Korean J Physiol Pharmacol
Vol 2: 753–761, December, 1998

Involvement of Adenosine in Cardioprotective Effect of Catecholamine Preconditioning in Ischemia-Reperfused Heart of Rat

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Preconditioning of a heart with small doses of catecholamines induces a tolerance against the subsequent lethal ischemia. The present study was performed to find a specific receptor pathway involved with the catecholamine preconditioning and to test if adenosine plays a role in this cardioprotective effect. Isolated rat hearts, pretreated with small doses of α- or β-adrenergic agonists/antagonists, were subjected to 20 minutes ischemia and 20 minutes reperfusion by Langendorff perfusion method. Cardiac mechanical functions, lactate dehydrogenase and adenosine release from the hearts were measured before and after the drug treatments and ischemia. In another series of experiments, adenosine A_1 or A_2 receptor blockers were treated prior to administration of adrenergic agonists. Pretreatments of a β-agonist, isoproterenol(10^{-9} ~10^{-7} M) markedly improved the post-ischemic mechanical function and reduced the lactate dehydrogenase release. Similar cardioprotective effect was observed with an α-agonist, phenylephrine pretreatment, but much higher concentration(10^{-4} M) was needed to achieve the same degree of cardioprotection. The cardioprotective effects of isoproterenol and phenylephrine pretreatments were blocked by a β_1-adrenergic receptor antagonist, atenolol, but not by an α_1-antagonist, prazosin. Adenosine release from the heart was increased by isoproterenol, and the increase was also blocked by atenolol, but not by prazosin. A selective A_1-adenosine receptor antagonist, 1,3-dipropyl-8-cyclopentyl xanthine (DPCPX) blocked the cardioprotection by isoproterenol pretreatment. These results suggest that catecholamine pretreatment protects rat myocardium against ischemia and reperfusion injury by mediation of β_1-adrenergic receptor pathway, and that adenosine is involved in this cardioprotective effect.

Key Words: Adenosine, Catecholamine preconditioning, Ischemia, Heart

INTRODUCTION

Sublethal, brief myocardial ischemia increases the tolerance of a heart to the subsequent lethal ischemia (Murry et al, 1986). This phenomenon termed "ischemic preconditioning" has been demonstrated to reduce the myocardial infarct size, decrease the development of reperfusion arrhythmias, and improve the post-ischemic functional recovery in various animal species and human hearts. Although the mechanism of its cardioprotective effect has not been clearly understood, the ischemic preconditioning is regarded as a potentially useful tool for myocardial protection against ischemia and reperfusion injury in some clinical situations such as coronary heart disease and open heart surgery.

More recently, it was proposed that pharmacological interventions that are able to mimic and/or activate the protective mechanisms responsible for the ischemic preconditioning would be a more attractive option without incurring possible harmful effects from even short-term ischemia during the process of ischemic preconditioning (Parratt, 1994). In this re-

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gard, numerous investigators reported that the preconditioning of a heart with small doses of catecholamines produced a cardioprotective effect against the post-ischemic reperfusion injury similar to that observed in the ischemic preconditioned hearts (Banerjee et al, 1993; Asimakis et al, 1994; Tosaki et al, 1995). However, the results from the investigations are equivocal, particularly with regard to adrenergic receptor pathway which can be involved in the catecholamine preconditioning. Banerjee et al (1993) and Tosaki et al (1995) concluded from their experiments using rat hearts that norepinephrine preconditioned the hearts by mediation of α_1-adrenergic receptor. Contrarily, Asimakis et al (1994) reported that the protective effect of catecholamine preconditioning was mainly due to β-adrenergic receptor stimulation.

A purine nucleoside, adenosine is released from the myocardium in response to a decrease in the oxygen supply/demand ratio, as seen in myocardial ischemia and hypoxia. This endogenous metabolite plays a multifaceted role in the protection of ischemic myocardium, manifested by coronary vasodilation which increases oxygen supply and by multiple effects which act in concert to decrease myocardial oxygen demand and to preserve cellular energy charge (Ely & Berne, 1992). Adenosine has been also implicated in the cardioprotective effect of ischemic preconditioning. Liu et al (1991) and Thornton et al (1992) indicated that the buildup of adenosine during the periods of ischemic preconditioning triggers the protective effect by A_1-receptor stimulation. Furthermore, intrvenous or intracoronary adenosine or the selecive A_1-receptor agonists conferred a similar protective effect against the subsequent prolonged ischemic episode (Liu et al, 1991; Pitarys II et al, 1991; Thornton et al, 1992; Mosca et al, 1994). Since adenosine production and release in ischemic hearts is accelerated by adrenergic stimulation through the mediation of either α- (Kitakaze et al, 1987) or β-adrenoceptor (Wangler et al, 1984; Richardt et al, 1994), we postulate that adenosine might play a role as a cardioprotective mediator in the catecholamine-preconditioned hearts also.

