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Adenosine's Effect on Myocardial Functional Recovery: Substrate or Signal?[1]

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During induced ischemia for cardiac surgery, nucleotides are degraded while being used to maintain myocyte integrity. The resulting nucleosides washout upon reperfusion, limiting nucleotide resynthesis resulting in poor postischemic cardiac function. We studied if the mechanism of the beneficial effect of adenosine, a nucleotide precursor, which is known to improve postischemic functional recovery is as a substrate for nucleotide resynthesis or by stimulation of adenosine A_1 or A_2 receptors. Isolated, retrograde-perfused rabbit hearts received cardioplegia as controls or cardioplegia containing 80 μM [*R*]-N^6-[1-methyl-2-phenylethyl]-adenosine, an A_1 receptor agonist, or 200 μM 5′-(*N*-ethylcarboxamido)adenosine, or 200 μM adenosine alone. To assess functional recovery developed pressure, max *dP/dt*, pressure–rate product, coronary flow, and myocardial oxygen consumption were compared after 120 min of 34°C global cardioplegic ischemia. Following ischemia and reperfusion, adenosine alone had better developed pressure, *dP/dt*, and pressure–rate product, while heart rates, wet weights, $\%H_2O$, end-diastolic volumes/pressures, and oxygen extraction were not significantly different between groups. While adenosine receptor stimulation may play a role, in this model the beneficial effect of adenosine on functional recovery appears to be mediated more by adenosine's role as a substrate for nucleotide resynthesis. © 1994 Academic Press, Inc.

INTRODUCTION

Many investigators, including our laboratory, have demonstrated that adenosine augmentation can improve postischemic myocardial functional recovery [1–6]. While it is postulated that adenosine is cardioprotective by being utilized as a substrate for nucleotide synthesis, recently there have been studies examining the effect of adenosine receptor stimulation upon myocardial ischemia [7, 8]. Many possible beneficial actions of adenosine are mediated by these extracellular surface receptors. The A_2 receptor augments intracellular cyclic AMP, mediating the vasodilating effects of adenosine and potentially enhancing the inotropic state of the heart [9]. A_2 receptor stimulation may also be cardioprotective by inhibition of neutrophil activity in ischemic areas. Conversely, the negative effects of adenosine on cardiac contractility and chronotropy are mediated by A_1 receptor stimulation [9], which could be cardioprotective during myocardial ischemia by downregulating energy demand. A_1 receptor stimulation is also thought to enhance myocyte glucose utilization [10]. To study if the beneficial cardioprotective effect of adenosine is mediated by stimulating A_1 or A_2 receptors, we added adenosine alone or [*R*]-N^6-[1-methyl-2-phenylethyl]-adenosine (PIA), a potent A_1 receptor agonist, or 5′-(*N*-ethylcarboxamido)adenosine (NECA), a potent A_2 receptor agonist, or adenosine plus NECA in a model of cardioplegic ischemia in isolated rabbit hearts and observed functional recovery compared to untreated controls.

MATERIALS AND METHODS

Preparation of isolated hearts. New Zealand white rabbits (male or female, 1.9–2.7 kg) were anesthetized with sodium pentobarbital (45 mg/kg, iv) and heparinized (700 units/kg, iv). The heart was rapidly excised and immersed in ice-cold physiologic salt solution (PSS) containing 118.0 m*M* NaCl, 4.0 m*M* KCl, 22.3 m*M* $NaHCO_3$, 11.1 m*M* glucose, 0.66 m*M* KH_2PO_4, 1.23 m*M* $MgCl_2$, and 2.38 m*M* $CaCl_2$. The aorta was cannulated in the Langendorff mode and perfused with PSS, equilibrated with 95% O_2–5% CO_2 at 37°C and a pH of 7.4. Perfusion pressure was maintained at 80 mm Hg. An incision was made in the left atrium and a fluid-filled latex balloon was passed through the mitral orifice and placed in the left ventricle. The balloon was connected to a pressure transducer for continuous measurement of left ventricular pressure and its first derivative (dP/dt).

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The superior and inferior vena cava and azygous vein were ligated. The pulmonary artery was cannulated to enable timed collection measurements of coronary flow and the cannula was connected to an oxygen meter (chemical microsensor; Diamond Electro-Tech, Inc., Ann Arbor, MI) for continuous measurement of the partial pressure of oxygen in the coronary effluent.

