Effect of Preconditioning Ischemia on Endothelial Dysfunction Produced by Ischemia-Reperfusion in Rabbit Coronary Artery

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= ABSTRACT =

This study was designed to test whether or not 1) ischemia-reperfusion attenuates endothelium-dependent relaxation of coronary arteries and 2) preconditioning protects the arterial endothelium from ischemia-reperfusion injury. In anesthetized open chest rabbits, branches of the left circumflex artery were exposed to different combinations of the experimental conditions; ischemia (15 minutes), ischemia (15 minutes)-reperfusion (10 minutes), preconditioning ischemia, and preconditioning followed by ischemia-reperfusion. Preconditioning consisted of 3 occlusions of 2-min duration, each followed by a 5-min reperfusion. Rings of the artery exposed to the experimental condition and of normal left anterior descending coronary artery were prepared and suspended for isometric force measurement in organ chambers containing Krebs Ringer bicarbonate solution. The rings were contracted with 29.6 mM KCl. Ischemia alone did not attenuate endothelium-dependent relaxation by acetylcholine. However, ischemia-reperfusion significantly impaired endothelium-dependent relaxation. Endothelium-independent relaxation by sodium nitroprusside was not impaired by ischemia-reperfusion and the constrictive response to acetylcholine was not altered in reperfused rings without endothelium, compared with control rings. Arterial rings exposed to preconditioning followed by ischemia-reperfusion exhibited impaired endothelium-dependent relaxation by acetylcholine. However, although preconditioning not followed by ischemia-reperfusion, attenuated endothelium-dependent relaxation at low concentrations of acetylcholine, the magnitude of the impairment by preconditioning followed by ischemia-reperfusion was significantly less than that of the impairment by ischemia-reperfusion alone. These data demonstrate that ischemia-reperfusion significantly attenuates endothelium-dependent relaxation by producing endothelial dysfunction and preconditioning protects the endothelium of coronary arteries from ischemia-reperfusion injury.

Key Words: Coronary artery, Endothelium-dependent relaxation, Ischemia-reperfusion injury, Preconditioning ischemia

INTRODUCTION

Endothelium has an obligatory role in the regulation of vascular tone to a variety of vasodilators. Structural or functional damage of endothelial cells enhances vasoconstrictive responses and impairs vasodilatory responses to a variety of vasoactive agents. Impairment of endothelial function of cor-

onary arteries may lead to a propensity for cor-
onary artery spasm (Vanhoutte & Shimokawa,
1989). Coronary artery spasm is frequently iden-
tified as a cause or precipitating factor for various
cardiac diseases, such as acute myocardial infarc-
tion, variant angina, and unstable angina (Roberts
et al, 1982; Koiwaya et al, 1982; Epstein et al,
1988).

Coronary arterial occlusion and reperfusion has
been shown to cause functional or structural
damage to myocardial cells (Blumgart et al, 1941;
Braunwald & Kloner, 1982; Virmani et al, 1990)
and attenuates endothelium-dependent dilatation of
coronary arteries (Ku, 1982; Pearson et al, 1990;
Kim et al, 1992). Recent studies have demon-
strated that myocardial damage produced by
ischemia-reperfusion can be significantly reduced
by preconditioning the heart with an episode or
episodes of brief ischemia. Preconditioning has
been shown to delay the onset of necrosis (Murry
et al, 1986) and preserve levels of high-energy
phosphate intermediates compared with a con-
tinuous occlusion of similar duration (Hoffmeister
et al, 1986). Preconditioning reduces myocardial
infarct size (Cohen et al, 1991; Murry et al, 1986)
the number and severity of reperfusion arrhythmias
(Shiki & Hearse, 1987: Hagar et al, 1991).
However, it is not clearly determined yet whether
the protective effect of preconditioning ischemia
occurs in coronary endothelium.

The present study was designed to examine the
effect of ischemia-reperfusion on coronary endo-
thelium and to evaluate the effect of precon-
ditioning ischemia on endothelial cell injury after
ischemia-reperfusion.

