Moderately ischemic model: Perfusion pressure was 30 cm H_2O during ischemia.

were divided into two groups: 1) Untreated group (n = 8): After 30 min baseline period, the perfusion pressure was lowered from 100 cm H_2O to 50 cm H_2O for 90 min. The perfusion pressure was then restored to 100 cm H_2O, and the heart was reperfused for 60 min; 2) SPT treated group (n = 8): The protocol for this group was identical to that of the untreated group except that SPT was included in the perfusate to yield 100 μM concentration of SPT from 20 min prior to ischemia until the end of the experiment.

Moderate ischemia: This ischemic model was produced by reducing coronary perfusion pressure for 90 min followed by 60 min reperfusion. Perfusion pressure was first lowered to 50 cm H_2O for 5 min and then further lowered to 30 cm H_2O for 85 min. Upon reperfusion, perfusion pressure was gradually increased from 30 cm H_2O to 50 cm H_2O for 5 min and then further restored the perfusion pressure to the baseline level (100 cm H_2O). The hearts subjected to moderate ischemia were also divided into two groups. 1) Untreated group (n = 8): The protocol for this group was same as that of the untreated hearts in the mildly ischemic group except that perfusion pressure was 30 cm H_2O during ischemia; 2) SPT treated group (n = 8): The protocol for this group was same as that of the untreated group above except that SPT was included in the perfusate to yield 100 μM concentration of SPT from 20 min prior to ischemia until the end of the experiment.

Data reduction and statistical analyses

Results are expressed as mean ± SEM. Coronary flow, MVO_2, lactate release and purine nucleoside release are expressed per gram wet heart mass per min. Hemodynamic and

ventricular function data were recorded every 10 min during the experiment protocol. The average values for these measurements during baseline, ischemia and reperfusion were calculated and compared. For mild ischemic data and for moderate ischemic data, differences between the two treatment conditions (untreated and SPT treated) and among three protocol conditions (baseline, ischemia, and reperfusion) for each variable were analyzed by two factor ANOVAs with repeated measurements. Again, for untreated data and for SPT treated data, differences between the two ischemic conditions (mild and moderate ischemia) and among three protocol conditions (baseline, ischemia, and reperfusion) for each variable were analyzed by the same method. The two factor ANOVAs were followed by Student-Newman-Keuls test if the P value for either factor was less than 0.05. The independent student's *t* test was used to assess differences between two groups at respective time points. For time control data, the differeces between untreated and SPT treated were analyzed by two factor ANOVA with repeated measurements. A probability value < 0.05 was considered statistically significant.

All statistical analysis techniques utilized have been described by Keppel.[50] Statistical analyses and graphical presentations were performed with Microsoft Excel (version 5.0, Microsoft Company) and Sigmastat (Jandel Company).

Chemicals

8-*p*-sulfophenyl theophyline was obtained from Research Biochemicals International (MA, USA). It is water soluble and was diluted to a concentration of 100 μM in perfusate.

Adenosine and inosine were obtained from Sigma Chemical Company (MO, USA) and were stored in a refrigerator.

RESULTS

Data from 40 rabbit hearts are presented. The average body weight and mean heart wet mass were 2147 ± 135 g and 4.2 ± 0.5 g, respectively. No significant difference among groups were observed in body weight and heart wet mass.

Stability of the preparation

The protocol for this study required that the preparations remain stable mechanically and metabolically for a period of 3 hr. For the 3 hr time control experiment, hemodynamic data and metabolic data were summarized in Table 1. In the time control (TC) group and the SPT time control (SPT TC) group (perfusion pressure at 100 cm H_2O for 3 hr), a progressive but very slight reduction of pressure-rate product was recorded over time. 1) Compared to the beginning of the protocol, PRP was 99 ± 1 % (TC) and 99 ± 1% (SPT TC) after 1 hr, 95 ± 2% (TC) and 99 ± 1% (SPT TC) after 2 hr, and 93 ± 5% (TC) and 97 ± 1% (SPT TC) after 3 hr. 2) Compared to the beginning of the protocol, MVO_2 was 101 ± 4 % (TC) and 98 ± 3 (SPT TC) after 1 hr, 96 ± 5% (TC) and 99 ± 4% (SPT TC) after 2 hr, and 92 ± 9% (TC) and 95 ± 2% (SPT TC) after 3 hr. 3) Compared to the beginning of the protocol, coronary flow was 96 ± 3% (TC) and 100 ± 1% (SPT TC) after 1 hr, 92 ± 5% (TC) and 99 ± 1% (SPT TC) after 2 hr, and 89 ± 7% (TC) and 95 ± 2% (SPT TC) after 3 hr. 4) Compared to the

Table. 1 Stability of Preparation

	PRP mmHg/min	MVO_2 µl/min/g	CF ml/min/g	V_{lac} µmol/min/g	$V_{ado+ino}$ nmol/min/g
10 min					
TC	15.3 ± 0.6	50 ± 7	4.8 ± 0.5	0.17 ± 0.07	1.78 ± 0.60
SPT TC	14.5 ± 0.6	42 ± 1	4.4 ± 0.2	0.63 ± 0.07*	2.68 ± 0.46
60 min					
TC	15.1 ± 0.7	50 ± 6	4.7 ± 0.6	0.21 ± 0.10	1.41 ± 0.73
SPT TC	14.4 ± 0.5	41 ± 1	4.3 ± 0.2	0.67 ± 0.07*	2.19 ± 0.98
120 min					
TC	14.5 ± 0.6	47 ± 5	4.5 ± 0.6	0.17 ± 0.03	0.77 ± 0.23
SPT TC	14.4 ± 0.5	41 ± 1	4.2 ± 0.2	0.60 ± 0.10*	1.68 ± 0.22
180 min					
TC	14.2 ± 0.3	44 ± 5	4.2 ± 0.6	0.11 ± 0.01	0.74 ± 0.23
SPT TC	14.0 ± 0.4	40 ± 1	4.0 ± 0.2	0.66 ± 0.09*	1.64 ± 0.29

PRP: Pressure-Rate Product, $\times 10^3$; V_{lac}: Lactate Release;
$V_{ado+ino}$: Purine Nucleoside Release (Ado + Ino);
TC: Time Control (n = 4);
SPT TC: SPT Treated Time Control (n = 4);
*$P<0.05$ *vs.* TC.

beginning of the protocol, lactate release was 117 ± 34 % (TC) and 112 ± 23% (SPT TC) after 1 hr, 98 ± 20% (TC) and 102 ± 29% (SPT TC) after 2 hr, and 69 ± 13% (TC) and 109 ± 23% (SPT TC) after 3 hr. 5) Compared to the beginning of the protocol, purine nucleoside release was 72 ± 14 % (TC) and 76 ± 25% (SPT TC) after 1 hr, 44 ± 3% (TC) and 64 ± 9% (SPT TC) after 2 hr, and 41 ± 3% (TC) and 66 ± 18% (SPT TC) after 3 hr. The mechanical and metabolic variables of the time control group were not statistically different from those of the SPT treated time control group except for lactate release. Lactate release in the SPT treated time control group was significantly higher than that in the untreated time control group ($P < 0.05$) at all times as shown in Table 1.

Mechanical Function

During the 30 min baseline period (pre-ischemia), LVDP, HR, coronary flow, dP/dt_{max} and dP/dt_{min} were stable in all groups. Values of these variables measured during the time course of the experiment in the different groups are presented in Table 2.

Left ventricular developed pressure: 1) Mildly ischemic model: The baseline value was 88 ± 4 mmHg in the untreated group and 92 ± 3 mmHg in the SPT treated group ($P > 0.05$). During 90 min of mild ischemia (perfusion pressure 50 cm H_2O), the average LVDP was 68 ± 4 mmHg, 78 ± 3% of baseline in the untreated group, and 72 ± 4 mmHg, 79 ± 3% of baseline in the SPT treated group ($P > 0.05$). During reperfusion, the average LVDP recovered to 81 ± 5 mmHg, 93 ± 4% of baseline level in the untreated hearts and to 80 ± 4 mmHg, 87 ± 3% of baseline level in the SPT treated hearts, respectively ($P > 0.05$).

