Journal of Korean Medical Science
Vol. 10, No. 2, 121-131 April 1995

Relation between Ischemic Preconditioning and the Duration of Sustained Ischemia

Dae-Joong Kim, M.D.,* Hyun Kim, M.S.,* Ji-In Park, M.D.,** Tae-Sub Shim, M.D.,**
Bong-Jin Rah, M.D.,* and Ho-Dirk Kim, M.D.*

Departments of Histology and Pediatrics***,
College of Medicine, Chung-Ang University, Seoul, Korea

It has been reported that repetitive brief periods of ischemia and reperfusion (ischemic preconditioning, IP) cause a significant reduction in the extent of myocardial necrosis or in the incidence of reperfusion arrhythmias in rat heart. However, recent reports have stated that IP effect is diminished or lost in the canine or bovine heart if ischemia (mostly regional) is sustained for 40 min or longer. The main objective of this study is to assess whether IP provides myocardial protection in prolonged sustained ischemia under the condition of global ischemia in isolated rabbit heart. The hearts were subjected to 10~60 min sustained ischemia (SI) followed by 60 min reperfusion with (IP heart) or without IP (ISCH heart). IP was induced by 4 cycles of 5 min global ischemia and 5 min reperfusion. Left ventricular function (LVF), extent of infarction (EI) and ultrastructural changes were examined. As a whole, the LVF began to recover on reperfusion but there was no significant difference in the funtional parameters. However, extracellular Ca^{2+} concentration was lower in the ISCH hearts ($p<0.05$) and the EI was significantly different between the hearts which had received 60 min SI (67% in the ISCH versus 32% in the IP heart, $p<0.01$). Ultrastructural changes were homogeneous in the ISCH hearts and became irreversible in accordance with increase of the duration of ischemia, while these changes were heterogeneous and restricted in the IP heart. These results suggest that IP does not attenuate the postischemic dysfunction in prolonged ischemia but it can provide an infarct size-limiting effect and delay ultrastructural changes. This cardioprotective effect may be related to calcium homeostasis.

Key Words : *Calcium, Infarct size, Ischemia and reperfusion, Ischemic preconditioning, Isolated rabbit heart, Left ventricular function, Ultrastructure*

INTRODUCTION

Although myocardial ischemia is a main cause of myocardial necrosis, one or more brief (2~5 min) periods of ischemia followed by reperfusion (ischemic preconditioning, IP) before a more prolonged period of regional ischemia have protective effect on a more prolonged period of ischemia. Murry and co-workers (1986) employed four 5 min periods of ischemia and reperfusion prior to 40 min coronary artery occlusion in their first studies on canine hearts, which resulted in

Address for correspondence : *Ho-Dirk Kim, M.D., Department of Histology, College of Medicine, Chung-Ang University, Seoul, 156-756, Korea. Tel : (02) 820-5644, Fax : (02) 815-4814.*

a significant reduction in the extent of myocardial necrosis. Since then, the cardioprotective effect of IP has been demonstrated in other laboratories and in other species including the pigs, rabbits and rats (Schott et al., 1990 ; Iwamoto et al., 1991 ; Cave and Hearse, 1992 ; Kim et al., 1992). The protective effect of IP is, however, diminished or lost if the reperfusion period between the initial short period of ischemia and the sustained prolonged ischemia is extended to 2 or 3 hours or if the duration of the prolonged period of ischemia is 40 min or greater (Miura et al., 1990 ; Murry et al., 1990, 1991 ; Van Winkle et al., 1991). Therefore, IP can be considered to have a time-limited or transient protective effect which slows the development of ischemic injury thus extending the duration of ischemia that can be tolerated before irreversible injury occurs (Cave and Hearse, 1992).

To date, most studies of IP have employed regional ischemia and have used either infarct size or the incidence of reperfusion-induced arrhythmias as end points for the assessment of protection. Few have investigated the ability of IP to enhance post-ischemic recovery of contractile function (Omar et al., 1991 ; Cave and Hearse, 1992 ; Kim et al., 1992). If IP can be induced under conditions of global ischemia and if it can enhance the rate or extent of post-ischemic recovery contractile function it might find a novel role in cardiac surgery. In other words, IP can protect against injury during the hypothermic ischemia as used in surgically-induced ischemic arrest.

The main objective of this study was to test whether IP can provide myocardial protection in global ischemia in isolated rabbit heart. Another objective was to assess whether IP's effect is related to Ca^{2+}.

MATERIALS AND METHODS

Materials

New Zealand White rabbits of either sex (1.5~2.0 kg bw ; n=53) were used in this study. They were kept in a constant condition and given balanced Purina animal chow and water *ad libitum*. Animal chow was restricted from 24 hours before sacrifice. All experiments were conducted in accordance with the guidelines for experimental and laboratory animal use from the American Physiological Society.

All chemicals except Ca^{2+} standard (Junsei Chemical, Japan) and Epon (Polyscience, USA) used in this study were purchased from Sigma Chemical (St Louis, USA).

Heart preparation

The animal was stunned by a blow to the neck 30 min after intraperitoneal injection of heparin (300 U/Kg), the heart rapidly excised from the thorax and attched to Langendorff apparatus (Size 5, Hugo Sachs Elektronik, Germnay) to be perfused with an oxygen-saturated modified Tyrode solution (containing in mM : NaCl 140.0, KCl 4.4, $CaCl_2$ 1.5, $MgCl_2$ 1.0, HEPES 3.0, and glucose 10.0 ; pH 7.4, 37℃) by non-recirculating Langendorff technique as previously described (Kim and Rah, 1988). Perfusion pressure and volume were maintained constantly 55~60 mmHg and 30~35 ml/min, respectively.

Experimental protocol

After stabilization of baseline hemodynamics, each experiment was performed as in the protocols (Fig. 1). In IP experiments, hearts were randomly selected and subjected to IP followed by sustained ischemia for 10 (IP+ISCH10, n=6), 20 (IP+ISCH20, n=5), 30 (IP+ISCH30, n=5) or 60 min (IP+ISCH60, n=7) and 60 min reperfusion. IP was induced by 4 cycles of 5 min global ischemia and 5 min reperfusion. Global ischemia was induced by interruption of retrograde aortic flow and reperfusion was done by release of the interruption of aortic flow. In control experiments, hearts were treated in the same way as in the IP experiments without IP(ISCH10, n=6 ; ISCH20, n=5; ISCH60, n=9). To determine the effect of IP itself on the heart, experiments were stopped after IP prior to sustained ischemina (IP-only, n=5).