MATERIAL AND METHODS

Animal preparation

Rabbits of either sex, weighing about 2.5 kg,

were anesthetized with intravenous pentobarbital
sodium (40 mg/kg), intubated, and ventilated with
room air via animal ventilator (Ugo Basile).
Ventilation rate was 35~40 breaths/min, tidal vol-
ume was 15 ml. The respiratory rate was adjusted
to keep the blood pH in the physiological range.
The chest was opened by median thoracostomy
and the pericardium was opened to expose the
heart. The first branch of left circumflex artery
(LCx) was isolated by an intramural stitch with 5-0
black silk (Fig. 1A). Coronary occlusion was
performed by passing a short length of poly-
ethylene tubing over the end of the suture and
clamping it against the heart. Coronary occlusion
was confirmed by darkening of the myocardium,
paradoxical wall motion of the ischemic myo-
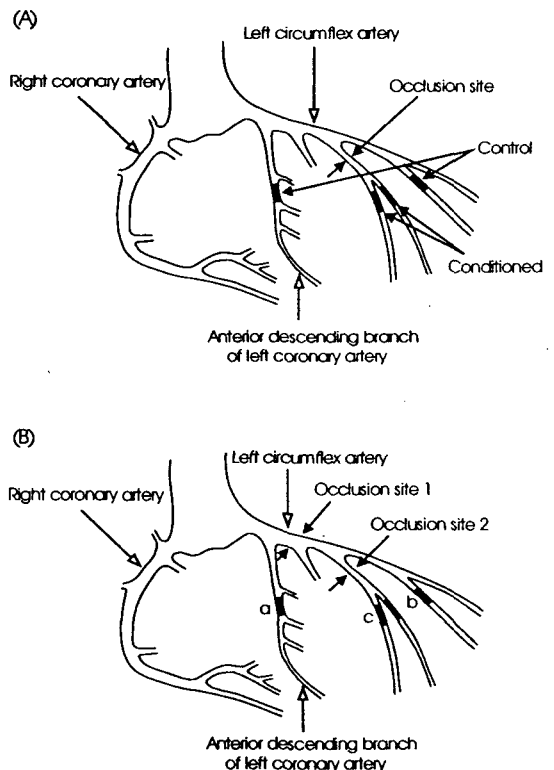cardium, and electrocardiographic evidence of

Fig. 1. Schematic presentation of coronary artery.

myocardial ischemia. Reperfusion was achieved by removing the clamp and confirmed by reactive hyperemia over the surface, electrocardiographic changes, and resumption of normal myocardial wall motion. Dysrhythmias that occurred were not treated. A ring of a branch of the left anterior descending coronary artery (LAD) or of nonoccluded branch of LCx was used as control.

In some experiments, the main trunk of LCx and first branch of LCx (or first and second branch of LCx) were isolated by intramural stitches with 5-0 black silk (Fig. 1B). At first, three occlusions of 2-minute duration, each followed by 5-minutes reperfusion (preconditioning ischemia) were done at the occlusion site 2, and then ischemia-reperfusion was performed by occlusion-reperfusion at the site 1. In the experimental condition, the branch below site 2 (c) was exposed to preconditioning ischemia followed by ischemia-reperfusion and a branch of LCx below site 1 except the branch below the site 2 (b) was exposed to ischemia-reperfusion. Rings of a branch of the nonoccluded LAD were used as control (a).
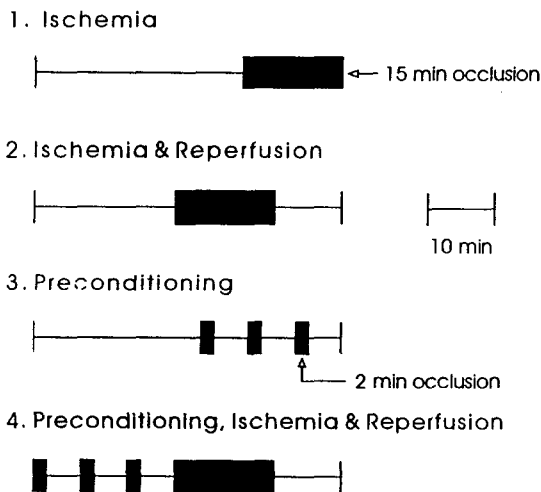

1. Ischemia

├──────────────████──◄── 15 min occlusion

2. Ischemia & Reperfusion

├───────████────┤   ├───┤

                          10 min

3. Preconditioning

├──────────█─█─█─┤
                ↑
                └── 2 min occlusion

4. Preconditioning, Ischemia & Reperfusion

█─█─█──████───┤

Fig. 2. Experimental protocol. The durations of experiments were equal in all cases.

In experimental protocol, depicted in Fig. 2, rabbits were randomized to receive one of the following experimental conditions: a period of preconditioning followed by 15-minute ischemia and 10 minutes of reperfusion or a control period of comparable length without any occlusion followed by ischemia only or preconditioning only or ischemia-reperfusion.