The analog signals were continuously recorded on a pressurized ink chart recorder (Model 2600S; Gould, Inc., Cleveland, OH) and on an on-line computer (AST Premium/386; AST Research Inc., Irvine, CA). To characterize cardiac function, developed pressure (DP) was defined as peak systolic pressure minus end-diastolic pressure. The product of heart rate (HR) and DP (pressure–rate product, PRP, mm Hg/min) was calculated to provide an estimate of changes in myocardial work. Myocardial oxygen consumption (MVO_2) was calculated as $MVO_2 = CF \times [(P_aO_2 - P_vO_2) \times (c/760)]$, where CF is coronary flow (ml/min/g), $(P_aO_2 - P_vO_2)$ is the difference in the partial pressure of oxygen (PO_2, mm Hg) between perfusate and coronary effluent flow, c is the Bunsen solubility coefficient of O_2 in perfusate at 37°C (22.7 μl $O_2 \cdot atm^{-1} \cdot ml^{-1}$ perfusate), and the PO_2 of the perfusate was 663 mm Hg. Coronary flow was measured by timed collections of the pulmonary effluent flow with a graduated cylinder. Oxygen extraction (O_2 EXT) was calculated as O_2 EXT = MVO_2/oxygen content in the perfusate. Following reperfusion, the hearts were removed from the perfusion column and water content determinations were made. The myocardium was weighed, desiccated for 48 hr at 80°C, and reweighed. Wet weight of the heart was determined after trimming the great vessels and fat and blot drying with eight-layer cotton gauze. Water content was determined using the formula [(1 − dry wt/wet wt) × 100 − %water]. This experimental design is well established and has been published previously [3, 4].

Preparation of cardioplegic solutions. Control hearts were treated with modified St. Thomas cardioplegic solution containing 109 m*M* Nacl, 25.5 m*M* KCl, 21.9 m*M* $NaHCO_3$, 16.0 m*M* $MgCl_2$, and 0.8 m*M* $CaCl_2$. In preliminary experiments a physiologic effect/dose-dependent curve was constructed for PIA in this model (5, 10, 20, 40, 80, 120, and 200 μM PIA, $n = 3$ each). We choose 80 μM to add to the modified St. Thomas cardioplegia, as at this dose maximal chronotropic and inotropic effects of PIA were noted, which were not enhanced at higher doses of PIA in this model. The adenosine (ADO) cardioplegia, the NECA cardioplegia, and the ADO + NECA solution were made by adding 200 μM ADO or 200 μM NECA or both to cardioplegia. All cardioplegic solutions were ice-cold (4°C) and equilibrated with 100% O_2. This method attempts to simulate the clinical condition of temperature swings with cardioplegia infusion during operative procedures.

Experimental protocol. After completing instrumentation and performing calibrations, left ventricular balloon volumes were varied over a range of values to construct modified left ventricular function curves. In this manner, it was possible to define a specific balloon volume that was associated with a developed pressure between 100 and 140 mm Hg. This volume was maintained the same during baseline and reperfusion conditions (isovolumic recovery). The intraventricular balloon volumes were not adjusted to produce specific end-diastolic pressures (rather, we defined a level of systolic pressure development), but end-diastolic pressures at baseline greater than 10 mm Hg were not considered acceptable. Hearts characterized by developed pressures less than 100 mm Hg or greater than 140 mm Hg were not used. Baseline data were obtained after an equilibration period of 30 min. There were seven hearts in the control group, seven hearts in the ADO group, and six hearts in PIA-, NECA-, and ADO + NECA-treated groups, respectively. During the baseline period, data were obtained with the hearts maintained at 37°C by an organ bath. During ischemia, the organ bath temperature was reduced to 34°C. Sixty milliliters of cardioplegia was injected into the aorta at a rate of 1 ml/sec and the PSS infusion was stopped to begin the 2-hr ischemic period. Fifteen milliliters of cardioplegia was injected every 30 min thereafter. Repeated administration of cardioplegic solution is commonly used clinically and is consistent with the experimental studies we and others have reported previously. Following the 2-hr ischemic period, hearts were reperfused with oxygenated PSS at 37°C and the water bath temperature was increased to 37°C. The intraventricular balloon remained deflated for 15 min to simulate the beating nonworking heart. Following this, the balloon was inflated to its specific isovolumic control and hemodynamic data were recorded every 15 min for 45 min to compare with baseline data, to determine functional recovery in each heart.