Table. 2 Hemodynamic data

	HR beats·min^{-1}	LVDP mmHg	dP/dt$_{max}$ mmHg·sec^{-1}	dP/dt$_{min}$ mmHg·sec^{-1}	LVEDP mmHg	CF ml·min^{-1}·g^{-1}
Baseline						
Untreated-m	176 ± 6	88 ± 4	1609 ± 160	1019 ± 150	0	5.2 ± 0.3
SPT-m	166 ± 3	92 ± 3	1341 ± 62	890 ± 44	0	4.9 ± 0.2
Untreated-s	155 ± 9	106 ± 4	1606 ± 78	1013 ± 23	1 ± 1	5.6 ± 0.4
SPT-s	169 ± 9	100 ± 5	1481 ± 85	1144 ± 108	2.3 ± 2	5.3 ± 0.2
I_{15}						
Untreated-m	152 ± 5	69 ± 4	916 ± 65	650 ± 117	0	3.4 ± 0.3
SPT-m	141 ± 8	75 ± 4	943 ± 42	730 ± 55	0	3.0 ± 0.1
Untreated-s	109 ± 7	62 ± 5	687 ± 72	537 ± 56	0	2.1 ± 0.3†
SPT-s	145 ± 11*	54 ± 3	762 ± 37	600 ± 26	0	1.9 ± 0.1†
I_{30}						
Untreated-m	148 ± 5	69 ± 4	908 ± 68	675 ± 145	0	3.2 ± 0.3
SPT-m	138 ± 9	73 ± 4	931 ± 42	718 ± 55	0	2.9 ± 0.1
Untreated-s	107 ± 5	61 ± 4	662 ± 60	537 ± 70	0	2.0 ± 0.3†
SPT-s	141 ± 11*	56 ± 3	750 ± 46	600 ± 42	0	1.9 ± 0.1†
I_{60}						
Untreated-m	147 ± 5	67 ± 4	883 ± 60	633 ± 115	0	3.1 ± 0.3
SPT-m	134 ± 8	71 ± 4	912 ± 47	693 ± 34	0	2.7 ± 0.1
Untreated-s	102 ± 6	59 ± 4	637 ± 65	512 ± 61	0	1.7 ± 0.3†
SPT-s	135 ± 11*	55 ± 3	712 ± 47	587 ± 40	0	1.8 ± 0.1†
I_{90}						
Untreated-m	136 ± 9	69 ± 4	866 ± 33	641 ± 96	0	2.9 ± 0.3
SPT-m	133 ± 8	69 ± 4	850 ± 32	662 ± 40	0	2.6 ± 0.1
Untreated-s	102 ± 6	58 ± 5	637 ± 65	512 ± 61	0	1.7 ± 0.3†
SPT-s	131 ± 11*	53 ± 3	662 ± 46	525 ± 45	0	1.7 ± 0.1†
R_{60}						
Untreated-m	166 ± 5	80 ± 5	1083 ± 48	800 ± 106	0	4.5 ± 0.2
SPT-m	152 ± 5	79 ± 4	1056 ± 66	743 ± 53	0	4.2 ± 0.2
Untreated-s	140 ± 8	89 ± 5	1200 ± 81	900 ± 35	2.1 ± 2	4.5 ± 0.3
SPT-s	146 ± 9	76 ± 3	1012 ± 61	850 ± 75	5.3 ± 2	4.2 ± 0.1

I_{15} = 15 min after ischemia; I_{30} = 30 min after ischemia; I_{60} = 60 min after ischemia; I_{90} = 90 min after ischemia; R_{60} = at 60 min of reperfusion. LVDP: left vetricular developed pressure; HR: heart rate; LVEDP: left ventricular end-diastolic pressure; CF: coronary flow.
Untreated-m: untreated group mild ischemia (n = 8);
SPT-m: SPT group mild ischemia (n = 8);
Untreated-s: untreated group moderate ischemia (n = 8);
SPT-s: SPT group moderate ischemia (n = 8);
*P<0.05 *vs*. untreated; †P<0.05 *vs*. mild ischemia.

2) Moderately ischemic model: The baseline value was 106 ± 4 mmHg in the untreated group and 100 ± 5 mmHg in the SPT treated group ($P > 0.05$). During 90 min of moderate ischemia (perfusion pressure 30 cm H_2O), the average LVDP was 60 ± 4 mmHg, 56 ± 3% of baseline in the untreated hearts and 55 ± 3 mmHg, 55 ± 2% of baseline in the SPT treated hearts ($P > 0.05$). During reperfusion, the average LVDP recovered to 88 ± 5 mmHg, 83 ± 2% of the baseline level in the untreated hearts and to 77 ± 3 mmHg, 78 ± 3% of baseline level in the SPT treated hearts ($P > 0.05$).

Compared to the mildly ischemic condition, LVDP of untreated hearts subjected to moderate ischemia was higher at baseline ($P < 0.05$), similar during ischemia ($P > 0.05$), and similar during reperfusion ($P > 0.05$). For treated hearts, the LVDP was similar at baseline ($P > 0.05$), lower during ischemia ($P < 0.05$) and similar during reperfusion ($P > 0.05$) compared to the mildly ischemic condition.

Heart rate: 1) Mildly ischemic model: The baseline value was 176 ± 6 beats · min^{-1} in the untreated group and 166 ± 3 beats · min^{-1} in the SPT treated group ($P > 0.05$). During 90 min of mild ischemia (perfusion pressure 50 cm H_2O), the average HR was 146 ± 5 beats · min^{-1}, 83 ± 4% of baseline in the untreated group, and 136 ± 8 beats · min^{-1}, 82 ± 5% of baseline in the SPT treated group ($P > 0.05$). During reperfusion, the average HR recovered to 163 ± 6 beats · min^{-1}, 92 ± 3% of baseline level in the untreated hearts and to 153 ± 6 beats · min^{-1}, 93 ± 6% of baseline level in the SPT treated hearts, respectively ($P > 0.05$).

2) Moderately ischemic model: The baseline value was 155 ± 9 beats · min^{-1} in the untreated group and 169 ± 9 beats · min^{-1} in the SPT treated group ($P > 0.05$). During 90 min of moderate ischemia (perfusion pressure 30 cm H_2O), the average HR was 105 ± 6 beats ·

min^{-1}, 69 ± 4% of baseline in the untreated hearts and 138 ± 11 beats · min^{-1}, 82 ± 4% of baseline in the SPT treated hearts ($P < 0.05$). During reperfusion, the average HR recovered to 140 ± 9 beats · min^{-1}, 90 ± 3% of the baseline level in the untreated hearts and to 145 ± 10 beats · min^{-1}, 86 ± 4% of baseline level in the SPT treated hearts ($P > 0.05$).

Compared to the mildly ischemic condition, HR of untreated hearts subjected to moderate ischemia was similar at baseline ($P > 0.05$), lower during ischemia ($P < 0.05$), and lower during reperfusion ($P < 0.05$). For treated hearts, the HR was similar at baseline ($P > 0.05$), similar during ischemia ($P > 0.05$) and similar during reperfusion ($P > 0.05$) compared to the mildly ischemic condition.

Pressure-rate product: 1) Mildly ischemic model: The baseline value was 15.5 ± 0.8 mmHg · min^{-1} ×10^3 in the untreated group and 15.2 ± 0.5 mmHg · min^{-1} ×10^3 in the SPT treated group ($P > 0.05$). During 90 min of mild ischemia (perfusion pressure 50 cm H_2O), the average pressure-rate product was 9.9 ± 0.6 mmHg · min^{-1} ×10^3, 64 ± 3% of baseline in the untreated group, and 9.7 ± 0.6 mmHg · min^{-1} ×10^3, 64 ± 3% of baseline in the SPT treated group ($P > 0.05$). During reperfusion, the average pressure-rate product recovered to 13.1 ± 0.6 mmHg · min^{-1} ×10^3, 85 ± 2% of baseline level in the untreated hearts and to 12.2 ± 0.8 mmHg · min^{-1} ×10^3, 81 ± 5% of baseline level in the SPT treated hearts, respectively ($P > 0.05$).

2) Moderately ischemic model: The baseline value was 16.3 ± 0.7 mmHg · min^{-1} ×10^3 in the untreated group and 16.7 ± 1.0 mmHg · min^{-1} ×10^3 in the SPT treated group ($P > 0.05$). During 90 min of moderate ischemia (perfusion pressure 30 cm H_2O), the average pressure-rate product was 6.3 ± 0.5 mmHg · min^{-1} ×10^3, 39 ± 4% of baseline in the untreated hearts

and 7.4 ± 0.4 mmHg · min^{-1} ×10^3, 44 ± 2% of baseline in the SPT treated hearts ($P > 0.05$). During reperfusion, the average pressure-rate product recovered to 12.2 ± 0.6 mmHg · min^{-1} ×10^3, 75 ± 3% of the baseline level in the untreated hearts and to 11.1 ± 0.7 mmHg · min^{-1} ×10^3, 67 ± 4% of baseline level in the SPT treated hearts ($P > 0.05$) as shown in Fig. 3.