In this study, therefore, we tried to identify the adrenoceptors responsible for the catecholamine preconditioning, and also to test whether adenosine is involved in the cardioprotective effect of catecholamine preconditioning in ischemia-reperfusion model of isolated rat hearts.

METHODS

Experimental protocol

Male Sprague-Dawley rats (200~250 g) were anesthetized with intravenous administration of pentobarbital sodium (30 mg/kg). Hearts were excised and immediately connected to aortic cannulae. Hearts were perfused by a constant pressure, non-recirculating Langendorff mode with Krebs-Henseleit buffer (KHB), containing: NaCl 118 mM, KCl 4.7 mM, $CaCl_2$ 1.25 mM, $MgSO_2$ 1.2 mM, glucose 10 mM, $NaHCO_3$ 25 mM, and KH_2PO_4 1.2 mM. The buffer solution was saturated with 95% O_2-5% CO_2 mixture at 37°C, and perfusion pressure was maintained at 80 cm H_2O. Left ventricular pressure was monitored continuously with a pressure transducer attached to a water-filled latex balloon inserted into the left ventricle. The balloon was initially inflated until the left ventricular end-diastolic pressure reached 5 mmHg. Each preparation was equilibrated for 20 minutes before experiment. After equilibration, hearts were subjected to global ischemia for 20 minutes, followed by reperfusion for 20 minutes. During ischemia, the hearts were immersed in buffer solution to maintain at 37°C. Left ventricular developed pressure (LVDP) was calculated from the difference between left ventricular end-systolic (LVESP) and end-diastolic pressure (LVEDP). The post-ischemic functional recovery was evaluated from the pressure-rate index (LVDP × Heart rate) expressed as percent of the preischemic value and LVEDP. Before and after the ischemia, coronary effluent was collected for the measurement of lactate dehydrogenase and adenosine release from the hearts.

Experimental groups and drug treatments

Experimental groups were divided into four; I) ischemia-reperfusion control, II) adrenergic agonists pretreated (catecholamine preconditioning) group, III) II puls adrenergic receptor antagonist, and IV) II plus adenosine receptor antagonist groups. Control hearts were subjected to 20 min ischemia, followed by 20 min reperfusion only. For catecholamine preconditioning, hearts were treated with various concentrations of an α-adrenergic agonist, phenylephrine (10^{-6}~10^{-4} M) or a β-adrenergic agonist, isoproterenol (10^{-9}~10^{-7} M) for 5 min, followed by 5 min washout, before the ischemia and reperfusion. In

group III, hearts were pretreated with a α_1-adrenoceptor antagonist, prazosin(10^{-6}, 10^{-5} M) or a β_1-antagonist, atenolol(10^{-6}, 10^{-5} M) for 10 minutes before the administrations of adrenergic agonists. In group IV, hearts were pretreated with an adenosine A_1-receptor antagonist, 1,3-dipropyl-8-cyclopentylxanthine (DPCPX, 2×10^{-5} M) or an A_2-receptor antagonist, 1,3- dimethyl-7-propylxanthine(DMPX, 3×10^{-6} M) for 10 minutes before the treatments of adrenergic agonists.

Measurements of lactate dehydrogenase and adenosine

Lactate dehydrogenase (LDH) released into the coronary perfusate was measured as an index of ischemia-reperfusion cadiomyocyte injury. Coronary effluent collected during the first 5 minutes of reperfusion was stored in ice. The LDH activity was assayed within 8 hours by the spectrophotometric method of Bergmeyer & Bernt (1974). An aliquot of the coronary effluent was added to a reaction mixture containing 48 mM KH_2PO_4 (pH 7.4), 0.6 mM pyruvate, and 0.18 mM NADH, and the rate of optical density change was measured at 340 nm and 25°C.