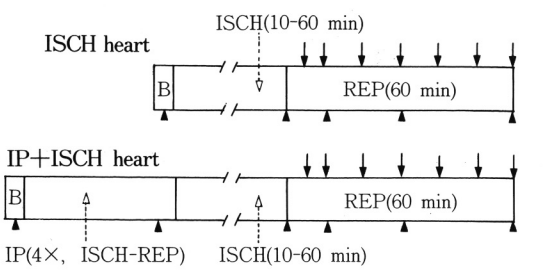

Fig. 1. Schematic illustration of the experimental protocol. Arrows indicate points of comparison of the left ventricular function between the control(ISCH heart) and the IP(IP+ISCH heart) hearts ; arrowheads indicate points of the extracellular fluid collection. B, baseline ; IP, ischemic preconditioning ; ISCH, global ischemia ; REP, reperfusion.

Measurement of the left ventricular function

After stabilization of baseline hemodynamics, a latex balloon-tipped cannula was inserted into the left ventricle via the left atrium and connected to a pressure transducer (Harvard Apparatus, Edenbridge, UK) to measure left ventricular developed pressure (LVDP, peak systolic pressure minus end-distolic pressure). The balloon was filled with saline using a screw-driven syringe until the left ventricular end-diastolic pressure (LVEDP) was 8~10 mmHg. Left ventricular systolic and diastolic pressures and surface electrocardiogram were continuously recorded on a 4-channel curvilinear polygraph (Harvard Apparatus, Edenbridge, UK). Left ventricular dP/dt (LV dP/dt) was also simultaneously recorded by electronic differentiation of the pressure pulse. All baseline measurements of each functional parameter from all experiments were collected and used to calculate the mean of baseline measurement.

Measurement of extracellular pH, Ca^{2+} concentration, and creatine kinase (CK) leakage

A small polyethylene catheter was inserted into the coronary sinus, connected to an air-tight sealed bottle, and extracellular fluid was timely collected for determination of pH and Ca^{2+} concentration. pH was measured with a pH electrode (Corning, USA) and Ca^{2+} was measured by inductively coupled plasma spectrometry (Jovin Yvon JY24, France; emission wave length, 393.366 nm; power, 1.0 kw; sample uptake rate, 1.2 ml/min; argon flow rate, sheath gas, 0.3 l/min, cooling gas, 12.0 l/min, and carrier gas, 0.3 l/min). Calcium standard solution was used for atomic absoprtion. CK leakage was determined by measurement of its activity in the timely collected coronary effluent with a dignostic kit.

Determination of the extent of infarction

At the end of each experiment, hearts were cut transversely 2~3 mm in thickness. The lower second and third slices which had been proved to show pronounced infarction were immersed in 1.5% triphenyltetrazolium chloride (in phosphate buffer) for 20 min at room temperature and overnight in 10% neutral buffered formalin to determine the extent of infarction (pale area). It has been proved that tetrazolium salt stains normal or viable cardiac myocytes (bright red) whereas infarcts are not stained (Klein et al., 1981). Heart slices were photographed on Ektachrome film (ISO 100), projected onto a screen and the outlines of the left ventricular cross sectional area (LVA) and infarct area (IA) were traced with a digitizer (Pias, Osaka, Japan). Extent of infarction was determined by image analysis system(Pias LA-555, Osaka, Japan) and expressed as % IA/LVA.

Electron microscopy

At the end of each experiment, a small piece of tissue was excised from the lateral free wall of the left ventricle and minced with a sharp clean razor blade. These tissue blocks were fixed in 3% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4, 4℃ for 6~8 hours and washed in veronal acetate buffer. After post-fixation in 1% osmium tetroxide, dehydration in graded ethanols and infiltration in propylene oxide, they were embedded in Epon 812. Thin sections were cut at 2 μm and stained with toluidine blue for light microscopy. Gold to silver sections were, then, cut and stained with aqueous uranyl acetate and lead citrate. Sections were examined with a transmission electron microscope (Philiops EM300, Eindhoven, The Natherlands or Jeol 200CX, Tokyo, Japan) at 60~80 kV.

Statistics

Data were expressed as mean±SEM. Differences between hearts were determined by use of two-way analysis of variance (ANOVA) and Turkey's multiple comparison and differences within the heart which received the same period of sustained ischemia were determined by t-test. A p value less than 0.05 was considered to be significant.

RESULTS

Left ventricular function

All hearts except for the ISCH60 heart recovered from sustained ischemia within 2 to 3 min on reperfusion. Two out of nine (22.2%) ISCH60 hearts did not recover by reperfusion. Functional data obtained during reperfusion from these two hearts were excluded for statistical analysis but the specimens were included for histological and electron microscopic examination.

The baseline LVDP, LV+dp/dt, LVEDP, and the heart rate (HIR) were 97.3±5.84 mmHg, 1649±161.1 mmHg/sec, 9.8±1.08 mmHg, and 111±6.5 bpm