Compared to the mildly ischemic condition, the pressure-rate product of untreated hearts subjected to moderate ischemia was similar at baseline ($P > 0.05$), lower during ischemia ($P < 0.05$), and similar during reperfusion ($P > 0.05$). For treated hearts, the pressure-rate product was similar at baseline ($P > 0.05$), lower during ischemia ($P < 0.05$) and similar during reperfusion ($P > 0.05$) compared to the mildly ischemic condition.

dP/dt_{max}: 1) Mildly ischemic model: The baseline value was 1609 ± 160 mmHg · sec^{-1} in the untreated group and 1341 ± 62 mmHg · sec^{-1} in the SPT treated group ($P > 0.05$). During 90 min of mild ischemia (perfusion pressure 50 cm H_2O), the average dP/dt_{max} was 1000 ± 91 mmHg · sec^{-1}, 63 ± 4% of baseline in the untreated group, and 909 ± 39 mmHg · sec^{-1}, 68 ± 1% of baseline in the SPT treated group ($P > 0.05$). During reperfusion, the average dP/dt_{max} recovered to 1156 ± 59 mmHg · sec^{-1}, 74 ± 4% of baseline level in the untreated hearts and to 1069 ± 63 mmHg · sec^{-1}, 80 ± 4% of baseline level in the SPT treated hearts, respectively ($P > 0.05$).

2) Moderately ischemic model: The baseline value was 1606 ± 78 mmHg · sec^{-1} in the untreated group and 1480 ± 85 mmHg · sec^{-1} in the SPT treated group ($P > 0.05$). During 90 min of moderate ischemia (perfusion pressure 30 cm H_2O), the average dP/dt_{max} was 656 ± 64 mmHg · sec^{-1}, 41 ± 3% of baseline in the untreated hearts and 722 ± 40 mmHg · sec^{-1},

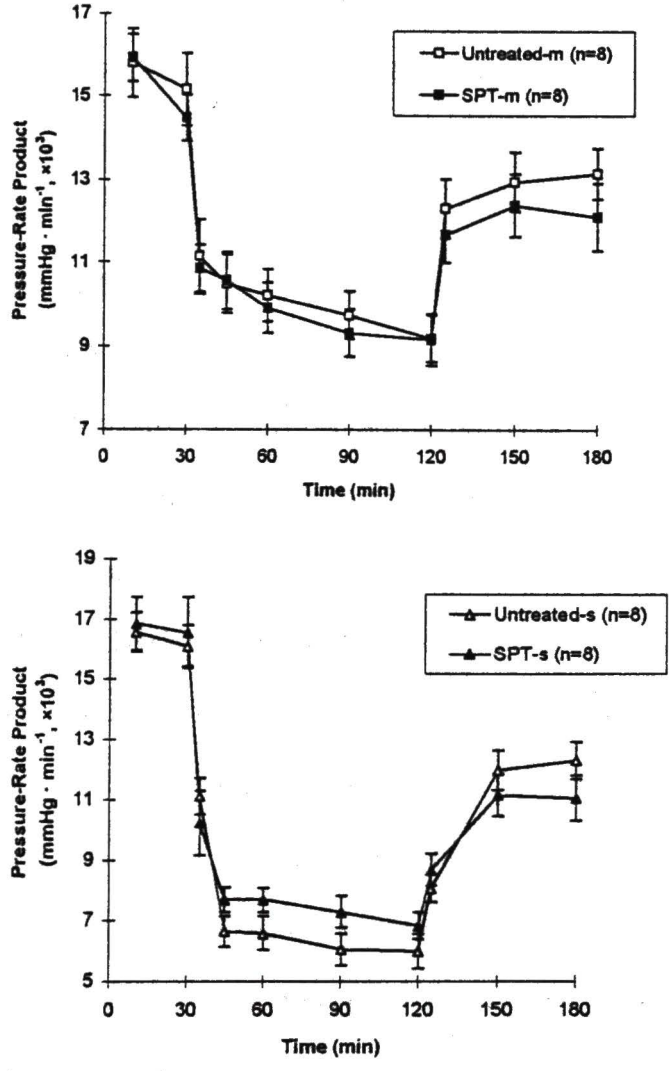

Figure 3. Pressure-rate product as a function of time. Results are expressed in absolute terms in mildly ischemic model (upper panel) and moderately ischemic model (bottom panel). From 1 to 30 min is baseline period; from 31 to 120 is ischemic period; from 121 to 180 is reperfusion period. Error bar = SEM.

49 ± 3% of baseline in the SPT treated hearts ($P > 0.05$). During reperfusion, the average dP/dt_{max} recovered to 1112 ± 107 mmHg · sec^{-1}, 69 ± 5% of the baseline level in the untreated hearts and to 1025 ± 56 mmHg · sec^{-1}, 70 ± 3% of baseline level in the SPT treated hearts ($P > 0.05$).

Compared to the mildly ischemic condition, dP/dt_{max} of untreated hearts subjected to moderate ischemia was similar at baseline ($P > 0.05$), lower during ischemia ($P < 0.05$), and similar during reperfusion ($P > 0.05$). For treated hearts, the dP/dt_{max} was similar at baseline ($P > 0.05$), lower during ischemia ($P < 0.05$) and similar during reperfusion ($P > 0.05$) compared to the mildly ischemic condition.

dP/dt_{min}: 1) Mildly ischemic model: The baseline value was 1081 ± 150 mmHg · sec^{-1} in the untreated group and 890 ± 44 mmHg · sec^{-1} in the SPT treated group ($P > 0.05$). During 90 min of mild ischemia (perfusion pressure 50 cm H_2O), the average dP/dt_{min} was 697 ± 92 mmHg · sec^{-1}, 70 ± 5% of baseline in the untreated group, and 702 ± 45 mmHg · sec^{-1}, 79 ± 4% of baseline in the SPT treated group ($P > 0.05$). During reperfusion, the average dP/dt_{min} recovered to 809 ± 78 mmHg · sec^{-1}, 84 ± 6% of baseline level in the untreated hearts and to 744 ± 53 mmHg · sec^{-1}, 84 ± 6% of baseline level in the SPT treated hearts, respectively ($P > 0.05$).

2) Moderately ischemic model: The baseline value was 1012 ± 23 mmHg · sec^{-1} in the untreated group and 1144 ± 107 mmHg · sec^{-1} in the SPT treated group ($P > 0.05$). During 90 min of moderate ischemia (perfusion pressure 30 cm H_2O), the average dP/dt_{min} was 525 ± 62 mmHg · sec^{-1}, 52 ± 6% of baseline in the untreated hearts and 578 ± 35 mmHg · sec^{-1},

52 ± 2% of baseline in the SPT treated hearts ($P > 0.05$). During reperfusion, the average dP/dt_{min} recovered to 900 ± 62 mmHg · sec^{-1}, 83 ± 6% of the baseline level in the untreated hearts and to 850 ± 76 mmHg · sec^{-1}, 75 ± 6% of baseline level in the SPT treated hearts ($P > 0.05$).

Compared to the mildly ischemic condition, dP/dt_{min} of untreated hearts subjected to moderate ischemia was similar at baseline ($P > 0.05$), similar during ischemia ($P > 0.05$), and similar during reperfusion ($P > 0.05$). For treated hearts, the dP/dt_{min} was higher at baseline ($P < 0.05$), higher during ischemia ($P < 0.05$) and similar during reperfusion ($P > 0.05$) compared to the mildly ischemic condition.

Coronary flow, Oxygen consumption and myocardial efficiency

Coronary flow: 1) Mildly ischemic model: The baseline value was 5.2 ± 0.3 ml · min^{-1} · g^{-1} in the untreated group and 5.0 ± 0.1 ml · min^{-1} · g^{-1} in the SPT treated group ($P > 0.05$). During 90 min of mild ischemia (perfusion pressure 50 cm H_2O), the average coronary flow was 3.1 ± 0.3 ml · min^{-1} · g^{-1}, 60 ± 3% of baseline in the untreated group, and 2.8 ± 0.1 ml · min^{-1} · g^{-1}, 56 ± 2% of baseline in the SPT treated group ($P > 0.05$). During reperfusion, the average coronary flow recovered to 4.6 ± 0.2 ml · min^{-1} · g^{-1}, 88 ± 3% of baseline level in the untreated hearts and to 4.2 ± 2 ml · min^{-1} · g^{-1}, 84 ± 3% of baseline level in the SPT treated hearts, respectively ($P > 0.05$).

2) Moderately ischemic model: The baseline value was 5.6 ± 0.3 ml · min^{-1} · g^{-1} in the untreated group and 5.5 ± 0.3 ml · min^{-1} · g^{-1} in the SPT treated group ($P > 0.05$). During 90 min of moderate ischemia (perfusion pressure 30 cm H_2O), the average coronary flow was

1.9 ± 0.3 ml · min^{-1} · g^{-1}, $33 \pm 3\%$ of baseline in the untreated hearts and 1.9 ± 0.1 ml · min^{-1} · g^{-1}, $35 \pm 2\%$ of baseline in the SPT treated hearts ($P > 0.05$). During reperfusion, the average coronary flow recovered to 4.4 ± 0.3 ml · min^{-1} · g^{-1}, $80 \pm 5\%$ of the baseline level in the untreated hearts and to 4.3 ± 0.1 ml · min^{-1} · g^{-1}, $78 \pm 4\%$ of baseline level in the SPT treated hearts ($P > 0.05$) as shown in Fig. 4.