Adenosine production and release from the heart was determined to investigate the involvement of this endogenous metabolite in the catecholamine preconditioning. Coronary effluent was collected during the periods of agonist administraion (5 min) and washout (5 min) before the ischemia. Adenosine level in the coronary effluent was assayed by a high performance liquid chromatography (HPLC) method (Gruber et al, 1989). An aliquot of sample (20 μL) was loaded onto a C-18 reverse phase column (Rainin, USA). Mobile phase was composed of 10 mM phosphate buffer (pH 2.9): 1% (v/v) acetonitrile. Adenosine was eluted at 16~18 minutes, and its identity was confirmed with authentic adenosine.

Data analysis

The results represent the mean±SE of at least five individual determinations. Differences between experiments were evaluated by Student's two-tailed unpaired t-test and considered significant when $p<0.05$.

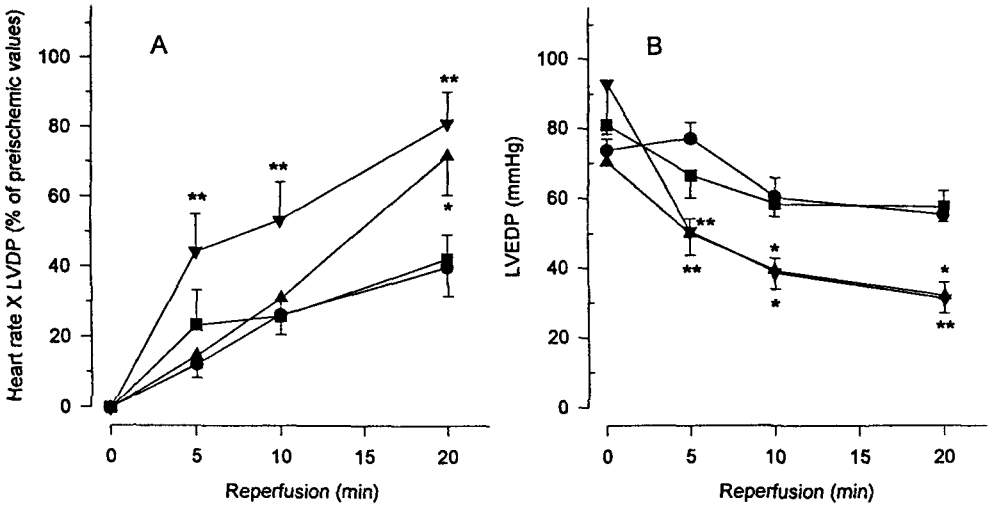

Fig. 1. Post-ischemic recovery of left ventricular function in isoproterenol pretreated rat hearts. Control hearts (●) were subjected to 20 min global ischemia and 20 min reperfusion. The hearts were treated with isoproterenol for 5 min, followed by 5 min washout, before the ischemia and repefusion. The concentrations of isoproterenol administered were 10^{-9} M(■), 10^{-8} M (▲) and 10^{-7} M (▼). Panel A; Post-ischemic recovery of the pressure-rate index (left ventricular developed pressure, LVDP × heart rate). Panel B; Post-ischemic left ventricular end-diastolic pressure (LVEDP). Values represent mean±SEM of 5~6 experiments.*: $p<0.05$, **: $p<0.01$ vs control.

