BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1995 Nov; 16 (6): 505-508

Protective effect of preconditioning on ischemic heart and characterization of adenosine receptors in ischemic rabbit hearts[1]

LI Yu-Long, HE Rui-Rong (*Department of Physiology, Institute of Basic Medicine, Hebei Medical College, Shijiazhuang 050017, China*)

AIM: To assess the role of adenosine receptors in the cardioprotective effect of preconditioning and the characterization of adenosine receptors in heart. **METHODS:** In 18 anesthetized rabbits, myocardial ischemia was induced by occlusion of left descending coronary artery. ST segment elevation in epicardial electrogram was used as a criterion of acute ischemic injury. Myocardial adenosine receptors were measured using [^{3}H]adenosine ligand. **RESULTS:** After 60-min ischemia, epicardial ST segment elevation was higher in ischemia group (13.9 ± 0.6 mV) than in the ischemic preconditioning group (3.1 ± 0.5 mV). In membranes of the ventricular myocytes prepared from ischemic-preconditioning group, the density of adenosine receptors was higher than that of ischemia and sham ischemia groups (B_{max} being 602 ± 40, 348 ± 28, and 335 ± 30 pmol/g protein, respectively). Affinity of the receptors for the [^{3}H] adenosine was not different in the 3 groups (K_d being 181 ± 18, 169 ± 21, and 196 ± 24 nmol·L^{-1}, respectively). **CONCLUSION:** Activation of adenosine receptors along with an increase in adenosine receptor density during ischemic-preconditioning provides the basis for adenosine to exert its protective effect on the ischemic heart.

KEY WORDS heart; myocardial ischemia; electrocardiography; cell membrane; radioligand assay; purinergic P_1 receptors

Exposure of the myocardium to a brief transient ischemia prior to a longer ischemia delayed myocardial necrosis during the longer ischemia, termed "preconditioning"[1]. The preconditioning effect is mediated by activation of the adenosine (Ade) A_1 receptors[2,3]. During anoxia and ischemia, Ade release increased about 50 times more than that during normoxia[4]. Endogenous Ade activates the Ade A_1 receptors. The level of endogenous Ade release was important in the infarct size limiting effect of preconditioning[5]. We hypothesized that preconditioning induced not only an increase in endogenous Ade release, but also the changes of Ade receptors including maximal density of binding sites (B_{max}) and apparent affinity of binding sites (K_d), and the latter was involved in the protective effect of ischemic preconditioning. To examine this hypothesis, the protective effect of preconditioning on the ischemic heart injury and characterization of Ade receptors were investigated.

MATERIALS AND METHODS

Preparation of rabbit ischemic heart model

Eighteen rabbits of either sex, weighting $3.5 \pm s$ 0.5 kg, were anesthetized with sodium pentobarbital 30 mg·kg^{-1}. Rabbits were intubated and ventilated with a Harvard model 607 animal respirator (12 mL·kg^{-1}, 30 breaths·min^{-1}). A thoracotomy was made in the left 4 intercostal space, and the heart was suspended in a pericardial cradle. A silk suture was positioned around the left descending coronary artery and the ends of suture were threaded through a small plastic tube. Myocardial ischemia was induced by pulling the suture and reperfusion was achieved by loosening the

[1] Project supported by Natural Science Foundation of Hebei Province, № 393125.
Received 1994-09-20 Accepted 1995-04-05

suture. At the end of experiment, the left decending coronary artery was ligated at the original site, and a diluted carbon ink was injected via aorta. The ischemic myocardium was excised to determine the Ade receptors.

Evaluation of acute ischemic heart injury The ST segment elevation in epicardial electrogram was used as a criterion of acute ischemic injury[2]. The cardiac color changed from red to cyanosis was served as marker of acute ischemic injury. To record the epicardial electrogram, a wick electrode was used. The cardioelectric signal was recorded on a polygraph (RM-6000) through a bioelectric amplifier (AB-620G).