After exposure to an experimental condition according to the experimental protocol, the animal was exsanguinated, and the beating heart was quickly removed and immersed in cold, oxygenated modified Krebs Ringer bicarbonate solution. The investigation conforms with the Guide for the care and use of laboratory animals published by US National Institutes of Health (NIH publication No 85-23, revised 1985).

In vitro experiment

2 rings of the occluded LCx were taken at least 5 mm distal to the occlusion site. 2 Rings of the nonoccluded branches of LCx or of a branch of LAD were used as control. Preliminary experiments (data not shown) demonstrated no difference in endothelium-dependent responses between LCx and LAD.

Mechanical responses were recorded from the ring segments ($1.0 \sim 1.5$ mm). Each ring was suspended by two L-shaped stainless steel pins (diameter, 80 μ m): one pin was anchored in organ chamber (0.5 ml) and the other connected to a mechano-transducer (Grass, FT-03), which was connected to a three dimensional manipulator. The rings were mounted under optimal resting tensions ($0.2 \sim 0.5$g) and the muscle chamber was perfused with modified Krebs Ringer bicarbonate solution maintained at 36.5℃, at a constant flow rate of 4 ml/min using peristaltic pump. The optimal resting tensions were determined by comparing the tension developed by high K^+ solution under different resting tension. The tissues were equilibrated for

60 min at the optimal resting tension for maximal tension development in response to high-K^+ solution. Cumulative relaxation curve to acetylcholine was obtained in each ring. Endothelial cells were removed by gently rubbing with a stainless steel pin and successful removal of functional endothelial cells was assumed from the absence of any detectable relaxation by acetylcholine (from 10^{-8} M to 10^{-6} M) in preparations precontracted with high-K^+ solution. Cumulative contraction curve to acetylcholine and relaxation curve to sodium nitroprusside were obtained in denuded rings.

Solutions and drugs

The ionic composition of the Krebs Ringer bicarbonate solution was as follows (in mM): NaCl 118.3, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4 1.22, $CaCl_2$ 2.5, $NaHCO_3$ 25.0, CaEDTA 0.016, and glucose 11.1. The solution was aerated with 95% O_2-5% CO_2 (pH 7.3-7.4). High-K^+ solution (29.6 mM KCl) was prepared by replacing NaCl with KCl.

Drugs used were acetylcholine chloride and sodium nitroprusside (all from Sigma, U.S.A.).

Statistics

Experimental values were expressed as means $\pm$ SEM for n separate experiments. Statistical significances were determined using paired Student's t-test, and probabilities of less than 5 % (p<0.05) were considered significant.

RESULTS

The Effect of ischemia-reperfusion on endothelium-dependent relaxation

Figure 3A showes the effect of ischemia-reperfusion on endothelium-dependent relaxation to acetylcholine. After ischemia-reperfusion, endothelium-dependent relaxation to acetylcholine in the contracted rings with high-K^+ solution was sig-

nificantly attenuated. Figure 3B shows the effect of ischemia on endothelium-dependent relaxation to acetylcholine. There were no significant differences between the magnitudes of the relaxations in control and in the rings exposed to ischemia (p>0.05). After ischemia-reperfusion, contractile responses to acetylcholine were augmented, compared with those of control. However, when the endothelium was denuded by gentle rubbing with a cotton ball, there was no significant difference in the contractile responses to acetylcholine between the arterial rings of the two groups (Fig. 3C). Additionally, there was no significant difference in response to sodium nitroprusside between control and reperfused rings without endothelium (Fig. 3D).

The effect of preconditioning ischemia followed by ischemia-reperfusion on endothleium-dependent relaxation

In the group treated with preconditioning ischemia, not followed by ischemia-reperfusion, there was a significant difference in relaxation to acetylcholine at low concentration between control and preconditioned rings (Fig. 4A). The endothelium-dependent relaxation of the rings exposed to preconditioning ischemia followed by ischemia-reperfusion was significantly attenuated, compared with that of control (Fig. 4B).