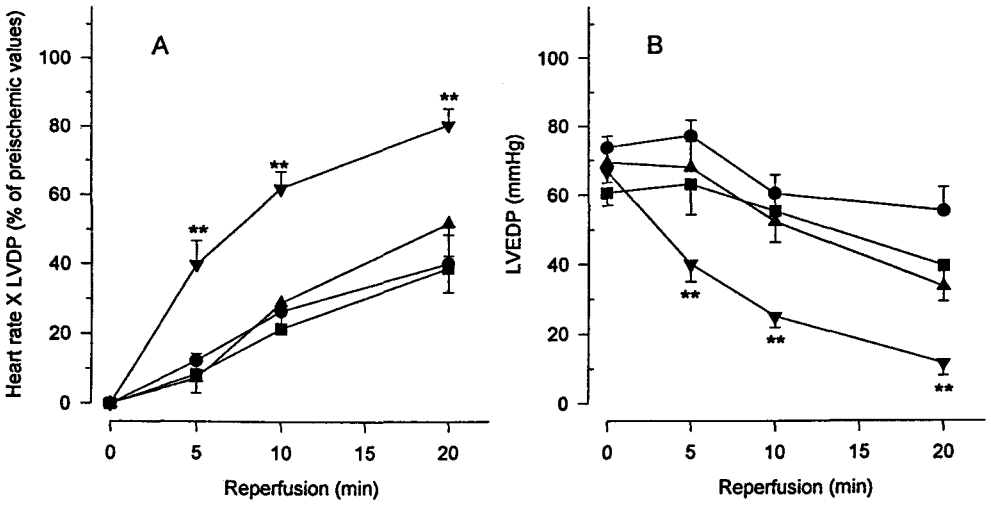

Fig. 2. Post-ischemic recovery of left ventricular function in phenylephrine pretreated rat hearts. Control hearts (●) were subjected to 20 min global ischemia and 20 min reperfusion. The hearts were treated with phenylephrine for 5 min, followed by 5 min washout, before the ischemia and repefusion. The concentrations of phenylephrine administered were 10^{-9} M(■), 10^{-8} M(▲) and 10^{-7} M (▼). Panel A; Post-ischemic recovery of the pressure-rate index (left ventricular developed pressure, LVDP × heart rate). Panel B; Post-ischemic left ventricular end-diastolic pressure (LVEDP). Values represent mean±SEM of 5~6 experiments. *: $p<0.05$, **: $p<0.01$ vs control.

RESULTS

Effects of adrenergic agonists pretreatments on post-ischemic functional recovery and LDH release

The pressure-rate index (LVDP × heart rate) of control hearts after 20 min of reperfusion following 20 min ischemia showed 40.3% of the preischemic value. LVEDP increased immediately after the onset of reperfusion and decreased gradually thereafter to 55.3 mmHg at the end of the reperfusion. Pretreatment with isoproterenol resulted in a significant improvement in post-ischemic cardiac functional recovery and diastolic dysfunction. In the 10^{-7} M isoproterenol-treated group, the pressure-rate index was 81.6% (vs control, $p<0.01$) of the preischemic value, and LVEDP was attenuated to 31.1 mmHg (vs control, $p<0.01$) at the end of the reperfusion. The improvement of the post-ischemic functional recovery and diastolic dysfunction by isoproterenol was dose-dependant in the range of 10^{-9}~10^{-7} M (Fig. 1). Phenylephrine pretreatment also affored a protection to the ischemic-reperfused hearts. However, this effect was not apparent until the concentration was increased much higher level (10^{-4} M) than that of isoproterenol. At the end of the reperfusion, percent recovery of the pressure-rate index was 80.2% (vs control, $p<0.01$) in 10^{-4} M phenylephrine pretreated hearts, but it was not significantly improved in 10^{-6} M and 10^{-5} M pretreated groups. Attenuation of LVEDP was also significant only in 10^{-4} M pretreated hearts (19.5 mmHg vs control, $p<0.01$) (Fig. 2).

The cardioprotective effect estimated by LDH release during the reperfusion was consistent with the functional improvement. The release rate during the

Table 1. Lactate dehydrogenase release during early 5 min of reperfusion

Catecholamine		LDH[a] (mU/min/g wet wt)
Ischemic control		78.0±10.5
Isoproterenol	10^{-9} M	53.1±4.4
	10^{-8} M	33.4±3.6*
	10^{-7} M	37.1±2.2*
Phenylephrine	10^{-6} M	93.3±5.3
	10^{-5} M	83.1±4.2
	10^{-4} M	47.5±4.9*

[a]: Mean±SEM, n=5~6
*: $p<0.05$ vs Ischemic control

first 5 min of reperfusion was 78.0±10.5 mU/min/g wet weight in the control heart, and it was significantly reduced to 37.1±2.2 ($p<0.05$) and 47.5±4.9 ($p<0.05$) mU/min/g wet weight in isoproterenol (10^{-7} M) and phenylephrine (10^{-4} M) treated groups, respectively (Table 1).

Influence of adrenergic antagonists on the cardioprotective effects of various agonists

Changes in the cardioprotective effects of isoproterenol and phenylephrine pretreatments were observed under the blockade of α_1- or β_1-adrenergic receptors with selective antagonists. Atenolol, a β_1-adrenergic antagonist, prevented the beneficial effects of isoproterenol. Atenolol(10^{-6} M) blocked the improvement of pressure-rate index, the attenuation of LVEDP and the reduction of LDH release in isoproterenol(10^{-7} M) treated hearts (Fig. 3, Table 2). Interestingly, the cardioprotective effects of high concentration of phenylephrine(10^{-4} M) pretreatment were also blocked by atenolol(10^{-5} M) (Fig. 4, Table 2). Meanwhile, prazosin, an α_1-adrenergic antagonist (10^{-6}, 10^{-5} M) did not prevent the cardioprotective effects of either isoproterenol or phenylephrine in terms of the improvement of pressure-rate index and the attenuations of LVEDP and LDH release (Fig. 3 & 4, Table 2).

