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主論文

SAKAGOSHI -1-

Alterations in the beta-adrenergic receptor system  
after hypothermic ischemia in hearts with  
preischemic beta-receptor desensitization

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- 4) reperfusion
- 5) desensitization

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## ABSTRACT

Although hypothermic cardioplegic arrest is a basic method of myocardial protection in cardiac surgery, the beta-adrenergic receptor (BAR) system has been little investigated in the heart subjected to hypothermic ischemia. Additionally, although the hypothermic arrest is often induced in hearts with preischemic desensitization of the BAR system by preceding congestive heart failure, the functional state of the BAR system after ischemia has not been studied in these hearts. We investigated alterations in the BAR system after hypothermic ischemia in normal rat hearts and in those with preischemic desensitization of the BAR system, produced with isoproterenol (ISP: 400  $\mu\text{g}/\text{kg}/\text{hr}$  for 24 hrs). Both normal and BAR-desensitized hearts were isolated, and subjected either to 40 min of hypothermic ( $10^{\circ}\text{C}$ ) global ischemia followed by 40 min of reperfusion or subjected to time-matched aerobic perfusion with modified Krebs-Henseleit solution. At the end of perfusion, 1) BAR binding properties with [ $^3\text{H}$ ]CGP-12177 and adenylate cyclase activity were measured in crude membrane fraction, and 2) the inotropic response to ISP ( $\Delta \text{LV}+\text{dP}/\text{dt}_{\text{max}}$ ) was evaluated in an isovolumetric contracting heart preparation. Following reperfusion, normal hearts without desensitized BAR, showed a higher  $B_{\text{max}}$  value than those of nonischemic time-matched hearts ( $41.8 \pm 3.1$  vs  $35.4 \pm 2.4$  fmol/mg protein,  $p < 0.05$ ), whereas the  $K_d$  value was in a similar range in the two groups. Basal adenylate cyclase activity and activities

after stimulation by 10 mM sodium fluoride (NaF) and 100  $\mu$ M forskolin were in a comparable degree between reperfused and nonischemic time-matched hearts, while the maximal ISP-stimulated adenylate cyclase activity obtained by 10-100  $\mu$ M of ISP was higher in the former (51.2 $\pm$ 3.1 vs 40.3 $\pm$ 4.7 pmol cAMP/min/mg protein,  $p < 0.05$ ). The maximal inotropic response to ISP (maximal  $\Delta$  LV+dP/dt<sub>max</sub>) was higher in reperfused hearts than nonischemic time-matched hearts (1650 $\pm$ 59 vs 1490 $\pm$ 83 mmHg/sec,  $p < 0.05$ ). On the other hand, in BAR-desensitized hearts where BAR density, maximal ISP-stimulated adenylate cyclase activity and maximal inotropic response to ISP were all depressed, B<sub>max</sub> value was not different between the reperfused and nonischemic time-matched hearts (23.6 $\pm$ 2.9 vs 23.9 $\pm$ 2.8 fmol/mg protein), as was the K<sub>d</sub> value. Adenylate cyclase activities under basal and stimulated conditions were in a similar range between the two groups. The ISP-stimulated maximal enzyme activity was also similar between these two groups (31.2 $\pm$ 5.3 vs 30.2 $\pm$ 4.1 pmol cAMP/min/mg protein), while maximal  $\Delta$  LV+dP/dt<sub>max</sub> was smaller in reperfused hearts than nonischemic hearts (726 $\pm$ 123 vs 1092 $\pm$ 105 mmHg/sec,  $p < 0.05$ ).

This study demonstrated that myocardial BAR density and BAR-mediated responses increased at reperfusion following hypothermic global ischemia in normal hearts, whereas these phenomena were not observed in preischemic BAR-desensitized hearts.

### INTRODUCTION

There have been various studies about alterations in myocardial beta-adrenergic receptor (BAR) system after myocardial ischemia in normal hearts, and it has been demonstrated that BAR density and BAR-mediated physiological responses increased after ischemia in this setting [12, 13, 15, 16]. This functional enhancement of BAR system has been suggested to relate to postischemic cell injury and ventricular arrhythmia [17, 19, 20] or to compensate for postischemic contractile dysfunction (8).