Radioligand binding of Ade receptors To determine the effects of preconditioning on Ade receptor density and affinity in ventricular myocytes, radioligand binding studies using [^{3}H]Ade ligand were carried out in ventricular myocyte membranes from control, ischemic, and preconditioning groups. Myocardium (0.5 g) was chilled in 15 mL of ice-cold Tris-HCl buffer 50 mmol·L^{-1} (pH 7.4), minced and homogenized in a glass potter at 400×g for 1 min. The supernatant was filtered through gauze and centrifuged at 100 000×g for 30 min. The pellet was resuspended in Tris-HCl buffer 50 mmol·L^{-1} and recentrifuged at 100 000×g for 30 min. Ade deaminase 300 U·L^{-1} was added. Membranes were preincubated with the enzyme and stirred at 4 ℃ for 40 min. Myocardial Ade receptors were measured using [^{3}H]Ade as radiolabeled ligand (specific activity 666 PBq·mol^{-1}·L^{-1}). Membranes were incubated with [^{3}H]Ade (20 — 800 nmol·L^{-1}). The assay was performed in 500 μL of incubation buffer. The incubation was carried out at 22 ℃ for 120 min to allow complete equilibration of the receptors with the radioligand. The reaction was terminated by rapid vacuum filtration through Hongguang-69 type microfibre filters. The filters were washed immediately thrice with 6 mL of ice-cold incubation buffer. All experiments were performed in triplicate. Filters were dried at 90 ℃ and placed in 4 mL scintillation fluid [POP (2, 5-diphenyloxazole) + POPOP (1,4-di-2,5-phenyloxazol- benzene)], and radioactivity was determined in a liquid scintillation counter (Tri-CARB4530 type, Packard). Specific binding was defined as the difference of binding in the absence and the presence of Ade 800 μmol·L^{-1}. The maximal density (B_{max}) and apparent affinity (K_d) of binding sites were obtained in individual experiments from Scatchard plots determined by linear regression analysis. Protein was determined according to Lowry *et al*.

Protocols The rabbits were randomized into 3 groups: 1) control group ($n=6$) — a 60-min sham-occlusion; 2) ischemic group ($n=6$) — a single 60-min occlusion; 3) preconditioned group ($n=6$) — four times of 5-min episodes of ischemia, at intervals of 5-min reperfusion, followed by a sustained occlusion for 60 min. Four occlusions were chosen to enhance formation and subsequent washout of ischemic catabolites[1]. Reflow for 5-min was adopted to ensure that the adenylate charge ([ATP + 1/2 · ADP]/[ATP + ADP + AMP]) was restored and lactate was washed out[7].

The results expressed as $\bar{x} \pm s$ were analyzed using t test.

RESULTS

Effect of preconditionong on cardiac ischemia In control (sham ischemia) group, the ST segment elevation in epicardial electrogram was not affected (0.9±0.1 mV before sham ischemia *vs* 1.0±0.2 mV after sham ischemia). In ischemic group, the ST segment after 60 min of ischemia was markedly elevated (1.2 ± 0.2 mV before ischemia *vs* 13.9 ±0.6 mV after ischemia, $P<0.01$). In preconditioning group, the ST segment was also elevated after 60 min of ischemia (1.3±0.2 mV before ischemic preconditioning *vs* 3.1 ±0.5 mV after ischemic preconditioning, $P<0.05$); however the ST segment elevation after 60 min of ischmeia in preconditioning group was lower than that in ischemic group ($P<0.01$) (Tab 1).

Effects of preconditioning on Ade receptor density and affinity in ventricular myocytes The density of Ade receptors in preconditioning group was higher than that in ischemic and control groups (602±40, 348±28 and 335±30 pmol/g protein, respectively,