Compared to the mildly ischemic condition, coronary flow of untreated hearts subjected to moderate ischemia was similar at baseline ($P > 0.05$), lower during ischemia ($P < 0.05$), and similar during reperfusion ($P > 0.05$). For treated hearts, the coronary flow was similar at baseline ($P > 0.05$), lower during ischemia ($P < 0.05$) and similar during reperfusion ($P > 0.05$) compared to the mildly ischemic condition.

MVO_2: 1) Mildly ischemic model: The baseline value was 53 ± 4 µl · min^{-1} · g^{-1} in the untreated group and 48 ± 3 µl · min^{-1} · g^{-1} in the SPT treated group ($P > 0.05$). During 90 min of mild ischemia (perfusion pressure 50 cm H_2O), the average MVO_2 was 33 ± 3 µl · min^{-1} · g^{-1}, $63 \pm 3\%$ of baseline in the untreated group, and 29 ± 2 µl · min^{-1} · g^{-1}, $60 \pm 2\%$ of baseline in the SPT treated group ($P > 0.05$). During reperfusion, the average MVO_2 recovered to 48 ± 3 µl · min^{-1} · g^{-1}, $92 \pm 6\%$ of baseline level in the untreated hearts and to 42 ± 2 µl · min^{-1} · g^{-1}, $87 \pm 2\%$ of baseline level in the SPT treated hearts, respectively ($P > 0.05$ vs. untreated hearts).

2) Moderately ischemic model: The baseline value was 54 ± 3 µl · min^{-1} · g^{-1} in the untreated group and 55 ± 2 µl · min^{-1} · g^{-1} in the SPT treated group ($P > 0.05$). During 90 min of moderate ischemia (perfusion pressure 30 cm H_2O), the average MVO_2 was 24 ± 3 µl ·

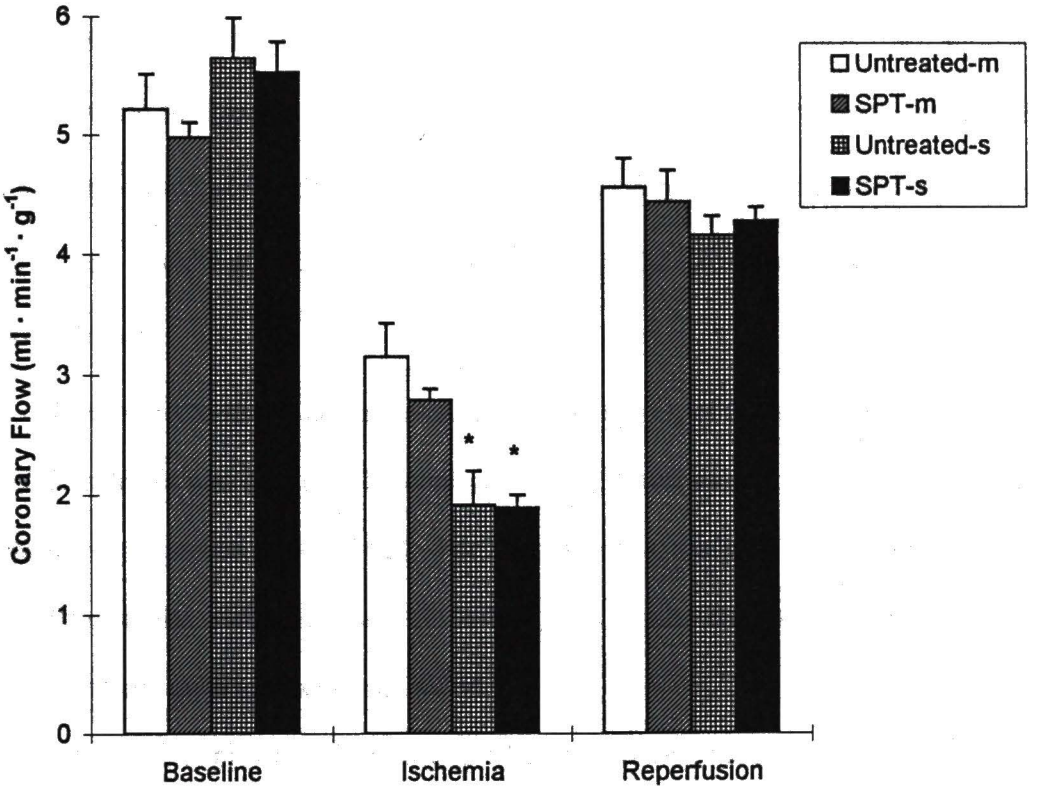

Figure 4. Values of coronary flow in baseline, ischemia and reperfusion in two ischemic models. Values are means, error bar = SEM.
*P<0.05 *vs.* untreated and SPT treated hearts in mildly ischemic model respectively.

$min^{-1} \cdot g^{-1}$, 43 ± 4% of baseline in the untreated hearts and 25 ± 2 $\mu l \cdot min^{-1} \cdot g^{-1}$, 47 ± 5% of baseline in the SPT treated hearts ($P > 0.05$). During reperfusion, the average MVO_2 recovered to 42 ± 3 $\mu l \cdot min^{-1} \cdot g^{-1}$, 78 ± 6% of the baseline level in the untreated hearts and to 46 ± 2 $\mu l \cdot min^{-1} \cdot g^{-1}$, 85 ± 5% of baseline level in the SPT treated hearts ($P > 0.05$) as shown in Fig. 5.

Compared to the mildly ischemic condition, MVO_2 of untreated hearts subjected to moderate ischemia was similar at baseline ($P > 0.05$), lower during ischemia ($P < 0.05$), and similar during reperfusion ($P > 0.05$). For treated hearts, the MVO_2 was similar at baseline ($P > 0.05$), similar during ischemia ($P > 0.05$) and similar during reperfusion ($P > 0.05$) compared to the mildly ischemic condition.

Myocardial efficiency: Myocardial efficiency was estimated as the ratio of work per unit oxygen use (and calculated as LVDP times heart rate divided by MVO_2). 1) Mildly ischemic model: The baseline value was 296 ± 13 mmHg $\cdot g^{-1} \cdot \mu l^{-1} O_2$ in the untreated group and 318 ± 9 mmHg $\cdot g^{-1} \cdot \mu l^{-1} O_2$ in the SPT treated group ($P > 0.05$). During 90 min of mild ischemia (perfusion pressure 50 cm H_2O), the average myocardial efficiency was 309 ± 22 mmHg $\cdot g^{-1} \cdot \mu l^{-1} O_2$, 104 ± 5% of baseline in the untreated group, and 343 ± 23 mmHg $\cdot g^{-1} \cdot \mu l^{-1} O_2$, 107 ± 5% of baseline in the SPT treated group ($P > 0.05$). During reperfusion, the average myocardial efficiency recovered to 284 ± 20 mmHg $\cdot g^{-1} \cdot \mu l^{-1} O_2$, 96 ± 7% of baseline level in the untreated hearts and to 298 ± 21 mmHg $\cdot g^{-1} \cdot \mu l^{-1} O_2$, 93 ± 5% of baseline level in the SPT treated hearts, respectively ($P > 0.05$).

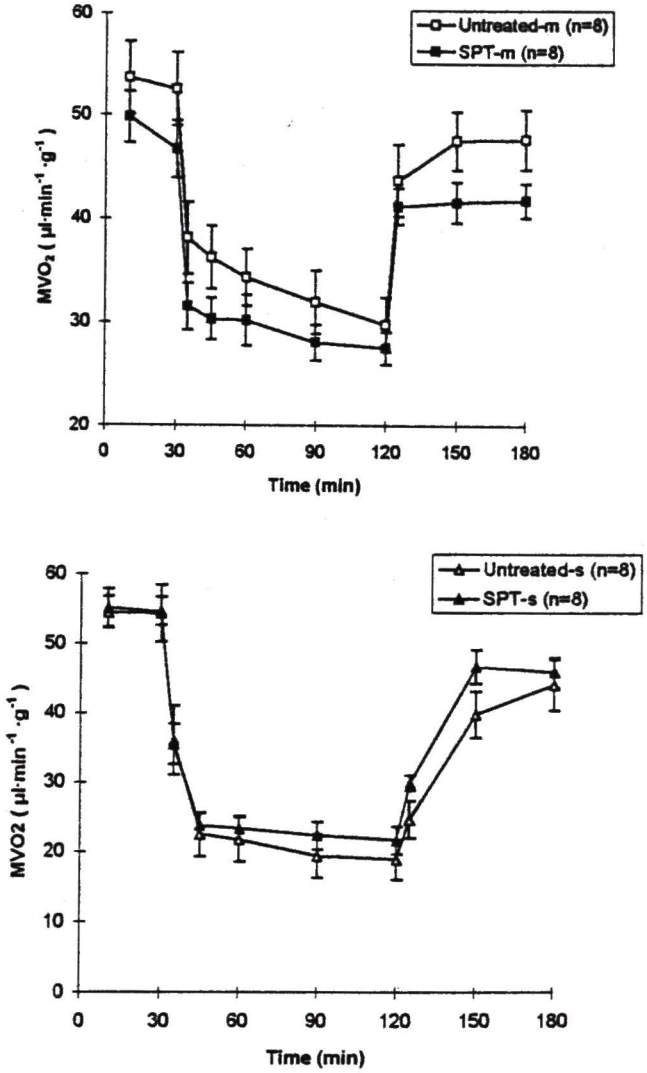

Figure 5. Myocardial oxygen consumption in mildly ischemic model (upper panel) and moderately ischemic model (bottom panel). Values are means, error bar = SEM. No significant differences between the untreated and SPT treated groups either in mildly ischemic model (upper panel) and moderately ischemic model (bottom panel).