Comparison the effect of ischemia-reperfusion on the endothelium-dependent relaxation and that of preconditioning ischemia followed by ischemia-reperfusion

Fig. 5 shows the effect of preconditioning on endothelial cell injury after ischemia-reperfusion. The endothelium-dependent relaxations were impaired in the rings exposed to preconditioning followed by ischemia-reperfusion or ischemia-reperfusion, compared with that of the control. However, the
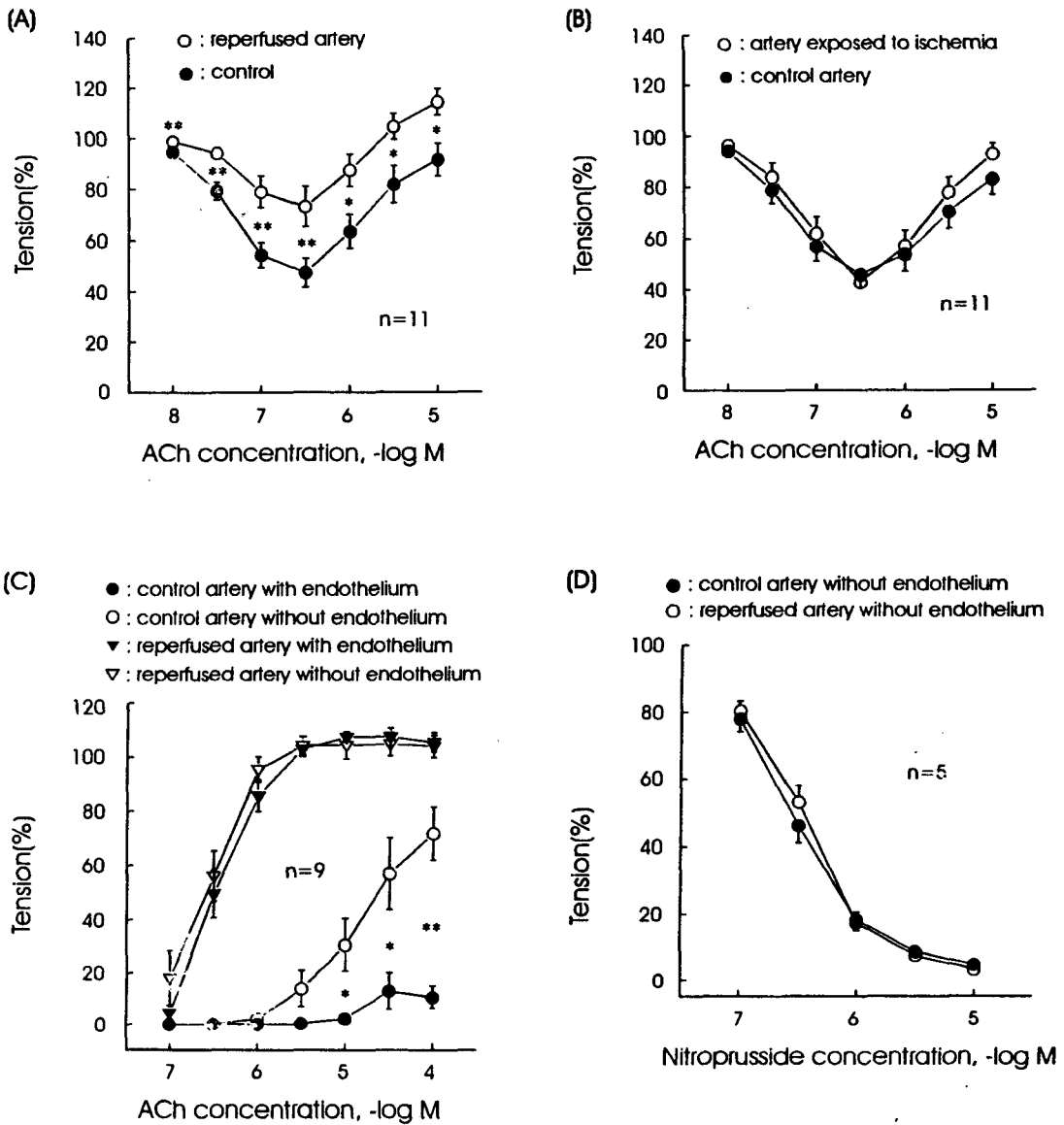
(A)

O : reperfused artery
● : control

Tension(%)

n=11

ACh concentration, -log M

(B)

O : artery exposed to ischemia
● : control artery

Tension(%)

n=11

ACh concentration, -log M

(C)

● : control artery with endothelium
O : control artery without endothelium
▼ : reperfused artery with endothelium
▽ : reperfused artery without endothelium

Tension(%)

n=9

ACh concentration, -log M

(D)