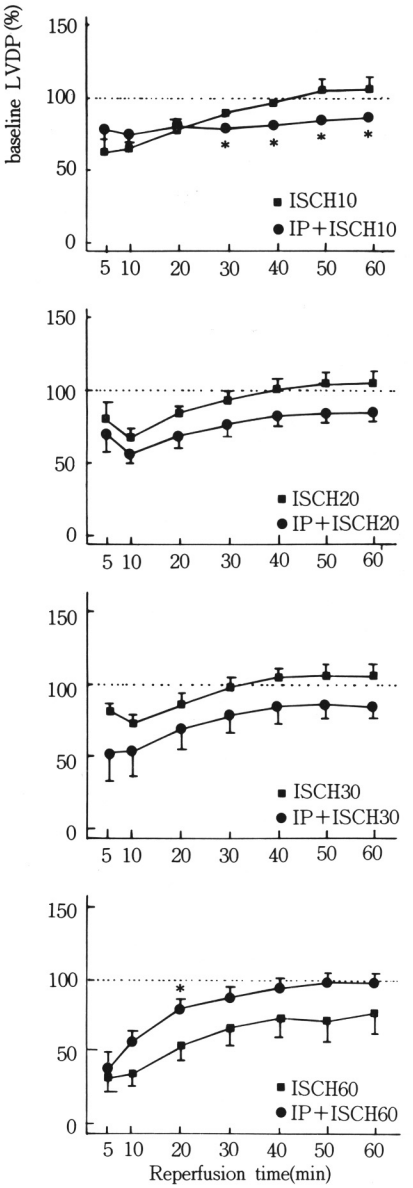

Fig. 2. Recovery rate of the left ventricular developed pressure(LVDP) during reperfusion. Data are expressed as mean ±SEM. Dotted lines indicate preischemic baseline. *p< 0.05, control(ISCH) vs IP(IP+ISCH) hearts.

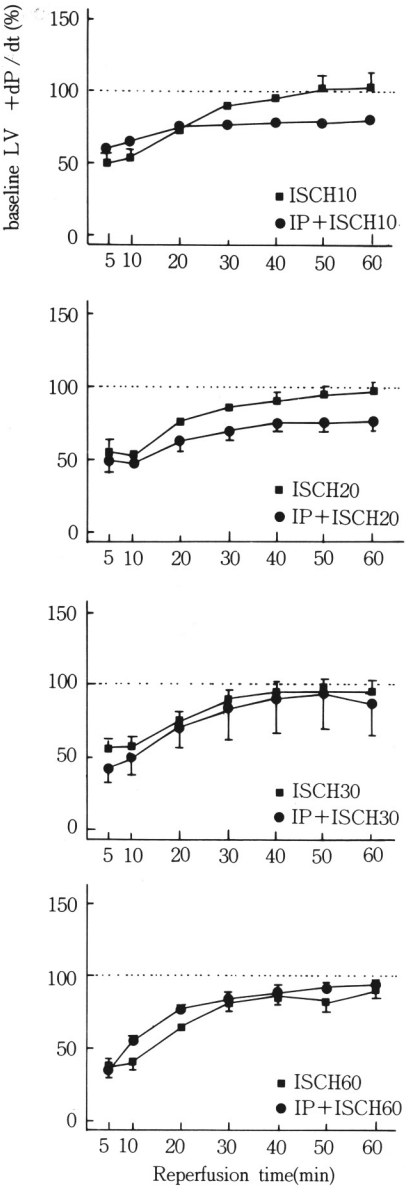

Fig. 3. Recovery rate of the left ventricular(LV) +dP/dt during reperfusion. Data are expressed as mean±SEM. Dotted lines indicate preischemic baseline.

(beats per minute), respectively. % baseline LVDP, % baseline LV+dp/dt, and HR were slightly higher on reperfusion in the ISCH heart than in the IP heart but there was no significant difference between hearts which received 10~30 min sustained ischemia (Fig. 2, 3, and 4). IP hearts which received 10 min sustanined ischemia showed significantly depressed % baseline LVDP (Fig. 2). This result indicate that IP can cause myocardial stunning. However, when the heart was subjected to 60 min sustained ischemia,

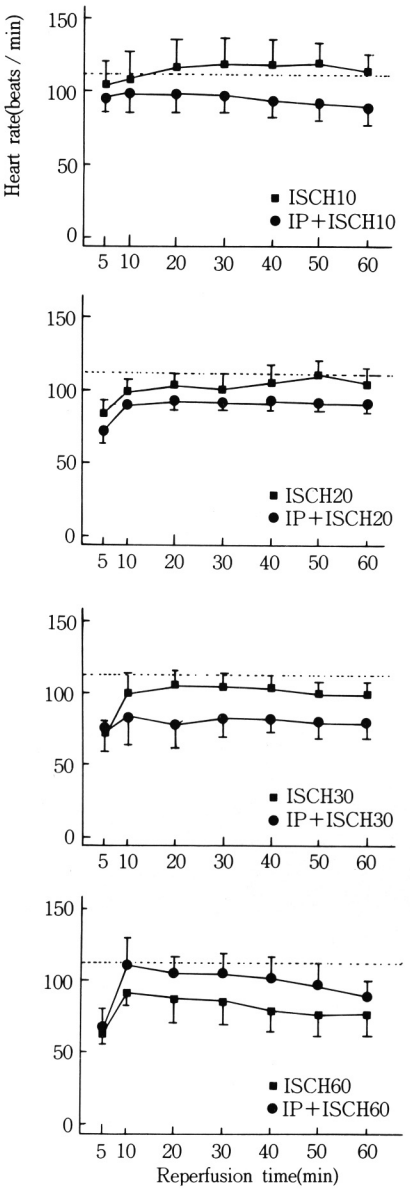

Fig. 4. Changes in the heart rate during reperfusion. Data are expressed as mean±SEM. Dotted lines indicate preischemic baseline.

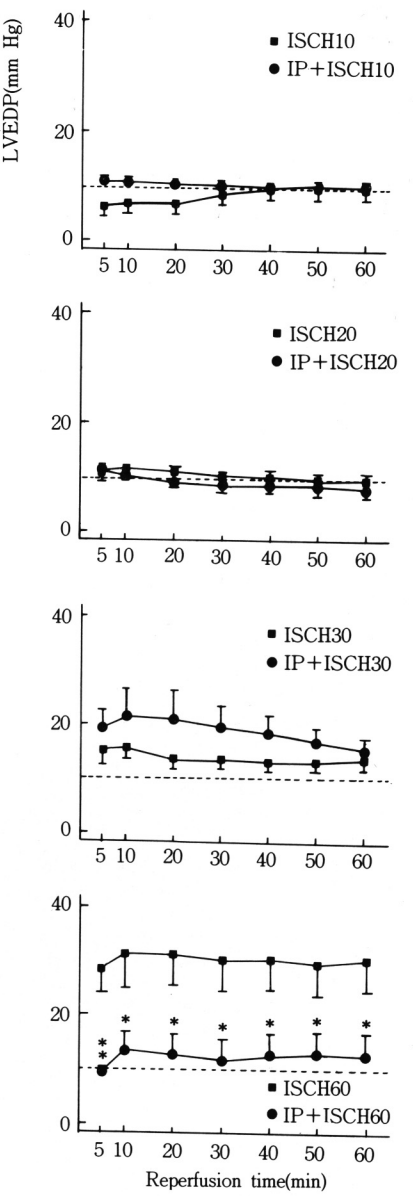

Fig. 5. Changes in the left ventricular end-diastolic pressure(LVEDP) during reperfusion. Data are expressed as mean ±SEM. Dotted lines indicate preischemic baseline. *$p<0.05$, **$p<0.01$, control(ISCH) vs IP(IP+ISCH) hearts.