Adenosine release and influence of adenosine receptor antagonists in isoproterenol pretreated hearts

We tried to test the possible involvement of adenosine in the cardioprotective effect of catecholamine preconditioning. Since, as seen in the above

Table 2. Effects of adrenergic antagonists on LDH release in catecholamine-pretreated hearts

Catecholamine	Antagonist	LDH[a] (mU/min/g wet wt)
Ischemic control		78.0±10.5
Isoproterenol, 10^{-7} M	–	37.1±2.2
	Prazosin, 10^{-6} M	39.0±6.5
	Atenolol, 10^{-6} M	65.5±8.7*
Phenylephrine, 10^{-4} M	–	47.5±4.9
	Prazosin, 10^{-5} M	47.7±4.9
	Atenolol, 10^{-5} M	81.9±11.0*

[a]: Mean±SEM, n=5~6
*: $p<0.05$ vs catecholamine only

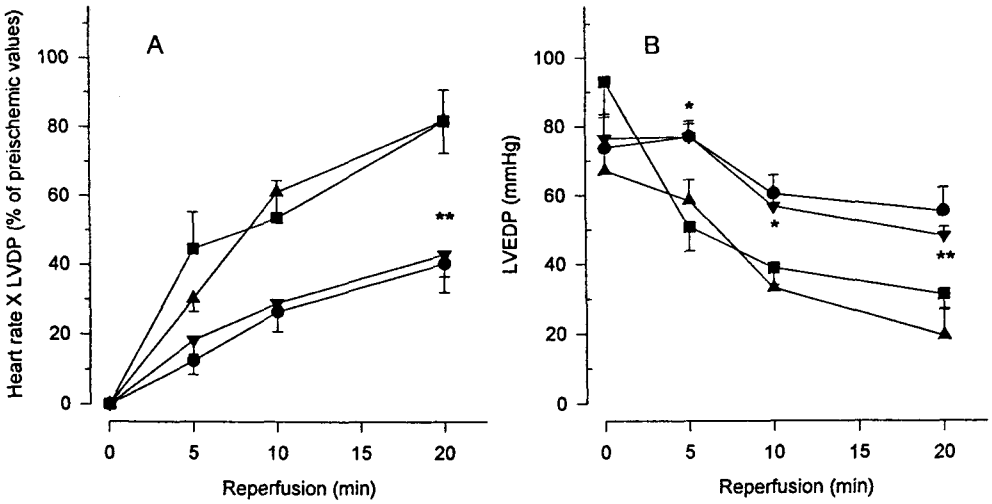

Fig. 3. Effects of adrenergic antagonists on post-ischemic functional recovery in isoproterenol pretreated rat hearts. Control hearts (●) were subjected to 20 min global ischemia and 20 min reperfusion. Hearts were pretreated with a α_1-adrenoceptor antagonist, prazosin (10^{-6} M, ▲) or a β_1-antagonist, atenolol (10^{-6} M, ▼) for 10 minutes before the administration of isoproterenol(10^{-7} M, ■). Panel A; Post-ischemic recovery of the pressure-rate index (left ventricular developed pressure, LVDP × heart rate). Panel B; Post-ischemic left ventricular end-diastolic pressure (LVEDP). Values represent mean± SEM of 5~6 experiments. *: $p<0.05$, **: $p<0.01$ vs isoproterenol.