● : control artery without endothelium
O : reperfused artery without endothelium

Tension(%)

n=5

Nitroprusside concentration, -log M

*Fig. 3. Graphs showing effect of ischemia-reperfusion (A) or ischemia (B) on endothelium-dependent relaxation, concentration-response curves to acetylcholine in control and reperfused arterial rings with and without endothelium (C), and concentration-response curves to sodium nitroprusside in control and reperfused arterial rings without endothelium (D). Values are means ± SEM and are expressed as percent of the initial contraction in panel A, C, and D. In panel C, values are expressed as percent of contraction to 29.6 mM KCl. * p<0.05, ** p<0.01 between control and reperfused arterial rings. In pannel C, * and ** mean significance between control and reperfused arterial rings with endothelium.*

magnitude of the impairment was significantly greater in the rings exposed ischemia-reperfusion, compared with that of the impairment of rings

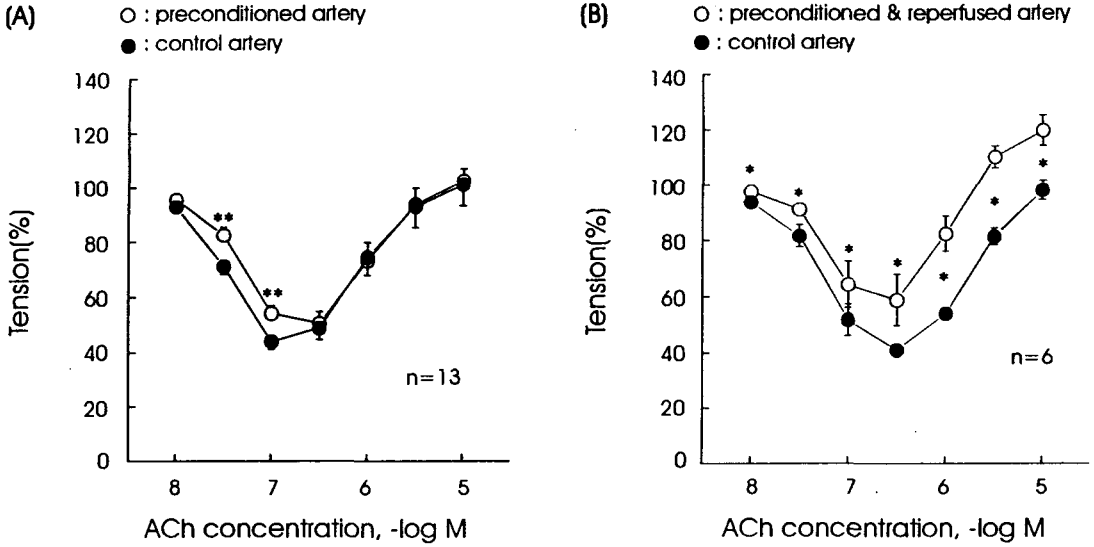exposed to preconditioning ischemia followed by ischemia-reperfusion.

(A)   ○ : preconditioned artery
       ● : control artery

(B)   ○ : preconditioned & reperfused artery
       ● : control artery

Fig. 4. Graphs showing effects of preconditioning, not followed by ischemia-reperfusion (A) and of preconditioning followed by ischemia-reperfusion on endothelium-dependent relaxation. Values are means ± SEM and are expressed as percent of the initial contraction. *p<0.05 and **p<0.01 between control and arterial rings exposed to preconditioning followed or not followed by ischemia-reperfusion.

▼ : reperfused artery
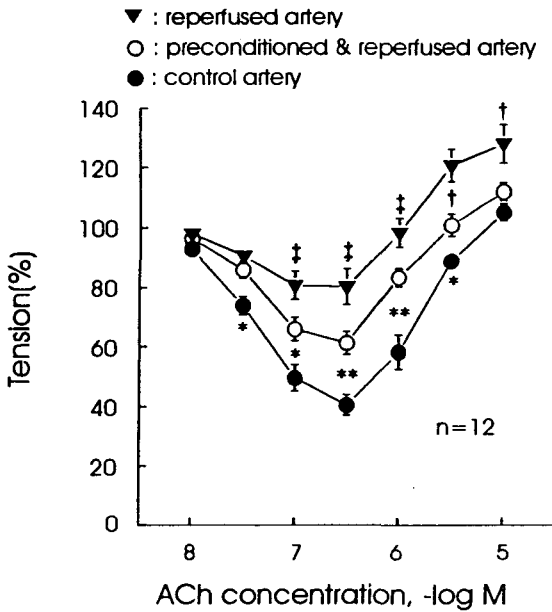○ : preconditioned & reperfused artery
● : control artery

Fig. 5. Comparison of the magnitude of impairment of endothelium-dependent relaxation by ischemia-reperfusion with that of impairment of endothelium-dependent relaxation after preconditioning followed by ischemia-reperfusion. Values are means ± SEM and are expressed as percent of the initial contraction. * p<0.05, ** p<0.01 between control and arterial rings exposed to preconditioning followed by ischemia-reperfusion and †P<0.05, †p<0.01 between rings exposed to ischemia-reperfusion and preconditioning followed by ischemia-reperfusion.