the IP (IP+ISCH60) heart showed a tendency of higher % baseline LVDP and HR on reperfusion than in the ISCH (ISCH60) heart (Fig. 2 and 4). The LVEDP was not changed in 10 or 20 min sustained ischemia with or without IP. After 30 min sustained ischemia, LVEDP increased on reperfusion in the IP heart but there was no significant difference between the ISCH and the IP hearts. After 60 min sustained ischemia, LVEDP in the ISCH heart was significantly elevated on reperfusion but changed only slightly in the IP heart

(Fig. 5, $p<0.01$ at 5 min, $p<0.05$, after 5 min, on reperfusion).

Extracellular pH, Ca^{2+} concentration, and CK leakage

Baseline pH, Ca^{2+} concentration, and CK leakage was 7.24±0.01, 63.94±2.34 mg/l, and 1.03±0.13 U/l, respectively. After 10 or 20 min sustained ischemia, Ca^{2+} concentration significantly increased on reperfusion in the IP hearts ($p<0.05$), while Ca^{2+} concentration was not changed on reperfusion after 30 or 60 min sustained ischemia. However, it was significantly lower in the ISCH heart than in the IP heart (Fig. 6, $p<0.01$). pH abruptly dropped during sustained ischemia but it reached to the baseline on reperfusion (data are not shown). CK leakage was nearly at the baseline on reperfusion except the 60 min sustained ischemic hearts, however, there was no significant difference (deta are not shown). These results indicate that pH and CK leakage do not correlate with IP effect. Therefore, pH and CK leakage will not be discussed further in this study.

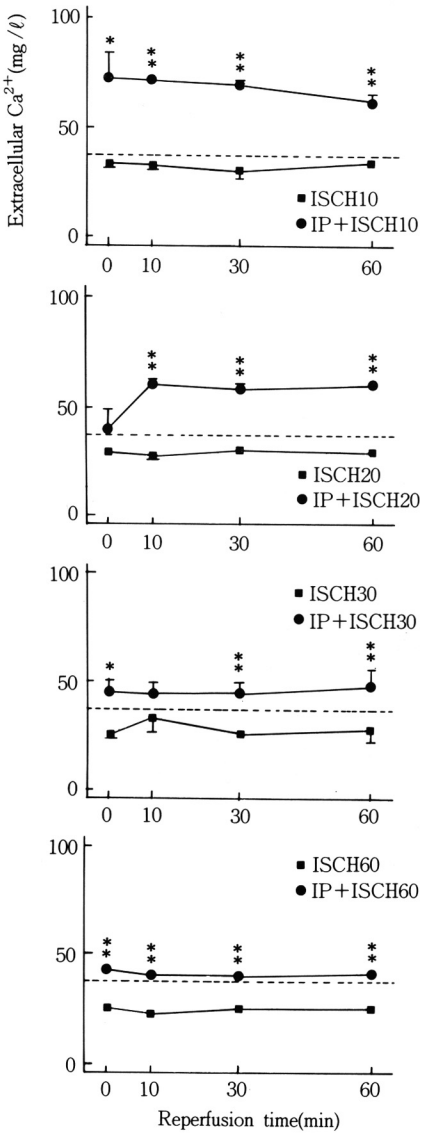

Fig. 6. Changes in the extracellular Ca^{2+} concentration during reperfusion. Data are expressed as mean±SEM. Dotted lines indicate preischemic baseline. *$p<0.05$, **$p<0.01$, control(ISCH) vs IP(IP+ISCH) hearts.

Extent of infarction (macroscopic findings)

Infarction was small and confined to the posterior or

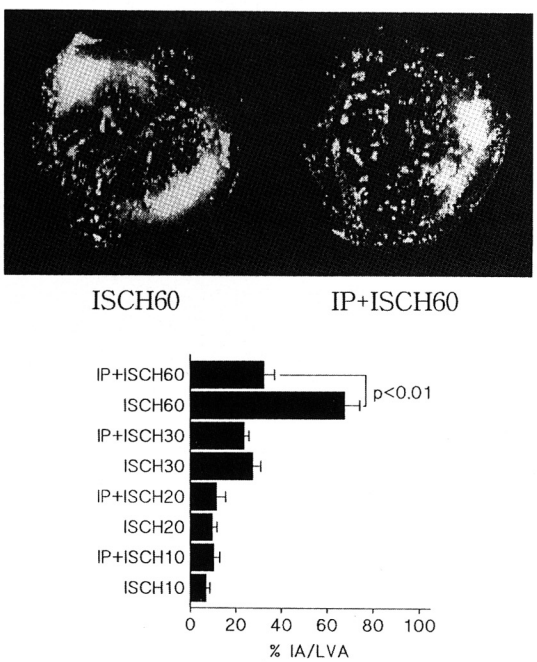

Fig. 7. *Upper*: The extent of infarction reconstructed by image analyzer. Widespread infarction(pale area) is found in the antero-lateral and posterior area of the left ventricular free wall in the control(ISCH60) while the IP(IP+ISCH60) heart shows infarction limited to the lateral area. *Lower*: Comparison of the extent of infarction(infarct area/left ventricular area, LA/LVA) between the controls(ISCH) and the IP(IP+ISCH) hearts.

antero-lateral free wall of the left ventricle. Extent of infarction (IA/LVA) increased nearly 2-fold after 30 min sustained ischemia with or without IP. However, there was no significant difference between the ISCH and the IP hearts. The infarction was more pronounced and extended to the posterior and the antero-lateral free wall of the left ventricle after 60 min sustained ischemia. IA/LVA was almost 67.3% in the ISCH60 heart. In contrast, IA/LVA was only 32.3% ($p < 0.01$) and the infarction was still confined to the antero-lateral free wall in the IP+ISCH60 heart (Fig. 7).