2) Moderately ischemic model: The baseline value was 308 ± 23 mmHg · g^{-1} · μl^{-1} O_2 in the untreated group and 309 ± 24 mmHg · g^{-1} · μl^{-1} O_2 in the SPT treated group ($P > 0.05$). During 90 min of moderate ischemia (perfusion pressure 30 cm H_2O), the average myocardial efficiency was 324 ± 23 mmHg · g^{-1} · μl^{-1} O_2, 106 ± 5% of baseline in the untreated hearts and 331 ± 22 mmHg · g^{-1} · μl^{-1} O_2, 112 ± 11% of baseline in the SPT treated hearts ($P > 0.05$). During reperfusion, the average myocardial efficiency recovered to 300 ± 22 mmHg · g^{-1} · μl^{-1} O_2, 98 ± 5% of the baseline level in the untreated hearts and to 240 ± 11 mmHg · g^{-1} · μl^{-1} O_2, 81 ± 7% of baseline level in the SPT treated hearts ($P < 0.05$) as shown in Fig. 6.

Compared to the mildly ischemic condition, myocardial efficiency of untreated hearts subjected to moderate ischemia was similar at baseline ($P > 0.05$), similar during ischemia ($P > 0.05$), and similar during reperfusion ($P > 0.05$). For treated hearts, the myocardial efficiency was similar at baseline ($P > 0.05$), similar during ischemia ($P > 0.05$) and lower during reperfusion ($P < 0.05$) compared to the mildly ischemic condition.

Lactate Release

Anaerobic glycolytic rate and the level of ischemic stress were assessed by sequential analysis of lactate release from coronary effluent. 1) Mildly ischemic model: The baseline value was 0.18 ± 0.02 μmol · min^{-1} · g^{-1} in the untreated group and 0.34 ± 0.05 μmol · min^{-1} · g^{-1} in the SPT treated group ($P < 0.05$). During 90 min of mild ischemia (perfusion pressure 50 cm H_2O), the average lactate release was 0.27 ± 0.05 μmol · min^{-1} · g^{-1}, 152 ± 24% of baseline in the untreated group, and 0.68 ± 0.08 μmol · min^{-1} · g^{-1}, 213 ± 24% of baseline in

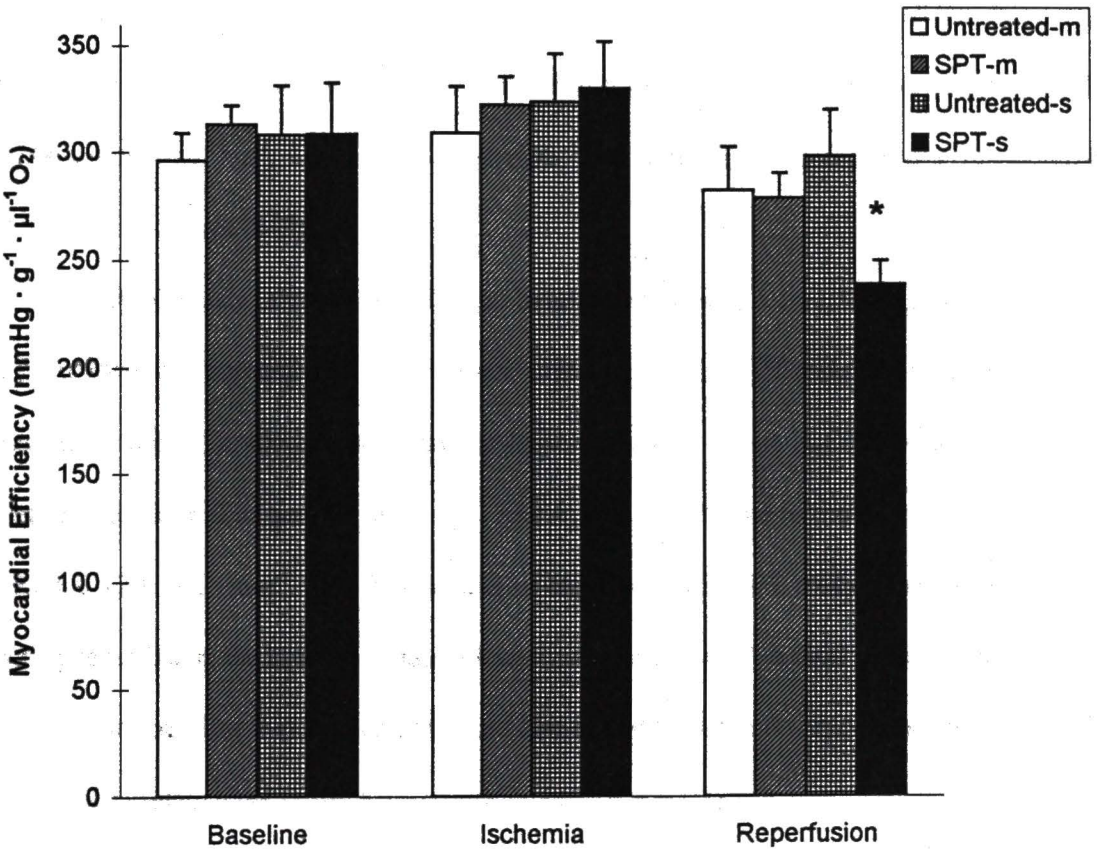

Figure 6. Myocardial efficiency in baseline, ischemia, and reperfusion in two ischemic models. Values are means, error bar = SEM.
*P<0.05 *vs.* untreated during reperfusion from moderate ischemia.

the SPT treated group ($P < 0.05$). During reperfusion, the average lactate release recovered to 0.17 ± 0.03 μmol · min^{-1} · g^{-1}, 95 ± 18% of baseline level in the untreated hearts and to 0.48 ± 0.06 μmol · min^{-1} · g^{-1}, 161 ± 31% of baseline level in the SPT treated hearts, respectively ($P < 0.05$) as shown in Fig. 7.

2) Moderately ischemic model: The baseline value was 0.14 ± 0.02 μmol · min^{-1} · g^{-1} in the untreated group and 0.46 ± 0.08 μmol · min^{-1} · g^{-1} in the SPT treated group ($P < 0.05$). During 90 min of moderate ischemia (perfusion pressure 30 cm H_2O), the average lactate release was 0.36 ± 0.05 μmol · min^{-1} · g^{-1}, 259 ± 26% of baseline in the untreated hearts and 0.93 ± 0.12 μmol · min^{-1} · g^{-1}, 234 ± 42% of baseline in the SPT treated hearts ($P < 0.05$). During reperfusion, the average lactate release recovered to 0.13 ± 0.02 μmol · min^{-1} · g^{-1}, 99 ± 11% of the baseline level in the untreated hearts and to 0.401 ± 0.07 μmol · min^{-1} · g^{-1}, 97 ± 17% of baseline level in the SPT treated hearts ($P < 0.05$).

Compared to the mildly ischemic condition, lactate release of untreated hearts subjected to moderate ischemia was similar at baseline ($P > 0.05$), similar during ischemia ($P > 0.05$), and similar during reperfusion ($P > 0.05$). For treated hearts, the lactate release was similar at baseline ($P > 0.05$), similar during ischemia ($P > 0.05$) and similar during reperfusion ($P > 0.05$) compared to the mildly ischemic condition.