Statistical analysis. Values reported in the text and in Table 1 are means ± standard deviation. The Statview 512$^+$ Program (Brain Power, Inc., Calabasas, CA) was used for statistical analysis. Data were evaluated with repeated measures analysis of variance (ANOVA) within groups and single-factor analysis of variance across groups. When significant F values were obtained, Scheffe's test was used to distinguish which time periods or groups differed from one another significantly. Differences were considered significant when $P < 0.05$. All animals received humane care in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research.

RESULTS

There were also no significant differences in DP, $+dP/dt_{max}$, and MVO_2 among groups during baseline conditions. There were minor differences between some groups in regards to negative dP/dt_{max}, HR, PRP, and

CF; therefore, we assumed baseline values as 100% to compare the changes in the percentage recovery between the groups during reperfusion.

Following ischemia, there were no significant differences among the groups in terms of heart wet weight, end diastolic volume or myocardial water content. Hemodynamic results are summarized in Table 1. As shown, while there was an increase in the recovery of coronary flow in the A_2 receptor agonist treated groups (NECA and ADO + NECA), only ADO had a significant beneficial effect upon functional recovery.

DISCUSSION

Nucleotide levels decrease during myocardial ischemia due to obligatory utilization of ATP for the maintenance of cellular integrity [4, 5]. Since ATP is essential for myocardial contraction and relaxation at the actin and myosin level, any depletion of ATP has a detrimental effect upon postischemic myocardial functional recovery. Many investigations have demonstrated a favorable effect of adenosine on the recovery of ventricular function following ischemia [1–6]. In a previous study from this laboratory [4] a beneficial effect of adenosine on recovery of ventricular function was demonstrated in this same isolated rabbit heart model. Hearts receiving cardioplegia supplemented with 100, 200, or 400 μM adenosine had significantly increased recovery of function. In a follow-up study [3] it was shown that augmentation of myocardial adenosine with exogenous adenosine or preservation of endogenous adenosine during global ischemia improves functional recovery not by reducing depletion of ATP during ischemia, but by serving as substrate for repletion of ATP after reperfusion. In that study, during ischemia ATP decreased equally in all groups, indicating that augmenting myocardial adenosine had no effect on depletion of ATP. However, after reperfusion, ATP levels remained depressed in controls, but increased in the adenosine-augmented groups suggesting that ATP resynthesis capacity was intact and that the improved functional recovery noted was due to adenosine serving as the substrate for nucleotide resynthesis. This work confirmed that of others [1, 2, 5, 6, 11–13], demonstrating that adenosine acted as a substrate for maintaining tissue ATP levels and thereby enhanced functional recovery in global stunning models. However, while adenosine availability as a substrate may be an important determinant of postischemic metabolic and functional recovery, the exact role of adenosine in improving postischemic functional recovery remains controversial.

Besides serving as the substrate for nucleotide regeneration, adenosine has many actions which are mediated by receptors on the extracellular surface of the sarcolemma. Adenosine receptors have been found in the skin, neural tissue, vascular smooth muscle, endothelium, and myocardium [9, 14, 15]. These receptors are

TABLE 1

Functional Recovery (% of Preischemic Base Line, Mean ± SD)