Ultrastructure of the cardiac myocyte

Cardiac myocytes showed slight but reversible ultrastructural changes after ischemic preconditioning (IP-only) (Fig. 8). After 10 or 20 min sustained ischemia, both ISCH and IP hearts showed heterogeneous ultrastructural changes of slight to moderate degree of reversible injury. After 30 min sustained ischemia, the ISCH heart showed homogeneous ultrastructural changes of severe to irreversible degrees (Fig. 9A). In contrast, the IP (IP+ISCH30) hearts showed moderate to severe degrees of ultrastructual changes which were heteregeneous and reversible (Fig. 9B). After 60 min sustained ischemia, the ISCH hearts showed more pronounced ultrastructural changes of irreversible injury (Fig. 10A). Sarcolemma was separated from its attachment at the Z lines. Sarcomeres were severely contracted (to result in contraction bands) and myofibrillar damage was

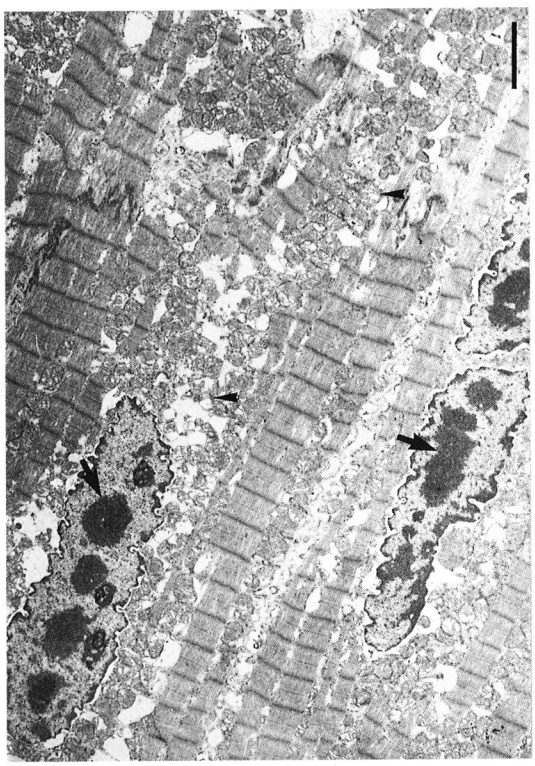

Fig. 8. Cardiac myocyte after IP. Ultrastructures are relatively intact. Nuclear chromatins are clumped(arrows) but not marginated ; in place, mitochondria are slightly swollen and show cleared matrices(arrowheads). Scale bar=2.4 μm.

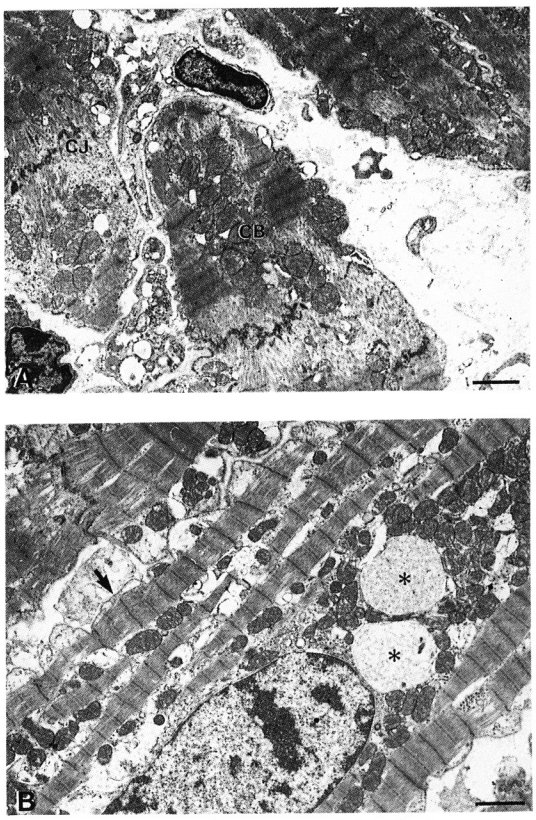

Fig. 9. (A) Cardiac myocyte after 30 min sustained ischemia and 60 min reperfusion without IP(ISCH30). Hypercontraction of sarcomeres results contracture bands(CB) and separation of cell junctions(CJ). Scale bar=1.6 μm. (B) Cardiac myocyte after 30 min sustained ischemia and 60 min reperfusion with IP(IP+ISCH30). There is some evidence of intracellular fluid accumulation(asterisks), however, mitochondria, nucleus and sarcomeres are relatively well preserved ; sarcolemma is scalloped but tightly apposed with the basal lamina(arrow). Scale bar=1.6 μm.