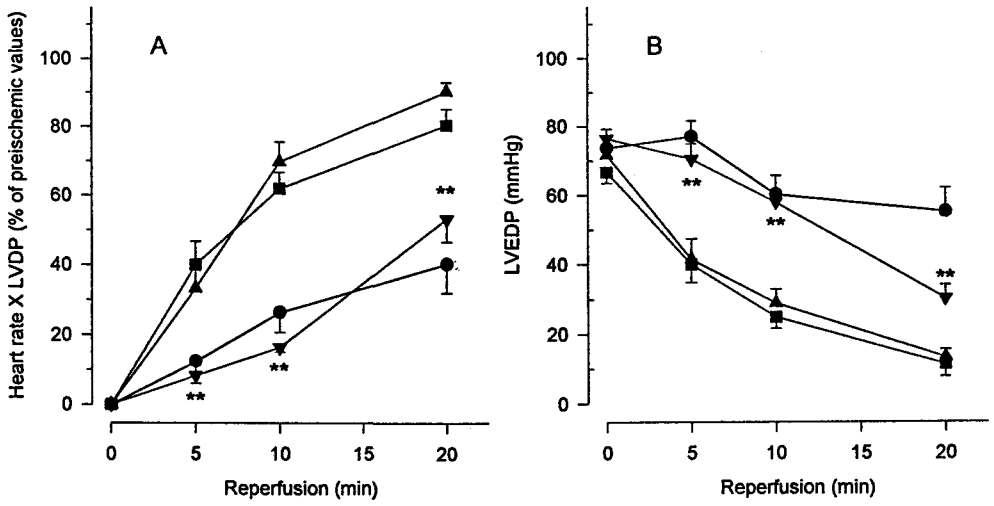

Fig. 4. Effects of adrenergic antagonists on post-ischemic functional recovery in phenylephrine pretreated rat hearts. Control hearts (●) were subjected to 20 min global ischemia and 20 min reperfusion. Hearts were pretreated with a α_1-adrenoceptor antagonist, prazosin(10^{-5} M, ▲) or a β_1-antagonist, atenolol (10^{-5} M, ▼) for 10 minutes before the administration of phenylphrine (10^{-4} M, ■). Panel A; Post-ischemic recovery of the pressure-rate index (left ventricular developed pressure, LVDP × heart rate). Panel B; Post-ischemic left ventricular end-diastolic pressure (LVEDP). Values represent mean± SEM of 5~6 experiments. *: $p<0.05$, **: $p<0.01$ vs phenylephrine.

Table 3. Adenosine release during pre-ischemic periods in isoproterenol-pretreated hearts

Catecholamine	Antagonist	Adenosinea (nmole/min/g wet wt)
Control	–	0.68±0.21
Isoproterenol		
10^{-9} M	–	0.61±0.07
10^{-8} M	–	5.68±1.50*
10^{-7} M	–	15.97±3.50*
10^{-7} M	Atenolol, 10^{-6} M	5.27±1.79*,+
10^{-7} M	Prazosin, 10^{-6} M	15.00±3.60

[a]: Mean±SEM, n=5~6
*: $p<0.05$ vs control, +: $p<0.05$ vs isoproterenol (10^{-7} M) only

Table 4. Eefect of adenosine antagonists on LDH release in catecholamine-pretreated hearts

Catecholamine	Antagonist	LDH[a] (mU/min/g wet wt)
Ischemic control	–	78.0±10.5
Isoproterenol,	–	37.1±2.2
10^{-7} M	DPCPX, 2×10^{-5} M	71.8±7.0*
	DMPX, 3×10^{-6} M	57.0±4.2*

[a]: Mean±SEM, n=5~6
*: $p<0.05$ vs isoproterenol only

experiments, adrenergic agonists preconditioned the hearts to produce a myocardial protection against ischemia-reperfusion injury probably by a mediation of β_1-adrenergic receptor pathway, we observed adenosine release and influence of adenosine receptor blockers on the cardioprotective effects in isoproterenol pretreated hearts. Adenosine release during the preischemic period was 0.68 nmole/min/g wet weight in the control hearts. The adenosine release was increased dose-dependently by isoproterenol pretreatment. Mean adenosine release by 10^{-7} M isoproterenol treatment was 15.97 nmole/min/g wet weight (vs control, $p<0.05$). The increase in adenosine release by isoproterenol was blocked by atenolol, while not by prazosin (Table 3).