In the clinical setting, myocardial ischemia often occurs in hearts with preischemic changes in BAR function. This has been demonstrated in acute myocardial infarction in hearts with enhanced BAR function due to preceding propranolol treatment [7, 21]. This issue has also been seen in cardiac surgery, where hypothermic ischemia is used as a routine procedure, and elective hypothermic ischemia has been often induced in BAR desensitized hearts by preceding congestive heart failure [1, 2].

Pretreatment with a beta-blocker and/or pre-existence of congestive heart failure may change the profile of the alterations of BAR system after myocardial ischemia, and Ohyanagi et al. [19] recently reported that the increase in BAR density was not observed after 60 min of normothermic ischemia in propranolol pretreated dog hearts. However, alterations in the BAR system have not been studied after ischemia either in hearts subjected to hypothermic ischemia or in hearts with preischemic

desensitization of BAR system.

Considering these situations, we evaluated alterations in myocardial BAR and BAR-mediated physiological responses in terms of adenylate cyclase activity and positive inotropic response after hypothermic ischemia using isolated rat hearts with or without preischemic desensitization of BAR system.

#### MATERIALS AND METHODS

##### Animals

Forty male Sprague-Dawley rats weighing 250-350 g were used in this study. Preischemic desensitization of the myocardial BAR system was induced in 20 of the animals with pretreatment with l-isoproterenol (ISP) according to the method of Chang et al. [3]. In brief, rats were anesthetized with ether and a polyethylene catheter was inserted into the right jugular vein. The free end of the catheter was brought subcutaneously to the back of the neck and led out of body. The catheter was protected by a flexible coiled steel spring and connected to the syringe-pump (model STC-521, Terumo, Japan) via the swivel-carriage apparatus. The syringe-pump was filled with ISP-HCl dissolved in acidified isotonic saline (0.001N HCl) containing 2 U/L of heparin sodium. The infusion rate of fluid from the syringe-pump was 0.4 ml/hr, delivering 400 µg/kg/hr of ISP for 24 hr (ISP-pretreated rats).

The remaining animals were given a similar infusion of acidified isotonic saline alone (non-treated rats).

### Isolated Heart Perfusion

#### Procedure

Animals were anesthetized with sodium pentobarbital (60 mg/kg i.p.) and heparinized. Within 10 min after 24 hr of ISP or saline infusion, hearts were rapidly excised and rinsed in ice-cold saline solution until contractions ceased and then subjected to non-recirculating Langendorff perfusion with modified Krebs-Henseleit solution containing, in mM: NaCl 119.0, NaHCO<sub>3</sub> 25, KCl 4.6, KH<sub>2</sub>PO<sub>4</sub> 1.2, MgSO<sub>4</sub> 1.2, CaCl<sub>2</sub> 1.25 and glucose 10.0 equilibrated with 95 % O<sub>2</sub>-5 % CO<sub>2</sub> (pH 7.4) (PO<sub>2</sub>>600mmHg) at 35°C. A polyester filter (0.8 um) was inserted into the perfusion circuit proximal to the heart to remove microparticulates in the buffer. After 5 min of stabilization, the coronary flow rate, controlled by a peristaltic pump, was adjusted such that the perfusion pressure equaled 60 mmHg. Coronary flow rate was similar between hearts from non-treated rats (14.0±1.3 ml/min, mean±SD) and those from ISP-pretreated rats (14.2±1.5 ml/min). Once adjusted, the coronary flow rate was kept constant throughout the experiment (except during hypothermic global ischemia; see below).

### Hypothermic global ischemia and reperfusion

After 10 min of Langendorff perfusion, some hearts were subjected to hypothermic global ischemia and reperfusion (reperfused hearts). Hypothermic global ischemia was induced as follows: the aortic root perfusion was interrupted and high potassium cold solution (cardioplegic solution: Na<sup>+</sup> 8mEq/L, K<sup>+</sup> 18mEq/L, glucose 73 g/L, insulin 12 U/L, 380 mOsm/L, pH 7.8, 4°C) was injected 10 mL/Kg through aortic root to induce rapid diastolic cardiac arrest. Myocardial temperature during arrest was maintained at 10°C. After 40 min of cardioplegic arrest, hearts were reperfused for 40 min at preischemic flow rates with KH solution at 35°C.