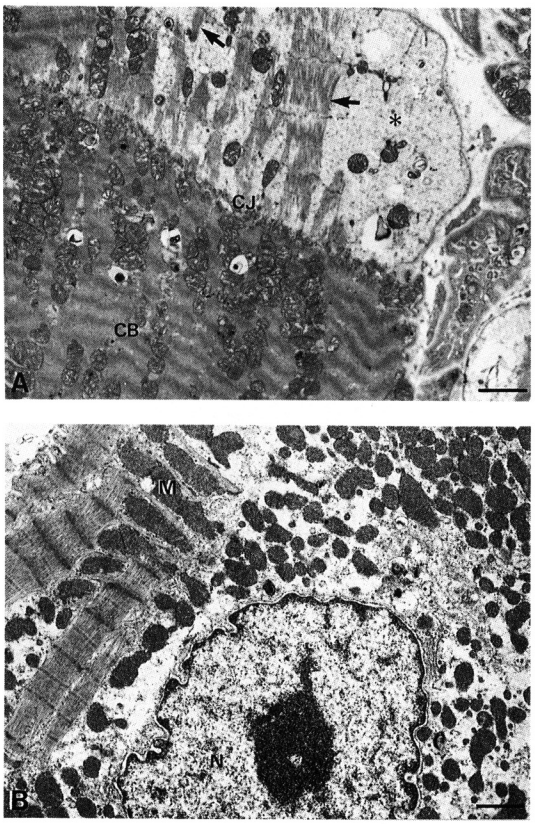

Fig. 10. (A) Cardiac myocyte after 60 min sustained ischemia and 60 min reperfusion without IP(ISCH60). Contracture band(CB), separation of cell junction(CJ), myofibrillar destruction(arrows) and intracellular fluid accumulation(asterisk) are evident. Scale bar=1.6 μm. (B) Cardiac myocyte after 60 min sustained ischemia and 60 min reperfusion with IP(IP+ISCH60). Sarcomeres are contracted but relatively intact, M, mitochondria ; N, nucleus. Scale bar=1.0 μm.

frequently found. Mitochondria were swollen and showed broken cristae. They contained amorphous matrix granules. Cell junctions were severely separated by hypercontraction of neighboring sarcomeres. In contrast, the IP (IP+ISCH60) hearts showed pronounced but heterogeneous ultrastructural changes. Numbers of cardiac myocytes exhibited irreversible ischemic injury but in places, the ultrastructure was relatively well preserved (Fig. 10B). They exhibited contraction bands but the ultrastructure of sarcomeres was relatively intact and myofibrillar damage was less pronounced compared to the ISCH60 hearts.

DISCUSSION

Effect of IP on the postischemic left ventricular function

In this study, the left ventricular function progressively recovered on reperfusion both in the ISCH and the IP hearts. Functional parameters including % baseline LVDP, % baseline +dp/dt, and HR in the ISCH hearts were slightly higher than in the IP hearts when the heart received 10~30 min of sustained ischemia but they tended to be reversed after 60 min of sustained ischemia. However, there were not any significant differences. These results mean that the effect of IP might be concealed by stunning because our protocol for IP, 4 cycles of 5 min ischemia and 5 min reperfusion (IP), may cause stunning and/or that the time window of sustained ischemia to see the effect of IP may be longer than 30 min. Our results are not consistent with those of Cave and Hearse (1992) who demonstrated that IP enhances coronary flow after longer periods of sustained ischemia (45~160 min) in isolated rat heart. They used one cycle of 5 min ischemia and 5 min reperfusion. However, it remains unclear whether a single brief period of ischemia can cause IP and reduce infarct size after a prolonged period of sustained ischemia. Because the threshold for IP is higher in rats than rabbits or dogs (Liu and Downey, 1992) and one cycle of 5 min ischemia and 5 min reperfusion is not sufficient to manifest the entire schema of myocardial stunning (Rah et al., 1994), one cycle of 5 min ischemia is not adequate to produce IP. Miyamae and co-workers (1993) stated that IP does not attenuate myocardial stunning from their study with pig hearts which had received IP followed by 15 min ischemia and 120 min reperfusion.

The LVEDP was elevated in 30 min of sustained ischemia both in the IP and the ISCH hearts but there was no significant difference between the two hearts as a whole. However, when the duration of sustained ischemia was 60 min, it was significantly elevated in the ISCH hearts ($p<0.05$). Cardiac muscle has a high resting tension at all lengths along its length-tension curve. The exact cause of the high diastolic stiffness of cardiac muscle is still not clearly understood, but some of the high resistance to stretch can be attributed to the extracellular matrix, notably collagen, that lies outside the myocardial cells (Weber, 1989). Cytoskeleton within the cells and cyclic interac-

tions between the thick and thin filaments initiated by cyclic release and uptake of calcium by the sarcoplasmic reticulum (Lakatta et al., 1985) also appear to contribute to the high diastolic stiffness of the myocardium. Therfore, LVEDP may be elevated due to ischemia in which extracellular matrix, cytoskeleton and ventricular geometry could be altered, and increase in ventricular wall thickness could occurr. These changes may be derived from calcium overload or hyperosmotic pressure from lactate accumulation within the cells. Contracture bands and subsarcolemmal bleb-like structures shown in this study support this suggestion.

Effect of IP on the infarct size and the ultrastructure of cardiac myocyte

In this study, myocardium sampled just after IP was indistinguishable from non-ischemic intact myocardium. This means IP does not cause myocardial injury as previously reported (Murry et al., 1990). The ultrastructural findings of this study are consistent with the previous reports (Murry et al., 1990 ; Kim et al., 1992), in that IP hearts developed ultrastructural injury more slowly than ISCH hearts. Infarction was small and the ultrastructural changes were reversible in the hearts which received 10 or 20 min sustained ischemia. However, in 30 min sustained ischemia, both IP and ISCH hearts exhibited larger infarction which was still confined to the posterior or antero-lateral free wall of the left ventricle. Although both IP and ISCH hearts began to exhibit irreversible ultrastructural changes including intramitochondrial flocculent densities or contracture bands of sarcomeres, the changes in ISCH hearts were homogeneous while those in IP hearts were heterogeneous. The ISCH hearts which received 60 min sustained ischemia showed widespred (extended) areas of infarction in the antero-lateral and posterior free wall and more pronounced ultrastructural changes. In contrast, in the IP hearts of 60 min sustained ischemia, the infarcted area was still confined to the aforementioned region and the ultrastructural changes were qualitatively the same as in the ISCH hearts. These findings suggest that IP can delay ischemic cell death.