DISCUSSION

We have demonstrated that ischemia-reperfusion significantly attenuates endothelium-dependent relaxation to acetylcholine in rabbit coronary arteries. We have also shown that preconditioning preserves endothelium-dependent relaxation.

Endothelium-dependent relaxation of coronary arteries to acetylcholine was significantly attenuated after ischemia-reperfusion. However, endothelium-dependent relaxation of coronary arteries was not attenuated after ischemia only. Thus the results indicate that the impairment of endothelium-dependent relaxation could be produced not by ischemia but by reperfusion. In addition, coronary artery exposed to ischemia-reperfusion retained the capacity to dilate to sodium nitroprusside, an endothelium-independent vasodilator, and the contractile response to acetylcholine was not altered in the reperfused rings without endothelium, compared with control without endothelium. These data suggest that the reduced endothelium-dependent relaxation to acetylcholine after ischemia-reperfusion was not the result of a nonspecific loss of vasodilatory capacity or due to damage of vascular smooth muscle but rather a specific attenuation of endothelium-mediated relaxation. The impairment of endothelium-dependent relaxation could be attributed to the damage of endothelium.

Preconditioning only, not followed by ischemia-reperfusion attenuated endothelium-dependent relaxation at low concentrations of acetylcholine. As preconditioning is a repetition of short duration ischemia-reperfusion, it could be suggested that ischemia-reperfusion injury is also produced by preconditioning only. Preconditioning followed by ischemia-reperfusion significantly impaired endothelium-dependent relaxation. However, although

preconditioning only attenuated endothelium-dependent relaxation and total duration of ischemia-reperfusion is longer in preconditioning followed by ischemia-reperfusion than in ischemia-reperfusion, the magnitude of the impairment was significantly lesser than that of the impairment after ischemia-reperfusion. Therefore, it could be concluded that preconditioning has a protective effect on endothelial ischemia-reperfusion injury.

Preconditioning is also thought to protect myocardial cells from ischemia-reperfusion injury. Although the mechanism(s) by which preconditioning protects myocardial cells from ischemia-reperfusion injury is not completely understood, there is a good evidence that preconditioning has a protective effect on the cells through A1 receptor activation (Liu et al, 1991) and adenosine is an initiator and mediator of preconditioning (Thornton et al, 1993). However, there is no evidence that the protective effect of preconditioning on vascular endothelium is also a receptor-mediated one with adenosine, acting via the A1 receptor and therefore it seems that the mechanism by which preconditioning protects myocardial cells is not the same as the mechanism of the protective effect of preconditioning on vascular endothelium.

Superoxide anions are known to inactivate endothelium-derived nitric oxide (EDNO), with attenuation of endothelium-dependent relaxation after ischemia-reperfusion of coronary arteries and EDNO is known to inactivate superoxide anion (Rubanyi & Vanhoutte, 1986; Stewart et al, 1988; VanBenthuysen et al, 1987). Infusion of NO generating systems before reperfusion inhibited neutrophil accumulation and retarded myocardial necrosis (Johnson et al, 1989; Johnson et al, 1990), which suggested that exogenously administered EDRF or NO exerts significant cardioprotective effects on ischemia-reperfusion injury. There is an evidence that the release of vasoactive autacoids, including NO, from vascular endothelial cells is enhanced in

hypoxia and that the release of NO is further enhanced on return to normoxia (Brown et al, 1993; Pearson et al, 1993). In addition to NO production, endothelium provents neutrophils from accumulating into myocardial cells, which causes myocardial necrosis after ischemia-reperfusion. Neutrophil accumulation by endothelial dysfunction leads to tissue injury (Bulkley, 1989). These data suggest that endothelium can prevent ischemia-reperfusion injury by producing NO and ischemia-reperfusion injury could be reduced by preserving endothelium and endothelial function.

Preservation of endothelial cell function may be one of the mechanisms by which preconditioning reduces the ischemia-reperfusion injury to myocardial cells, since it may improve perfusion to the area of ischemic myocardium and protect vascular as well as myocardial cells from ischemia-reperfusion injury.

ACKNOWLEDGEMENTS

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