The remaining hearts were perfused with KH solution for 90 min instead of 40 min of reperfusion following 40 min of hypothermic global ischemia (nonischemic hearts).

### Experimental allocations

Forty isolated hearts were allocated into four groups;

I-A: nonischemic hearts from non-treated rats (n=10);

I-B: reperfused hearts from non-treated rats (n=10);

II-A: nonischemic hearts from ISP-pretreated rats (n=10);

II-B: reperfused hearts from ISP-pretreated rats (n=10).

In each group, half of the hearts were randomly selected for biochemical assay of BAR-adenylate cyclase system with myocardial

crude membrane fraction and the remaining half were used for measurement of myocardial inotropic response to beta-adrenergic agonist with perfused whole heart.

#### Assay of myocardial BAR system

After 40 min of reperfusion in reperfused hearts (I-B, n=5; II-B, n=5) or after 90 min of aerobic perfusion in nonischemic hearts (I-A, n=5; II-A, n=5), the ventricle of each heart was dissected, frozen in liquid nitrogen and stored at  $-70^{\circ}\text{C}$ .

#### Preparation of myocardial membrane

The ventricles were weighed, minced and placed in 10 volume of ice-cold homogenization buffer (0.25M sucrose containing 5 mM Tris-HCl and 1 mM  $\text{MgCl}_2$ , pH 7.4). They were homogenized with a Polytron (Nihon seiki) three times for 15 sec at setting power 6. The homogenate was centrifuged at 800 g for 10 min. The supernatant was filtered through a single layer of cheese-cloth and centrifuged at 30000 g for 30 min. The pellet was suspended in 5 ml of Tris-HCl buffer (50 mM Tris-HCl, 10 mM  $\text{MgCl}_2$ , pH 7.4) and recentrifuged at 30000 g for 30 min. The resultant pellets were resuspended in the Tris-HCl buffer for adenylate cyclase assay and BAR binding study with [ $^3\text{H}$ ]CGP-12177. All procedures were carried out at  $0-4^{\circ}\text{C}$ .

### Radioligand binding assay

The assay buffer was 50 mM Tris-HCl and 10 mM MgCl<sub>2</sub>, pH 7.4. For measurement of BAR density, duplicate tubes containing six increasing concentrations of [<sup>3</sup>H]CGP-12177 from 0.3 nM to 5.0 nM with or without 1 μM l-alprenolol in a total volume of 1 ml were prepared. The assay was begun with the addition of 50 μl of membrane preparation containing about 200 μg membrane protein, and incubated for 10 min at 37°C. These conditions allowed complete equilibration of the receptor with the radioligand. The reaction was terminated by rapid vacuum filtration through Whatman GF/F filters. The filters were washed immediately three times with 4 ml ice-cold assay buffer. Filters were dried at 90°C and placed in 10 ml of Triton/toluene based scintillation cocktail (ACS-II, Amersham), and radioactivity was determined in a liquid scintillation spectrophotometry. The equilibrium dissociation constant (K<sub>d</sub>) and the maximal number of binding sites (B<sub>max</sub>) were determined from Scatchard analysis. Protein concentration was determined by the method of Lowry using bovine serum albumin as the protein standard [11].

### Adenylate cyclase assay

Adenylate cyclase assay was performed in a 100 μl assay with 50-80 μg of membrane. The incubations were carried out at 37°C for 7 min. The final concentrations were 50 mM Tris-HCl, pH 7.4,

10 mM MgCl<sub>2</sub>, 0.1 mM EGTA, 100 μM [α-<sup>32</sup>P]ATP (0.2 to 0.4 μCi per tube), 1 mM adenosine 3', 5'-cyclic monophosphate (cAMP), 1 mM 3-isobutyl-1-methylxanthine (IBMX), 1 μM GTP, 0.4 mg/ml creatine phosphokinase, 6.5 mM creatine phosphate. The other compound listed were used at the following concentration: ISP 1 nM to 1 mM, sodium fluoride (NaF) 10 mM, and forskolin 100 μM. The [α-<sup>32</sup>P]cyclic AMP formed was isolated as described by Watanabe and Jakobs [27]. All assays were performed in duplicate, and the activity was linear with respect to time at least for 9 min and protein concentration.