Possible mechanisms of the cardioprotective effect of IP

It has been well established that ischemic myocardial injuries are closely related with depletion of high energy phosphate (Reimer et al., 1981) and increased intracellular Ca^{2+} (Jennings et al., 1985 ; Tani and Neely, 1988 ; Steenbergen et al., 1990). In case of severe ischemia we could find a lot of contracture band, elevation of LVEDP, and significant decrease in extracellular Ca^{2+}. We think that these changes were derived from derangement of intracellular Ca^{2+}. In isolated hearts perfused with physiological solution, a few structures (eg, endothelial cells, fibroblasts) in the extracellular space in which Ca^{2+} could be bound are available. Therfore, decreased extracellular Ca^{2+} may reflect calcium overload. Murphy and co-workers (1988) stated that increased intracellular free Ca^{2+} activates Ca^{2+}-dependent degenerative process to result in irreversible changes. From this point of view, cellular injury can be suppressed or delayed by reduction of intracellular Ca^{2+} (Kohmoto and Barry, 1989 ; Steenbergen et al., 1990) as seen in the IP hearts in this study. Therefore, the infarct-limiting effect of IP may be closely related to intracellular Ca^{2+} control, however, its precise mechanism is still unclear. Volovsek and co-workers (1992) stated that the rate of ATP depletion and intracellular lactate accumulation were lowered by IP. It could be suspected that slow ATP depletion lowers accumulation of lactates, a byproduct of glycolysis, in the cardiac myocytes. In fact, ischemic cells demand anaerobic glycolysis to maintain adequate ATP amount but ATP by anaerobic glycolysis is not sufficient for inhibition of ATP depletion. Therefore, the cardioprotective effect of IP may be derived rather from reduction of ATP demand in the ischemic cells than from ATP preservation (Swain et al., 1984). It is likely that the delay in cell injury could be resulted from the cardiac myocytes free from increased osmotic pressure by reduction of lactate accumulation within the cells, but the relation between glycogen depletion and the effect of IP is still not well understood.

Uraizee and co-workers (1987) and Murry and co-workers (1988) found that free radical scavengers such as SOD (superoxide dismutase) and catalase abolish the effect of IP and they proposed that oxygen radicals may play an important role in producing the effect of IP. In contrast, Iwamoto and co-workers (1991) found that free radical scavengers do not affect the extent of infarct size in rabbit hearts and they suggested that free radicals are not an important factor of IP. In addition, polymorphonuclear leukocyte (PMN) (Donnelly et al., 1989), stress protein (Mehta et al., 1988) and adenosine (Liu et al., 1991 ; Thornton et

al., 1992) have been proposed to explain the cardioprotective mechanism of IP. Donnelly and co-workers (1989) found that IP lowers the activity of myeloperoxidase whose resource is PMN. It has been reported that PMNs are closely related with the ischemic injury (Engler and Covell, 1987 ; De Servi et al., 1990 ; Kim et al., 1993) However, PMN may not be a key factor related to the effect of IP because there was no difference in numerical density of PMN between IP and non-IP pig hearts and PMN can be completely washed out in isolated perfused heart preparations as in this study.

It has been suspected that increased expression of stress protein gene by IP can cause amplification of the preventive mechanism against ischemia, thus, IP delays ischemic injury. However, the precise role of stress protein on IP is still unclear. Recently, Liu and co-workers (1991) and Thornton and co-workers (1992) reported that A1 adenosine receptors mediate the mechanism of IP in rabbit hearts. They could block the cardioprotective effect of IP in *in vivo* models by administration of adenosine receptor antagonists and mimic IP in isolated hearts by injection of either adenosine or an A1-selective adenosine receptor agonist. In contrast, Li and co-workers (1993) have found that adenosine agonist theophylline failed to abolish the cardioprotective effects of IP and intravenous adenosine was unable to protect the hearts and they came to the conclusion that the mechanism of IP is not mediated by adenosine receptors in the rat model.

In conclusion, our isolated rabbit heart model indicates that IP cannot attenuate postischemic dysfunction (stunning) in prolonged ischemia, but it has an infarct-limiting effect and this cardioprotective effect may be related in part to calcium homeostasis.

ACKNOWLEDGEMENT

This work was supported by grants (for basic medical science research) from the Ministry of Education, Republic of Korea, 1993 and 1994. Part of this work was presented at the XIIth World Congress of Cardiology and the XVIth Congress of the European Society of Cardiology, Berlin, Germany, September 10-14, 1994. The authors are grateful to Mr Eung-Sirk Lee, student of Chung-Ang University College of Medicine, for his secretarial support and artwork.

REFERENCES

Cave AC, Hearse DJ. *Ischemic preconditioning and contractile function : studies with normothermic and hypothermic global ischemia J Mol Cell Cardiol 1992 ; 24 : 1113-23.*

De Servi S, Mazzone A, Ricevuti G, Fioravanti A, Bramucci E, Angoli L, Stefano G, Specchia G. *Granulocyte activation after coronary angioplasty in humans. Circulation 1990 ; 82 : 140-6.*

Donnelly TJ, Sievers RE, Bunting S, Bullens SL, Parmley WW, Wolfe CL. *Myocardial protection with preconditioning during ischemia and reperfusion : assessment by ^{31}phosphorus magnetic resonance (abstract). Circ Res 1989 ; 37 : 255A.*

Engler RL, Covell JW. *Granulocytes cause reperfusion ventricular dysfunction after 15-minute ischemia in dog. Circ Res 1987 ; 61 : 20-8.*

Geft IL, Fishbein MC, Ninomiya K, Hashida J, Chaux E, Yano J, Y-Rit J, Genov T, Shell W, Ganz W. *Intermittent brief periods of ischemia have a cumulative effect and may cause myocardial necrosis. Circulation 1982 ; 66 : 1150-3.*

Hagar JM, Hale SL, Kloner RA. *Effect of preconditioning ischemia on reperfusion arrhythmias after coronary artery occlusion and reperfusion in the rat. Circulation 1991 ; 68 : 61-8.*

Iwamoto T, Miura T, Adachi T, Noto T, Ogawa, T, Tsuchida A, Iimura O. *Myocardial infarct size-limiting effect of ischemic preconditioning was not attenuated by oxygen free-radical scavengers in the rabbit. Circulation 1991 ; 83 : 1015-22.*

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

Kim HD, Rah BJ. *Effects of diltiazem on isoproterenol- or Ca-induced ventricular myocardial injuries in isolated perfused rabbit heart : an electron microscopic study. Anat Rec 1988 ; 222 : 260-71.*