Purine Nucleoside Release

The effects of adenosine receptor blockade and severity of ischemia on purine nucleoside release (Ado + Ino) are shown in Fig. 8. 1) Mildly ischemic model: The baseline

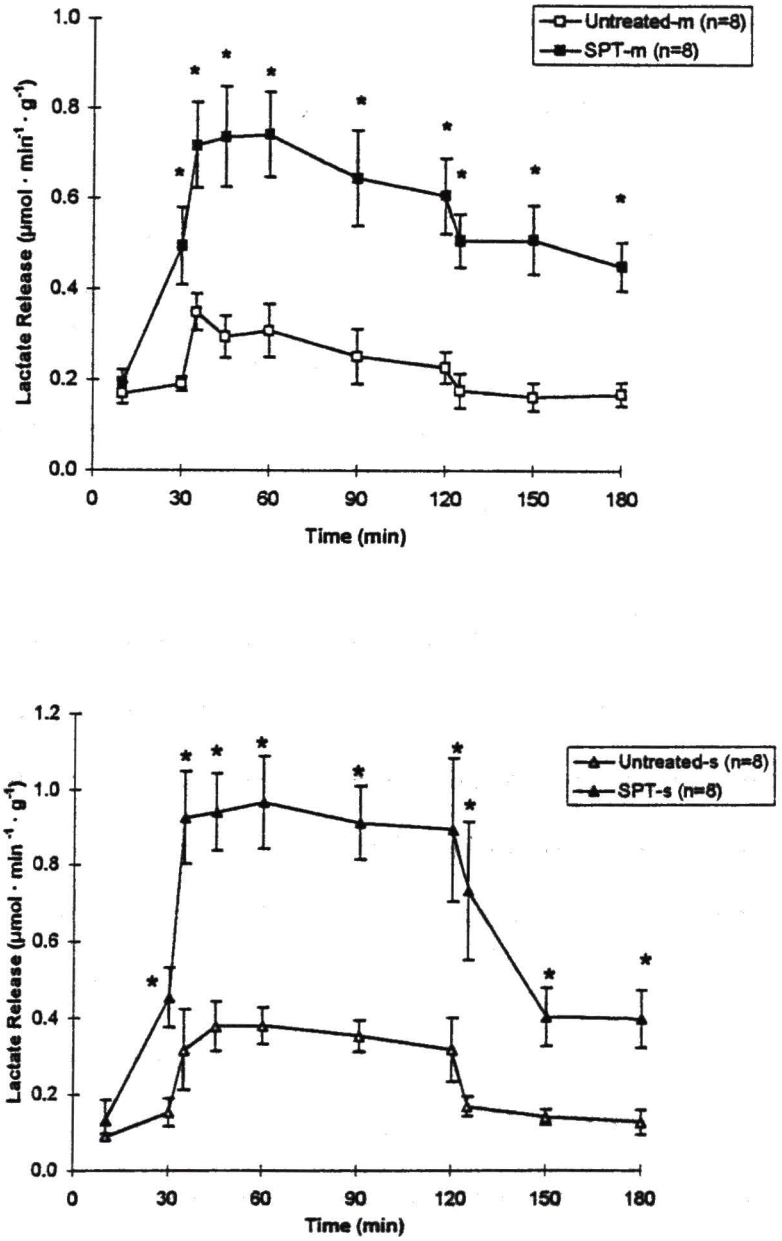

Figure 7. Effects of ischemia/reperfusion and adenosine receptor blocker SPT on lactate release. Lactate release increased during baseline, ischemia and reperfusion either in mildly ischemic model (upper panel) and moderately ischemic model (bottom panel).
*P<0.05 *vs.* untreated.

value was 2.39 ± 0.5 nmol · min^{-1} · g^{-1} in the untreated group and 2.43 ± 0.66 nmol · min^{-1} · g^{-1} in the SPT treated group ($P > 0.05$). At 5 min of mild ischemia (the peak values), purine nucleoside release was 3.7 ± 0.6 nmol · min^{-1} · g^{-1}, 171 ± 21% of baseline in the untreated group, and 5.6 ± 0.9 nmol · min^{-1} · g^{-1}, 289 ± 43% of baseline in the SPT treated group ($P > 0.05$). At 15 min of mild ischemia, purine nucleoside release was 2.1 ± 0.5 nmol · min^{-1} · g^{-1}, 96 ± 43% of baseline in the untreated group, and 2.5 ± 0.2 nmol · min^{-1} · g^{-1}, 180 ± 65% of baseline in the SPT treated group ($P > 0.05$). At 60 min of mild ischemia, purine nucleoside release was 1.4 ± 0.3 nmol · min^{-1} · g^{-1}, 66 ± 13% of baseline in the untreated group, and 1.5 ± 0.3 nmol · min^{-1} · g^{-1}, 103 ± 39% of baseline in the SPT treated group ($P > 0.05$). At 90 min of mild ischemia, purine nucleoside release was 1.2 ± 0.2 nmol · min^{-1} · g^{-1}, 62 ± 13% of baseline in the untreated group, and 1.3 ± 0.4 nmol · min^{-1} · g^{-1}, 80 ± 18% of baseline in the SPT treated group ($P > 0.05$). During reperfusion, the average purine nucleoside release recovered to 1.35 ± 0.2 nmol · min^{-1} · g^{-1}, 63 ± 7% of baseline level in the untreated hearts and to 1.2 ± 0.3 nmol · min^{-1} · g^{-1}, 73 ± 22% of baseline level in the SPT treated hearts, respectively ($P > 0.05$).

2) Moderately ischemic model: The baseline value was 1.86 ± 0.3 nmol · min^{-1} · g^{-1} in the untreated group and 2.2 ± 0.2 nmol · min^{-1} · g^{-1} in the SPT treated group ($P > 0.05$). At 5 min of mild ischemia (the peak values), purine nucleoside release was 3.3 ± 0.4 nmol · min^{-1} · g^{-1}, 199 ± 29% of baseline in the untreated group, and 6.5 ± 0.8 nmol · min^{-1} · g^{-1}, 346 ± 82% of baseline in the SPT treated group ($P < 0.05$). At 15 min of mild ischemia, purine nucleoside release was 2.4 ± 0.5 nmol · min^{-1} · g^{-1}, 138 ± 27% of baseline in the untreated group, and 4.1 ± 0.8 nmol · min^{-1} · g^{-1}, 205 ± 51% of baseline in the SPT treated group ($P <$

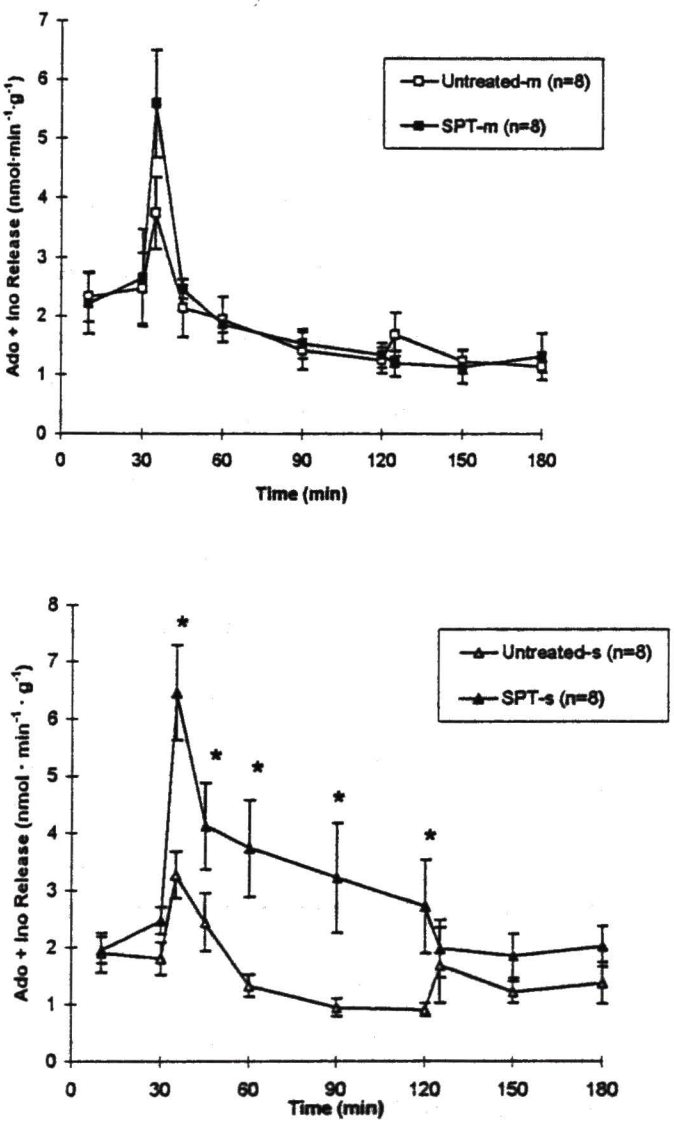

Figure 8. Effects of ischemia/reperfusion and adenosine receptor blocker SPT on purine nucleoside (Ado + Ino) release in mildly ischemic model (upper panel) and moderately ischemic model (bottom panel).
*P<0.05 *vs.* untreated.