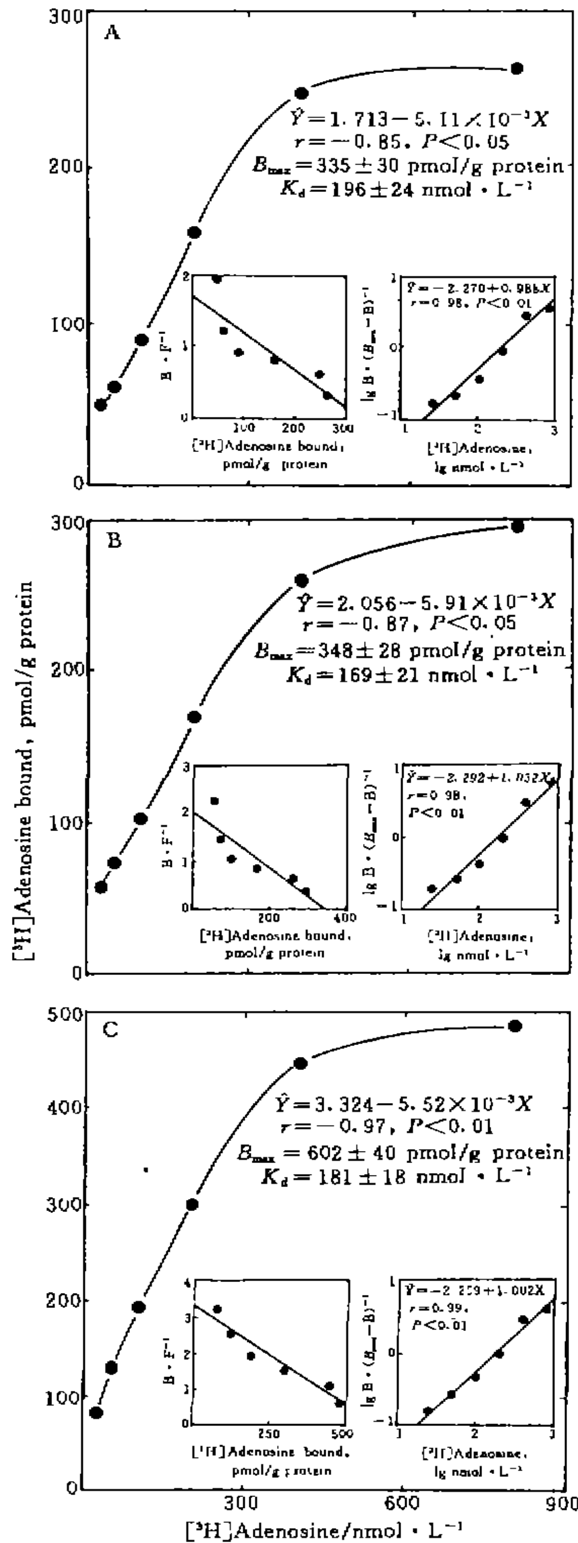

Fig 1. Binding of [³H]Ade to cardiac membranes of sham ischemic, ischemic, and ischemic-preconditioning rabbits. $n=6$. $\bar{x}\pm s$. A) sham ischemic group; B) Ischemic group; C) ischemic-preconditioning group.

Tab 1. Effect of preconditioning on ischemic heart injury in anesthetized rabbits. $n=6$. $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* control; $^dP>0.05$, $^eP<0.05$, $^fP<0.01$ *vs* sham ischemic group; $^gP>0.05$, $^iP<0.01$, *vs* ischemic group.

Group	ST segment/mV
Sham ischemia	
Control	0.9±0.1
Sham ischemic 60 min	1.0±0.2^a
Ischemia	
Control	1.2±0.2^d
Ischemia 60 min	13.9±0.6^{cf}
Preconditioning	
Control	1.3±0.2^{dg}
60 min ischemia after preconditioning	3.1±0.5^{bei}

$P<0.01$), while the density of Ade receptors in ischemic group was not much different from that in control group ($P>0.05$) (Fig 1). Affinity of the Ade receptors for the radioligand [³H]Ade was not different in all groups (control 196±24, ischemia 169±21, and preconditioning 181±18 nmol·L⁻¹).