Kim HD, Jung HL, Oh SH, Rah BJ. *Intravascular plugging in clinical experimental coronary artery occlusion and recanalization. Korean J Electr Microsc 1993 ; 23 : 139-63.*

Kim HD, Kim DJ, Lee JK, Shim TS, Rah BJ. *Effect of preconditioning on postischemic reperfused rabbit heart. Korean Circ J 1992 : 22 : 418-30.*

Klein HH, Puschmann S, Schaper J, Schaper W. *The mechanism of the tetrazolium reaction in identifying experimental myocardial infarction. Virchows Arch [Pathol Anat] 1981 ; 393 : 287-97.*

Kohmoto O, Barry WH. *Mechanism of protective effects of Ca^{2+} channel blockers on energy deprivation contracture in cultured ventricular myocytes. J Pharmacol Exp Ther 1989 ; 248 : 871-8.*

Lakatta EG, Capogrossi MC, Kort AA, Stern MD. *Spon-*

taneous myocardial Ca oscillation : an overview with emphasis on rynodine and caffeine. Fed Proc 1985 ; 44 : 2977-83.

Li Y, Kloner RA. *The cardioprotective effects of ischemic 'preconditioning' are not mediated by adenosine receptors in rat hearts. Circulation 1993 ; 87 : 1642-8.*

Liu Y, Downey JM. *Ischemic preconditioning protects against infarction in rat heart. Am J Physiol 1992 ; 263 : H1107-12.*

Liu GS, Stanley AWH, Downey JM. *Neutrophil free perfusion eliminates neutrophils but does not alter infarct size (abstract). Circulation 1991 (Suppl II) : II-281.*

Mehta HB, Popovich BK, Dillmann WH. *Ischemia induces changes in the level of mRNAs coding for stress protein 71 and creatine kinase M. Circ Res 1988; 63 : 512-7.*

Miura T, Ogawa T, Iwamoto T, Tsuchida A, Iimura O. *Infarct size limiting effect of preconditioning : its duration and "dose-response" relationship (abstract). Circulation 1990 82 (Suppl III) : III-271.*

Miyamae M, Fujiwara H, Kida M, Yokota R, Tanaka M, Katsuragawa M, Hasegawa K, Ohura M, Koga K, Yabuuchi Y, Sasayama S. *Preconditioning improves energy metabolism during reperfusion but does not attenuate myocardial stunning in porcine hearts. Circulation 1993 ; 88 : 223-34.*

Murphy E, Steenbergen C, LeFurgey A, Jacob R, Lobaugh LA, Lieberman M. *Cytosolic free calcium and myocardial cell injury. In : Lesmasters JJ, Hackenbrock CR, Thurman RG, Westrhoff HV, eds. Integration of Mitochondrial Function. New York, Plenum, 1988 ; 413-20.*

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

Murry CE, Richard VJ, Jennings RB, Reimer KA. *Myocardial protection is lost before contractile function recovers from ischemic preconditioning. Am J Physiol 1991 ; 260 : H796-804.*

Murry CE, Richard VJ, Jennings RB, Reimer KA. *Preconditioning with ischemia : is the protective effect mediated by free radical-induced myocardial stunning? (abstract). Circulation 1988 ; 78 (Suppl II) : II-77.*

Murry CE, Richard VJ, Reimer KA, Jennings RB. *Ischemic preconditioning slows energy metabolism and delays ultrastructural damage during a sustained ischemic episode. Circ Res 1990 ; 66 : 913-31.*

Omar B, Hanson A, Bose S, McCord J. *Reperfusion with pyruvate eliminates ischemic preconditioning : an apparent role for enhanced glycolysis (abstract). FASEB J 1991 ; 5 : A1257.*

Rah BJ, Kim HD, Park YW. *Effect of pretreatment with diltiazem on left ventricular function and intracellular calcium distribution in postischemic reperfused guinea-pig hearts. Coronary Artery Dis 1994 ; 5 : 415-23.*

Reimer KA, Jennings RB, Hill ML. *Total ischemia in dog hearts in vitro : II. High energy phosphate depletion and associated defects in energy metabolism, cell volume regulation, and sarcolemmal integrity. Circ Res 1981 ; 49 : 901-11.*

Schott RJ, Rhomann ER, Braun ER, Schaper W. *Ischemic preconditioning reduces infarct size in swine myocardium. Circ Res 1990 ; 66 : 1133-42.*

Steenbergen C, Murphy E, Watts JA, London RE. *Correlation between cytosolic free calcium, contracture, ATP, and irreversible ischemic injury in perfused rat heart. Circ Res 1990 ; 66 : 135-46.*

Swain JL, Sabina RL, Hines JJ, Greenfield Jr JC, Holmes EW. *Repetitive episodes of brief ischemia (12 min) do not produce a cumulative depletion of high energy phosphate compounds. Cardiovasc Res 1984 ; 18 : 264-9.*

Tani M, Neely JR. *Hearts from diabetic rats are more resistant to in vitro ischemia : possible role of altered* Ca^{2+} *metabolism. Circ Res 1988 ; 62 : 931-40.*

Thornton JD, Liu GS, Olsson RA, Downey JM. *Intravenous pretreatment with A1-selective adenosine analogues protects the heart against infarction. Circulation 1992 ; 85 : 659-65.*

Uraizee A, Reimer KA, Murry CE, Jennings RB. *Failure of superoxide dismutase to limit infarct size of myocardial infarction after 40 minutes of ischemia and 4 days of reperfusion in dogs. Circulation 1987 ; 75 : 1237-48.*

Van Winkle DM, Thornton J, Downey DM, Downey JM. *The natural history of preconditioning : cardioprotection depends on duration of transient ischemia and time to subsequent ischemia. Coronary Artery Dis 1991 ; 2 : 613-9.*

Volovsek A, Subramanian R, Reboussin D. *Effects of duration of ischemia during preconditioning on mechanical function, enzyme release and energy production in the isolated working rat heart. J Mol Cell Cardiol 1992 ; 24 : 1011-9.*

Weber KT. *Cardiac interstitium in health and disease : the fibrillar collagen network. J Am Coll Cardiol 1989 ; 13 : 1637-52.*