0.05). At 60 min of mild ischemia, purine nucleoside release was 0.9 ± 0.2 nmol · min^{-1} · g^{-1}, 56 ± 9% of baseline in the untreated group, and 3.2 ± 1.0 nmol · min^{-1} · g^{-1}, 179 ± 74% of baseline in the SPT treated group ($P < 0.05$). At 90 min of mild ischemia, purine nucleoside release was 0.9 ± 0.1 nmol · min^{-1} · g^{-1}, 56 ± 10% of baseline in the untreated group, and 2.7 ± 0.8 nmol · min^{-1} · g^{-1}, 139 ± 46% of baseline in the SPT treated group ($P < 0.05$). During reperfusion, the average purine nucleoside release recovered to 1.3 ± 0.3 nmol · min^{-1} · g^{-1}, 72 ± 9% of baseline level in the untreated hearts and to 1.9 ± 0.4 nmol · min^{-1} · g^{-1}, 94 ± 22% of baseline level in the SPT treated hearts, respectively ($P > 0.05$).

Compared to the mildly ischemic condition, purine nucleoside release of untreated hearts subjected to moderate ischemia was similar at baseline ($P > 0.05$), similar during ischemia ($P > 0.05$), and similar during reperfusion ($P > 0.05$). For treated hearts, the purine nucleoside release was similar at baseline ($P > 0.05$), similar during ischemia ($P > 0.05$) and similar during reperfusion ($P > 0.05$) compared to the mildly ischemic condition.

High Energy Phosphate and Lactate Content in Myocardial Tissue

Table 3. Presents that ATP contents were at the same levels in all groups at the end of reperfusion. CrP and creatine contents in SPT treated hearts were slightly higher than those in the untreated hearts, but no significant differences were detected ($P > 0.05$). Lactate contents in the SPT treated hearts also exhibited a tendency to be higher to the untreated hearts, both in mild ischemia and moderate ischemia, although significant differences were not obtained ($P > 0.05$).

Table 3. Contents of Myocardial Metabolites

	ATP	CrP	Creatine	Lactate
Untreated-m	21.4 ± 1.5	35.4 ± 3.7	42.2 ± 4.3	2.1 ± 0.6
SPT-m	20.6 ± 1.1	43.5 ± 3.8	49.7 ± 4.8	3.8 ± 0.8
Untreated-s	21.9 ± 1.9	36.7 ± 4.1	45.6 ± 6.0	1.7 ± 0.3
SPT-s	19.9 ± 1.3	38.1 ± 1.9	54.0 ± 4.2	3.6 ± 0.8

ATP: adenosine triphosphate; CrP: creatine phosphate;
Mean ± SEM ($\mu mol \cdot g^{-1}$ dry)
Untreated-m: untreated group mild ischemia (n = 8);
SPT-m: SPT group mild ischemia (n = 7);
Untreated-s: untreated group moderate ischemia (n = 7);
SPT-s: SPT group moderate ischemia (n = 7);

DISCUSSION

This study tested the effects of adenosine receptor blockade on contractile function, lactate release, and purine nucleoside release during mild and moderate ischemia and reperfusion in isolated rabbit hearts. The major findings of this study are: 1) Adenosine receptor blockade with SPT significantly stimulated glycolysis and increased lactate release in both normoxic and ischemic isolated rabbit myocardium; 2) During moderate ischemia, purine nucleoside release was increased in hearts treated with SPT. 3) Myocardial efficiency was significantly lower in the SPT treated hearts during reperfusion from moderate ischemia.

4) Heart rate decreased less in SPT treated hearts during moderate ischemia, but SPT had no effect on heart rate during mild ischemia. 5) Adenosine receptor blockade with SPT had no significant effect on contractile function, coronary flow and myocardial oxygen consumption during ischemia and reperfusion in the crystalloid perfused rabbit hearts.

Methodological considerations

The reasons for using the isolated rabbit heart model were: 1) Myocardial preconditioning has been found to be especially potent in rabbit myocardium, and adenosine is a mediator of this cardioprotective mechanism.[16] Liu *et al.*[16] reported that a 5 min intracoronary infusion of adenosine was as effective as 5 min of ischemia in protecting perfused isolated rabbit hearts against infarction from a 45 min ischemic insult. Adenosine receptors are known to vary widely between species.[66] This may account for the failure of adenosine to mimic preconditioning in the isolated rat heart.[15] Also, Cave *et al.*[15] pretreated isolated rat hearts perfused with adenosine-rich (100 μM) buffer for 5 min followed by 20 min global ischemia. The results suggested that adenosine pretreatment had no discernable effect on aortic flow, coronary flow, cardiac output and creatine kinase leakage. 2) Adenosine receptor activation has been found to be particularly effective in attenuating ischemic contracture and increasing ATP content of severe ischemia/reperfused rabbit hearts.[12] To date no studies have determined if adenosine receptor activation is cardioprotective in rabbit hearts subjected to prolonged, moderate ischemia, *i.e.* acute myocardial hibernation. 3) The Langendorff model allows isolated hearts to maintain stable contractile function and heart rate for an extended period. In the time control experiments, pressure-rate product, coronary

flow and MVO_2 were almost unchanged during the 3 hr protocol. Thus, the Langendorff heart model is suitable for the long protocol required for ischemia/reperfusion experiments. Moreover, left ventricular developed pressure was measured with a fluid-filled latex balloon inserted into the left ventricle, and the size of balloon was kept constant throughout the experimental protocol. Thus, the hearts beat isovolumicly throughout the protocol, and the preload on the left ventricle was unchanged during ischemia and reperfusion.

The purpose of the present study was to test the effects of adenosine receptor blockade on mechanical and metabolic responses during baseline, ischemia, and reperfusion. The isolated heart model provides a number of advantages to study intrinsic responses to ischemia without influence of autonomic nervous stimulation, increased concentrations of catecholamines and steroids.[14] Therefore, this is an ideal model to study the effects of adenosine receptor blockade during ischemia and reperfusion. The results from this model could reveal the role of endogenous adenosine in ischemia/reperfused rabbit hearts. In the present study, perfusion pressure was maintained constant to avoid confounding effects of changes in this important variable. Both mechanical function and metabolic variables remained essentially stable for 3 hr in the time control group and in the SPT treated time control group.

Effects of adenosine receptor blockade on myocardial functional recovery

Three methods are often been generally employed in attempting to reveal responses to endogenous adenosine on contractile function in isolated hearts. 1) Endogenous adenosine concentration is directly decreased by adenosine deaminase[57] or increased by adenosine

deaminase inhibitors;[37] 2) Endogenous adenosine concentration is increased by adenosine transport inhibitors;[24,27] 3) Endogenous adenosine receptors are blocked by its receptor blockers. In the present study, SPT was utilized to block adenosine receptors to reveal any potential cardioprotective effects of endogenous adenosine in mild or moderate ischemia/reperfused rabbit hearts. Interestingly, functional recovery in the present study was not changed by the adenosine receptor blockade, since contractile function assessed from pressure-rate product recovered to the same levels in untreated and SPT treated hearts during reperfusion, either after mild ischemia or moderate ischemia. Thus, endogenous adenosine appears not to significantly affect post-ischemic myocardial functional recovery in this isolated rabbit heart model. This result is consistent with the findings from our laboratory in an isolated guinea pig heart model,[41] and with the observations of Offstad and his coworkers[32] in red blood cell perfused piglet hearts. The present data demonstrated that heart rate of SPT treated hearts decreased less than untreated hearts during moderate ischemia. This result suggests that endogenous interstitial adenosine was elevated and mediated a portion of the bradycardia through its negative chronotropic action on the sinoatrial node during moderate ischemia. During ischemia, heart rate is also reduced by other factors, such as decreased intracellular pH, so the bradycardia was not completely blocked by adenosine receptor blockade.