	Rep 15 min	Rep 30 min	Rep 45 min
DP			
CONT	27 ± 16	37 ± 15	41 ± 16
NECA	25 ± 10	37 ± 11	39 ± 16
A&N	40 ± 11	49 ± 11	51 ± 9
PIA	33 ± 11	41 ± 12	45 ± 11
ADO	56 ± 16*	62 ± 10*	63 ± 8*
$+dP/dt_{max}$			
CONT	27 ± 17	37 ± 18	40 ± 19
NECA	29 ± 14	44 ± 18	47 ± 20
A&N	41 ± 13	51 ± 16	53 ± 17
PIA	35 ± 11	47 ± 16	43 ± 14
ADO	56 ± 12*	67 ± 10*	66 ± 6*
$-dP/dt_{max}$			
CONT	28 ± 15	39 ± 19	44 ± 16
NECA	27 ± 11	43 ± 15	47 ± 14
A&N	45 ± 11	57 ± 16	61 ± 15
PIA	37 ± 14	52 ± 16	55 ± 15
ADO	59 ± 15	67 ± 10*	69 ± 7*
HR			
CONT	92 ± 33	93 ± 19	93 ± 14
NECA	69 ± 13	83 ± 27	88 ± 30
A&N	73 ± 10	72 ± 13	75 ± 19
PIA	76 ± 15	84 ± 20	88 ± 25
ADO	95 ± 7	95 ± 8	95 ± 9
PRP			
CONT	26 ± 19	36 ± 18	39 ± 18
NECA	18 ± 9	31 ± 14	34 ± 14
A&N	29 ± 9	36 ± 13	39 ± 16
PIA	25 ± 10	32 ± 14	39 ± 11
ADO	50 ± 13*	58 ± 10*	60 ± 8*
CF			
CONT	76 ± 21	71 ± 15	65 ± 11
NECA	134 ± 24*	117 ± 19*	100 ± 14*
A&N	137 ± 49*	119 ± 34*	98 ± 32*
PIA	94 ± 19	84 ± 21	78 ± 18
ADO	98 ± 11	93 ± 7	87 ± 12*
MVO_2			
CONT	46 ± 19	45 ± 20	44 ± 20
NECA	53 ± 36	38 ± 27	38 ± 22
A&N	42 ± 17	38 ± 17	38 ± 16
PIA	39 ± 12	42 ± 13	44 ± 11
ADO	69 ± 22	76 ± 10	74 ± 8*
O_2 EXT			
CONT	59 ± 11	60 ± 20	66 ± 24
NECA	39 ± 21	31 ± 13	37 ± 18
A&N	34 ± 13	34 ± 13	41 ± 17
PIA	42 ± 12	54 ± 22	61 ± 20
ADO	52 ± 13	62 ± 10	65 ± 12

Note. Hemodynamics were determined at 45 min of reperfusion. CONT, control group (n = 7); NECA, NECA-treated group (n = 6); A&N, adenosine and NECA-treated group (n = 6); PIA, PIA-treated group (n = 6); and ADO, adenosine treated group (n = 7). DP, developed pressure (mm Hg); dP/dt_{max}, maximum of the first derivative of left ventricular pressure/sec (mm Hg/sec); HR, heart rate (beats/min); PRP, product of HR and DP (10^3 mm Hg/min); CF, coronary flow (ml/min/g); MVO_2, myocardial oxygen consumption (μl/min/g); O_2 EXT, oxygen extraction (ml/min/gm). * $P < 0.05$, compared with CONT.

coupled either in an inhibitory (A_1) or stimulatory (A_2) manner to adenylate cyclase via the guanine protein system. The vasodilating vascular smooth muscle effects of adenosine may be mediated by the A_2 receptor. In addition, there is evidence that A_2 receptors potentiate cAMP production and augment the inotropic state of the isolated perfused heart. A_2 receptors also have been shown to decrease neutrophil migration and infiltration [9, 16]. Conversely, the A_1 receptor mediates the antiadrenergic effects of adenosine on cardiac contractility and heart rate. This A_1 receptor action is thought to be due to an inhibition of β-adrenergic receptor mediated stimulation of adenylate cyclase via modulation of β-adrenergic receptor coupling [9, 15, 16]. A_1 receptor stimulation is also thought to enhance myocyte glucose utilization [10, 17].

While adenosine itself has been shown to reduce infarct size, improve ventricular function, and preserve myocyte ultrastructure following ischemia [7–9, 13], perhaps adenosine's protective role in myocardial ischemia may be mediated via A_1 and A_2 receptor modulation and not as a substrate. Investigators have studied A_1 and A_2 adenosine receptor agonists in rabbit hearts subjected to 30 min of coronary occlusion and 48 hr of reperfusion. They found that agonists of both adenosine receptors afforded cardioprotection. Other investigators have postulated that resistance to ischemic may be mediated by A_1 adenosine receptors and observed that infusion of adenosine or an A_1 agonist (PIA) equally enhanced resistance to ischemia in isolated hearts, while A1 receptor blocking agents abolished this effect [8].