An adenosine A_1-receptor antagonist, 1,3-dipropyl-8-cyclopentylxanthine (DPCPX) administered for 10 min before and during isoproterenol treatment prevented the cardioprotective effects of isoproterenol. DPCPX (2×10^{-5} M) blocked the improvement of

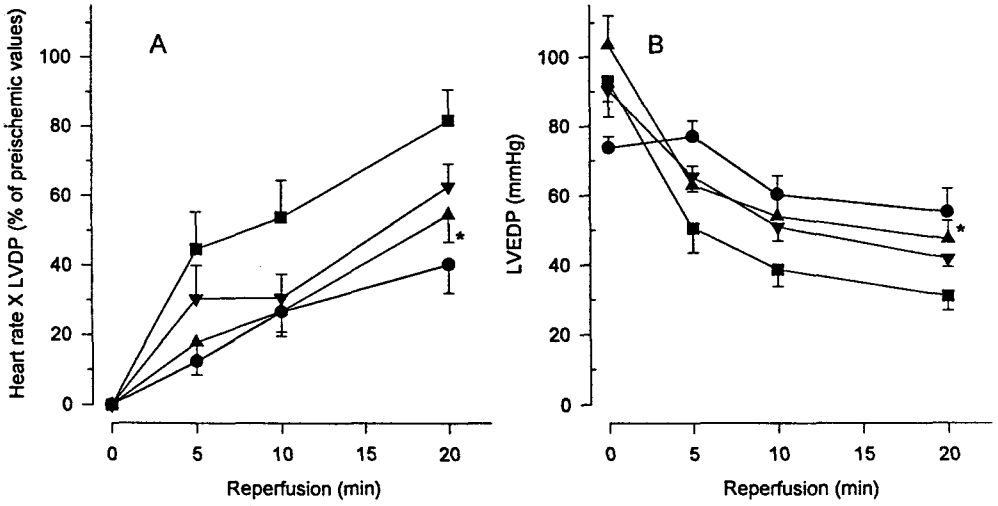

Fig. 5. Effects of adenosine antagonists on post-ischemic functional recovery in isoproterenol pretreated rat hearts. Control hearts (●) were subjected to 20 min global ischemia and 20 min reperfusion. Hearts were pretreated with an A_1-antagonist, DPCPX (3×10^{-6} M, ▲) or a A_2-antagonist, DMPX (2×10^{-5} M, ▼) for 10 minutes before the administration of isoproterenol(10^{-7} M, ■). Panel A; Post-ischemic recovery of the pressure-rate index (left ventricular developed pressure, LVDP × heart rate). Panel B; Post-ischemic left ventricular end-diastolic pressure (LVEDP). Values represent mean± SEM of 5~6 experiments. *: $p<0.05$ vs isoproterenol.

pressure-rate index and the attenuation of LVEDP and LDH release induced by isoproterenol (10^{-7} M) pretreatment (Fig. 5, Table 4). An A_2-receptor antagonist, 1,3-dimethyl-7-propylxanthine (DMPX, 3×10^{-6} M) also showed a similar blocking effect on the cardioprotective effects of isoproterenol pretreatment. However, the differences of functional parameters between the DMPX treated and the untreated hearts were statistically insignificant (Fig. 5). The reduction of LDH release in isoproterenol pretreated hearts was also blocked by DMPX, but not so markedly as by DPCPX (Table 4).

DISCUSSION

Short-term pretreatment of adrenergic agonists (catecholamine preconditioning), as well as ischemic preconditioning, can increase the tolerance of a heart against a subsequent prolonged episode of ischemia. However, the adrenergic receptor pathway involved in the cardioprotective effect has not been clearly defined (Banerjee et al, 1993; Asimakis et al, 1994; Tosaki et al, 1995). The present study shows that pretreatment with exogenous adrenergic agonists protects the rat hearts against the post-ischemic myocardial injuries probably by mediation of β_1-adrenoceptors. Banerjee et al (1993) reported that ischemic preconditioning in the rat heart is mediated by norepinephrine released during the periods of transient ischemia, which enhances the functional and metabolic recovery after an episode of prolonged myocardial ischemia. Moreover, they and Tosaki et al (1995) observed that exogenous norepinephrine and phenylephrine could precondition the heart and protect against a prolonged ischemia, which was blocked by α-adrenergic antagonists. Consequently, the investigators concluded that transient α-adrenergic receptor stimulation can precondition the rat heart to prevent the post-ischemic myocardial injuries. On the contrary, Asimakis et al (1994) reported that the cardioprotective effects of adrenergic agonists were due to β-adrenergic stimulation. They found that transient pretreatment with exogenous norepinephrine or isoproterenol, but not phenylephrine, could attenuate post-ischemic contractile dysfunctions in the isolated rat hearts, and that these effects were blocked by a β-antagonist propranolol, but not by an α-antagonist prazosin. In the present study, we also suggest that adrenergic agonists preconditioned the hearts most probably by a β_1-adrenergic mechanism because we observed, firstly, that isoproterenol, a pure β-ad-