Role of adenosine on glycolysis and lactate release

Myocardial lactate production is an index of myocardial ischemia or hypoxia. However, a quantitative relationship between the severity of ischemia and the amount of

lactate production has not been defined.[54] According to Opie,[42] glycolysis is inhibited by severe ischemia, whereas it is stimulated by mild ischemia. The critical factor is whether or not the coronary flow is adequate to wash out the accumulated lactate. In the present study, an isolated, globally ischemic heart preparation was used to allow the myocardial perfusion pressure to be precisely controlled, and two degrees of ischemia were produced. The results (Fig. 7) suggest that lactate release was not significantly different between mild ischemia and moderate ischemia either in SPT treated hearts or in untreated hearts. However, myocardial oxygen consumption and pressure-rate product decreased significantly with reduction in perfusion pressure in moderate ischemia, but no significant difference was detected between SPT treated and untreated hearts. This finding is consistent with results of Keller *et al.*[56], who measured lactate production in graded reduction of coronary pressure and flow in isolated rat hearts. They reported that lactate production was constant over the range of perfusion pressures from 66 to 36 mmHg.[56]

Lactate accumulation decreases contractile activity in the ischemic zone and promotes mitochondrial damage,[29] decreases the action potential duration[30] and inhibits glyceraldehyde-3-phosphate dehydrogenase, a key rate-controlling enzyme in the glycolysis pathway.[42] The mechanism of accumulated lactate inhibition of glyceraldehyde-3-phosphate dehydrogenase is through a feedback mechanism. Increased lactate production leads to decreased intracellular pH during ischemia, and glyceraldehyde-3-phosphate dehydrogenase is sensitive to a decrease in pH.[59] Another feedback inhibiting factor of this enzyme is an increase of NADH. Since lactate accumulates during ischemia, the equilibrium of the reaction catalyzed by lactate dehydrogenase will eventually favor a relative increase of lactate

to pyruvate with an increased NADH.[42,59] Lactate causes decrease of intracellular pH, swelling of myocardial cells, and damage to endothelial cells, resulting in impairment to ischemic myocardium.[31] However, there is also evidence that glycolysis can delay the onset of ischemic contracture, maintain ischemic cellular ion homeostasis, and reduce post-ischemic dysfunction.[12,34,37] Janier *et al.*[12] found that exogenous adenosine slightly elevates pre-ischemic glycogen content and maintains lactate production during ischemia to preserve tissue ATP content. Increased lactate production diminished ischemic contracture by stimulation of glycolysis during ischemia.[12]

There is controversy regarding the effects of adenosine on glycolysis and lactate release in normoxic and ischemic hearts. Wyatt *et al.*[34] found that exogenous adenosine stimulates glycolytic flux in normoxic myocardium and endogenous adenosine stimulates glycolytic flux in ischemic myocardium. This stimulation is mediated by adenosine A_1 receptors activation. Lasley *et al.*[37] observed that adenosine plus the adenosine deaminase inhibitor EHNA caused an increase in lactate release and a delayed onset of contracture during low flow ischemia, whereas adenosine receptor blockade markedly reduced lactate release and accelerated the onset of contracture in isolated perfused rat hearts. Moreover, when hearts were perfused with glucose-free perfusate, adenosine plus EHNA treatment had no effect on lactate release or time to onset of ischemic contracture.[37] However, Finegan *et al.*[39] reported that glycolysis was inhibited by adenosine pre-treatment in isolated working rat hearts. Dale *et al.*[40] reported inhibition of glycolysis by the adenosine A_1 receptor agonist PIA. In addition, adenosine deaminase enhances the effect of insulin on glucose uptake.[40] Adenosine elevated lactate production in isolated perfused guinea pig hearts under conditions

of normoxia but did not under hypoxia.[38] In a study of canine myocardium preconditioning, Vander Heide *et al.*[43] reported that adenosine slowed the rate of glycogen utilization and decreased lactate accumulation during the first 20 min of total ischemia. In our laboratory, Gao *et al.*[41] recently found that isolated guinea pig hearts treated with SPT increased ischemic lactate release by 2-fold compared to untreated hearts during ischemia. Thus, the importance of glycolysis and the possible modulation of this process by adenosine during ischemia and reperfusion still remains a complicated issue.

Adenosine receptor blockade and myocardial energy metabolism

In this study, purine nucleoside release increased during moderate ischemia in adenosine receptor blocker treated hearts. What regulates adenosine release during ischemia? One of major factors to control adenosine release is the oxygen supply/demand ratio.[52-53] Increased cardiac adenosine release is correlated with a decrease in the oxygen supply/demand ratio, which is reflected in the coronary sinus oxygen content.[28] In contrast, Gorman *et al.*[23] found that adenosine release did not correlate with the oxygen supply/demand ratio during intracoronary norepinephrine infusion in intact dog hearts. He *et al.*[33] observed a biphasic change in adenosine release occurred as the energy charge and phosphorylation potential fell in graded, hypoperfused guinea pig hearts and when 2-deoxyglucose was infused in rat hearts. 5'-nucleotidase is responsible for dephosphorylation of AMP to produce adenosine. A possible link between oxygen supply/demand ratio and 5'-nucleotidase activity is the phosphorylation potential.[52,55] During ischemia, decreased coronary flow lowers the oxygen supply/demand ratio, causing declination of cytosolic energy level, *i.e.* the ATP

phosphorylation potential. Headrick *et al.*[44] reported an inverse relationship between cytosolic energy level and adenosine release in guinea pig hearts. Moreover, degradation of ATP causes accumulation of AMP when cytosolic energy level falls. The accumulated AMP activates 5'-nucleotidase activity. Thus, it is concluded that ATP phosphorylation potential is negatively correlated with purine nucleoside release.[41,44]

Enhanced adenosine and inosine release by adenosine receptor blockade has been noted previously in dog heart *in situ*,[47] and in rat heart *in vitro*.[48] A feedback mechanism of stimulation of adenosine formation may be activated when adenosine receptors are blocked. Mckenzie *et al.*[47] examined the effect of locally administered theophylline on active hyperemia and myocardial adenosine production during intracoronary infusion of isoproterenol in the dog heart. Isoproterenol decreased coronary resistance and increased myocardial adenosine production. However, theophylline plus isoproterenol produced greater increases in myocardium adenosine production than isoproterenol alone. These findings suggest that the failure of theophylline to attenuate isoproterenol hyperemia in the dog heart results at least in part from an increase in adenosine concentration. Bünger *et al.*[49] demonstrated reversible competition between theophylline and adenosine in the coronary vasculature of the isolated guinea pig hearts. The mechanism of the increase in adenosine concentration when theophylline is superimposed on isoproterenol is not clear. Mckenzie *et al.*[47] suggested that increased production of adenosine subsequent to enhanced metabolic activity by infusion of isoproterenol might be due to greater adenosine concentration required to override the effect of adenosine receptor blockade with theophylline. Angello *et al.*[48] employed ^{31}P NMR to test the effect of adenosine receptor blockade with SPT on function

and metabolism in isolated rat hearts during low flow ischemia and reperfusion. The major effects of adenosine receptor blockade were significant reduction in post-ischemic CrP levels and an increase of post-ischemic P_i levels. ATP levels remained relatively unaffected by adenosine receptor blockade during ischemia and reperfusion. These results suggest that increased post-ischemic rephosphorylation of ATP was due to the dephosphorylation of CrP in the SPT treated hearts.

Mechanism by which endogenous adenosine improves myocardial efficiency

Exogenous adenosine treatment has been reported to increase myocardial efficiency in isolated rabbit hearts during ischemia and reperfusion.[12] Myocardial efficiency could be defined as the ratio of ventricular work to oxygen consumed.[58] In the present study, work was represented by the pressure-rate product. Heart rate could be a determinant of myocardial efficiency. Higher heart rate has been reported make the heart less efficient, because high heart rate seems to use more oxygen for a given amount of work.[58] However, Marshall *et al.* found that heart rate was not linearly related to efficiency in blood perfused rabbit hearts.[67] Suga *et al.* reported that heart rate had no effect on efficiency in isolated dog hearts.[68] In the present study, heart rate was not significantly different between SPT treated hearts and untreated hearts during reperfusion after moderate ischemia. Thus, the improved efficiency appears unrelated to heart rate in this isolated rabbit heart model. Laster *et al.*[60] found that external work declined and MVO_2 was unchanged compared to time control hearts during reperfusion after global low flow ischemia in crystalloid perfused rabbit hearts. This inefficiency might be related to an enhanced need for energy supply to maintain cytosolic

calcium levels during reperfusion.[60] A similar finding were also reported by Schipke *et al.*[61] in isolated blood perfused rabbit hearts. Calcium overload during reperfusion could potentially injury myocardium.[62] Energy is required for calcium uptake by the sarcoplasmic reticulum. Extra energy might be needed to remove excess cytosolic calcium. Moreover, adenosine A_1 receptor activation is well known to open ATP sensitive potassium channels (K_{ATP}) in myocardium.[45,63] Since opening of K_{ATP} channels would hyperpolarize myocyte membranes and shorten the action potential duration by reducing Ca^{2+} influx through voltage activated calcium channels.[46,64] This may be beneficial to the ischemic hearts. Additionally, activation of adenosine A_1 receptors inhibits adenylate cyclase through coupling to G_i protein, which in turn decreases cytosolic cAMP levels and attenuates calcium influx.[32,65] These effects of adenosine on calcium influx might be the mechanism by which endogenous adenosine increases myocardial efficiency during reperfusion.

CONCLUSION

Increased lactate release during baseline, ischemia and reperfusion suggests that adenosine receptor blockade stimulates glycolysis. Increased adenosine and inosine release during moderate ischemia in the SPT treated hearts indicates enhanced feedback stimulation of adenosine formation. During reperfusion from moderate ischemia, the protective action of adenosine is related to increased myocardial efficiency. Cardiac contractile function during ischemia and reperfusion is independent of adenosine receptor activation in this isolated rabbit heart model.

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