While these studies concentrated upon infarct sizing, others have examined the effect of adenosine receptors on functional recovery following ischemia. In a regional canine model, a potent adenosine A_1 receptor agonist significantly improved the recovery of regional contractile function without affecting coronary flow or blood pressure [18]. While no effect of A_2 receptor agonists upon recovery of contractile function was not noted, it was postulated that A_2 receptor stimulation could produce a beneficial effect via its potent coronary vasodilating activity or by inhibiting neutrophil function in ischemic areas. In further confirmation, Lasley and Mentzer [19] demonstrated that adenosine enhanced postischemic myocardial function in isolated rat hearts via activation of the adenosine A_1 receptor but not the A_2 receptor. These results imply that adenosine A_1 receptor activation may be important for preservation of myocardial contractile function.

The mechanisms by which activation of adenosine A_1 receptors could protect the heart against ischemic injury remain to be defined. It is known that endogenous catecholamines are released during myocardial ischemia. Activation of adenosine A_1 receptors have been shown to reduce catecholamine release [9]. Furthermore, adenosine A_1 receptor stimulation has been shown to decrease intracellular ATP loss, decrease intracellular cAMP levels, and produce a negative inotropic effect, potentially leading to a more favorable supply/demand ratio during ischemia [9, 15]. Activation of A_1 receptors also has been proposed to decrease free radical formation by reducing lipolysis, thus inhibiting the formation of lipid hydroperoxides and by decreasing the catecholamines available for autoperoxidation, reducing oxygen-derived free radical-induced damage [8]. Another possible mechanism by which activation of adenosine A_1 receptors could protect the ischemic myocardium is via opening K_{ATP} channels [20], as K_{ATP} channel openers have been shown to improve recovery of segment shortening in stunned myocardium [21].

Finally, other investigators have noted that adenosine stimulates glycolysis by activation of A_1 receptors [10, 17]. Following activation of A_1 receptors there is an increase in membrane transport of glucose, resulting in glucose influx and an increase in myocardial ATP production in isolated, perfused rat hearts. Adenosine A1 receptor agonists can delay the onset of ischemic contracture; however, in the absence of glucose this effect is lost [17].

However, the favorable effects of adenosine receptor agonists upon function may be limited and have mostly been noted in regional models. Furthermore, there are conflicting results regarding the cardioprotective effect of adenosine A_1 and A_2 receptors against ischemic damage, as other authors have noted that adenosine receptor antagonists have failed to abolish the cardioprotective effects of preconditioning and in certain models, intravenous adenosine was unable to be cardioprotective. Some authors have therefore concluded that adenosine's mechanism of ischemic resistance is not mediated by adenosine receptors and that ischemic resistance may be related to adenosine, muscarinic, and α-adrenergic receptor signal balance [22], acting as an "ischemic-mimetic," promoting eventual protein kinase C phosphorylation and, through an unknown mechanism, enhancement of protection against myocyte necrosis.

In this study, although the addition of NECA to the cardioprotective regime resulted in profound vasodilatation, NECA and adenosine together were not additive in their protection, but perhaps detrimental. This may have resulted from A_2-mediated potentiation of the adrenergic state, which could be postulated to be harmful in the immediate postischemic state in terms of energy balance. PIA was not combined with adenosine in this study due to PIA's anti-adrenergic effect on inotropy and chronotropy, but, conversely, this may be "protective" and will be examined in future studies.

In this model, while adenosine receptor stimulation may certainly play a role in cardioprotection, we found that despite maximal A_1 and A_2 receptor stimulation, as noted by the observed physiologic effects of potent A1 and A2 receptor agonists, the best functional recovery was seen with adenosine itself. We conclude that the beneficial effect of adenosine on functional recovery

may be mediated more by adenosine's role as a substrate for nucleotide resynthesis than as a receptor signal. As opposed to adenosine's role in ischemic preconditioning, this finding in the present study may be due to differing end points, as in infarction/preconditioning models the end point is myocyte necrosis, whereas for this surgically analagous model the end point is myocyte stunning. While static ATP levels certainly do not equate linearly with functional recovery following stunning, many investigators have shown that ATP and nucleotide resynthesis are intimately involved with postischemic recovery [1–6]. Additionally, although ATP levels were not obtained in this preliminary screening model, further studies to measure ATP and nucleotide resynthesis rates with A_1 and A_2 receptor stimulation are planned. Finally, adenosine's cardioprotective mechanism, while remaining controversial, continues to be a promising area for potential clinical application.

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