renergic agonist, preconditioned the hearts very effectively. Pretreatment with isoproterenol in the concentration range of 10^{-8} and 10^{-7} M induced a marked improvement of functional recovery and the attenuation of LDH release after 20 min ischemia followed by reperfusion. Secondly, pretreatment with phenylephrine, a pure α-agonist, did not show the cardioprotective effect until the concentration was increased to 10^{-4} M, which was more than 1,000 times that of isoproterenol. While Banerjee et al (1993) and Tosaki et al (1994) observed the cardioprotective effects in phenylephrine pretreated rat hearts as well as in norepinephrine pretreated hearts, Asimakis et al (1995) failed to observe any protective effect with the same concentrations of phenylephrine as we used, including 10^{-4} M. The reason for the discrepancies between our study and others is not readily apparent, though the experimental protocols, such as the ischemia and reperfusion times and the observed parameters, are different between studies. Thirdly, the cardioprotective effects of both isoproterenol and phenylephrine (10^{-4} M) were blocked by atenolol, a β_1-adrenergic antagonist, but not by prazosin, an α_1-antagonist. It is interesting that the effects of high concentraion of phenylephrine, a pure α-agonist, is blocked by a β_1-antagonist, but not by an α-antagonist. This is thought to be probably due to loss of receptor selectivity with the presence of a much higher concentration of the agonist at the receptor sites.

Adenosine production is accelerated in response to a decrease in the oxygen supply/demand ratio, as in the ischemic or hypoxic myocardium. Most of the intracellular adenosine is released out of the cells via the nucleoside transporter (Meghji et al, 1985), so that interstitial and venous adenosine concentrations are increased to play a protective role (Knabb et al, 1984; Van Wylen et al, 1992; Headrick et al, 1996). It has been known that adrenergic stimulation favors the adenosine release from the cardiomyocytes by mediation of either α-adrenergic (Kitakaze et al, 1987) or β-adrenergic receptors (Wangler RD et al, 1984; Fenton & Dobson Jr, 1993; Richardt et al, 1994). In the present study, we observed a dose-dependant increase in adenosine release by the isoproterenol treatment. The increase in adenosine release by isoproterenol was blocked by atenolol, indicating that the release is mediated by the β_1-adrenergic stimulation. Adenosine has been implicated in ischemic preconditioning, and the beneficial effects of the preconditioning could be prevented by the pretreatment with adenosine receptor antagonist 8-sulfophenyltheophylline in rabbit hearts (Liu et al, 1991). Furthermore, intravenous or intracoronary adenosine or the selective A_1-receptor agonists instead of the ischemic preconditioning protocol conferred a similar cardioprotective effect against the subsequent prolonged ischemic episode (Pitarys II et al, 1991; Thornton et al, 1992; Mosca et al, 1994). These observations suggest that endogenous adenosine is involved in the preconditioning effect by A_1-receptor activation. Although the involvement of adenosine in the ischemic preconditioning has been confirmed by many investigators in rabbit hearts, studies from several laboratories have shown that ischemic preconditioning is not mediated by adenosine in the rat hearts (Asimakis et al, 1993; Cave et al, 1993; Li & Kloner, 1993). In the present study, however, pretreatment of rat hearts with isoproterenol increased adenosine release in association with the prevention of the post-ischemic myocardial cellular injuries and dysfunctions. Furthermore, the cardioprotective effect of isoproterenol accompanied by an increased adenosine release was blocked not only by a β-adrenergic antagonist atenolol but also by a selective A_1-adenosine receptor blocker DPCPX. The protective effect of adenosine in the rat hearts against ischemic injury has been also demonstrated in other studies (Lasley et al, 1992). They observed that rat hearts treated with adenosine and R-phenylisopropyladenosine, an adenosine A_1-receptor agonist, exhibited a significantly greater TOIC (time to onset of ischemic contracture), a marker of myocardial ischemic injury, after a global normothermic ischemia. From this and other observations, we suggest that endogenous adenosine plays a role in attenuating myocardial ischemic damage in catecholamine pretreated rat hearts, and that the beneficial effect of adenosine is mediated by interaction with A_1-receptors.

ACKNOWLEDGEMENT

This work was supported by Basic Medical Research Grant from the Ministry of Education (1996), by a grant from Seoul National University Hospital Research Fund (1996) and by a grant from the Ministry of Science and Technology, ROK (1997).

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