DISCUSSION

Epicardial ST segment elevation shortly after artery occlusion correlated closely with subsequent depletion of myocardial creatinine phosphokinase (CPK) activity and with histological evidence of necrosis of subjacent myocardium[8]. In our experiment, epicardial ST segment elevation was significantly lower in ischemic preconditioning group than in ischemia group, indicating the cardioprotective effect of preconditioning. During anoxia and ischemia, endogenous Ade release increased[4,9]. Ade plays an important role in the ischemic preconditioning[10]. Our results

first demonstrated that the density of Ade receptors was also increased in the ischemic preconditioning and it is involved in the cardioprotective effect of preconditioning.

In conclusion, activation of Ade receptors along with an increase in Ade receptor density during ischemic preconditioning provides the basis for Ade to exert its protective effect against the ischemic heart injury.

REFERENCES

1 Murry CE, Jennings RB, Reimer KA. Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. Circulation 1986; **74**: 1124−36.

2 Liu GS, Thornton JD, Van Winkle DM, Stanley AWH, Olsson RA, Downey JM. Protection against infarction afforded by preconditioning is mediated by A_1 adenosine receptors in rabbit heart. Circulation 1991; **84**: 350−6.

3 Thornton JD, Liu GS, Olsson RA, Downey JM. Intravenous pretreatment with A_1-selective adenosine analogues protects the heart against infarction. Circulation 1992; **85**: 659−65.

4 Kitakaze M, Hori M, Kamada T. Role of adenosine and its interaction with α adrenoceptor activity in ischaemic and reperfusion injury of the myocardium. Cardiovasc Res 1993; **27**: 18−27.

5 Miura T, Iimura O. Infarct size limitation by preconditioning: its phenomenological features and the key role of adenosine. Cardiovasc Res 1993; **27**: 36−42.

6 Institute of Basic Medicine, Hebei Xinyi Medical College. Effect of clonidine on experimental myocardial ischemic injury: functional and morphological observations. Natl Med J China 1979; **59**: 302−5.

7 Jennings RB, Schaper J, Hill ML, Steenbergen C Jr, Reimer KA. Effect of reperfusion late in the phase of reversible ischemic injury. Changes in cell volume, electrolytes, metabolites, and ultrastructure. Circ Res 1985; **56**: 262−78.

8 Hillis DL, Askenazi J, Braunwald E, Radvany P, Muller JE, Fishbein MC, *et al*. Use of changes in the epicardial QRS complex to assess interventions which modify the extent of myocardial necrosis following coronary artery occlusion. Circulation 1976; **54**: 591−8.

9 Li YL, He RR. Endogenous adenosine and ATP-sensitive potassium channel modulate anoxia-induced electrophysiological changes of pacemaker cells in sinoatrial node of guinea pigs. Acta Pharmacol Sin 1995; **16**: 42−6.

10 Downey JM, Liu GS, Thornton JD. Adenosine and the anti-infarct effects of preconditioning. Cardiovasc Res 1993; **27**: 3−8.

505-508

预缺血对家兔缺血心脏的保护作用及其对心肌腺苷受体特性的影响

李玉龙，何瑞荣 （河北医学院基础医学研究所生理室，石家庄 050017，中国）

R965.2

目的：分析腺苷受体在预缺血对心肌保护中的作用和心肌腺苷受体的特性． **方法**：1）在18只麻醉家兔，结扎左冠脉前降支以诱发心肌缺血；2）心外膜ST段抬高作为急性心肌缺血的指标；3）用[³H]腺苷测定心肌腺苷受体． **结果**：1)缺血60 min时，心外膜ST段抬高(13.9±0.6 mV)明显高于预缺血后再缺血60 min时(3.1±0.5 mV)；2)在预缺血后再缺血心室肌细胞膜，腺苷受体密度(B_{max})高于单独缺血组和对照组(B_{max}分别为602±40，348±28和335±30 pmol/g protein)，但缺血组的心室肌细胞膜腺苷B_{max}未增高；3）各组的心室肌细胞膜的腺苷受体亲合力(K_d)无显著差异(K_d分别为181±18，169±21和196±24 nmol·L^{-1})． **结论**：预缺血时腺苷受体激活和腺苷受体数增多是腺苷对缺血心肌保护作用的基础.

关键词 心脏；心肌缺血；心电图；细胞膜；放射配位体测定；嘌呤受体