Since differences in receptor density and adenylate cyclase activity among any groups could conceivably be due to differences in membrane recovery, 5'-nucleotidase activity was measured according to the method of Newby et al. [9, 18] in membranes prepared as described above. Protein concentration was determined by the method of Lowry using bovine serum albumin as the protein standard [11].

#### Materials

[<sup>3</sup>H]CGP-12177 and [α-<sup>32</sup>P]ATP were obtained from New England Nuclear Medicine (Boston, MA, USA). All other chemicals used were from a standard source.

### Measurement of myocardial inotropic response to beta-adrenergic agonist

As described above, measurements of the inotropic response to a beta-adrenergic agonist were performed utilizing the hearts, which were not used for biochemical assay of myocardial BAR system. A small latex balloon tied to the end of a polyethylene tube was passed into the left ventricle (LV) of isolated perfused hearts through the mitral valve and connected to a Statham P-23DI pressure transducer. The balloon was filled with saline solution. 10 min after initiation of perfusion, the balloon volume was adjusted to an LV end-diastolic pressure (LVEDP) of 0-2 mmHg, then kept at the same volume throughout the experiment. Balloon pressure was electrically differentiated to yield  $LV+dP/dt_{max}$ . Our isolated rat heart preparation is stable for a minimum of 120 minutes.

Reperfused hearts (I-B, n=5; II-B, n=5) were allowed to beat spontaneously during the initial 10 min of perfusion and during the initial 30 min of reperfusion after 40 min of hypothermic ischemia. After 30 min of reperfusion, hearts were electrically paced at 330 beats per min (bpm) with square-wave pulses. In preliminary experiments, it was determined that at this rate pacing could be maintained in most hearts even with maximal beta-adrenergic stimulation. The applied voltage was about 10 % above that minimally required to pace the heart. Inotropic responses to beta-adrenergic agonist (ISP) were

measured with cumulative dose-response studies during 30 to 40 min of reperfusion, because intrinsic cardiac function, such as heart rate and  $LV+dP/dt_{max}$ , recovered to a new steady state in this period both in I-B and II-B. After measuring base-line values of  $LV+dP/dt_{max}$ , ISP (ranging from  $1 \times 10^{-11}$  to  $3 \times 10^{-9}$  moles in control hearts, and  $3 \times 10^{-11}$  to  $1 \times 10^{-8}$  moles in ISP-pretreated hearts) was injected as a bolus ( $10 \mu l$ ) directly into the perfusion stream about 2 cm above the orifice of the coronary arteries. Maximal responses to ISP were determined in each administered dose, and the increase in  $LV+dP/dt_{max}$  was calculated as the difference between the maximal value and the base-line value measured immediately before ISP stimulation.

Nonischemic hearts (I-A, n=5; II-A, n=5) were allowed to beat spontaneously during the initial 80 min of perfusion and then electrically paced at 330 bpm. In these hearts, inotropic responses to ISP were measured during 80-90 min of perfusion in the same manner as reperfused hearts.

### Statistical analysis

Data are presented as mean  $\pm$  SD. Two-tailed unpaired t test was used to analyze the difference between mean value in two groups in Figure 1-5. Values in Table 1 were evaluated with one-way analysis of variance. Tukey's multiple comparison test was applied only if F ratio from analysis of variance indicated that a significant difference was presented. Differences were

Table 1

considered to be significant at a value of  $p < 0.05$ .

## RESULTS

### Characterization of membrane preparation

The yield of membrane fragments obtained from the homogenate in I-A, I-B, II-A, and II-B was  $8.2 \pm 1.6$ ,  $8.7 \pm 3.7$ ,  $8.8 \pm 2.2$ , and  $8.3 \pm 1.1$  mg protein/g wet wt ( $n=5$ ) respectively. These values were not different from each other. 5'-Nucleotidase activity in I-A, I-B, II-A, and II-B was  $50.1 \pm 4.3$ ,  $48.7 \pm 4.6$ ,  $49.5 \pm 5.1$ , and  $52.2 \pm 4.7$  nmol Pi/min/mg protein ( $n=5$ , respectively). These values were also not different from each other. It is unlikely, therefore, that any change in  $B_{max}$  or adenylate cyclase activity among any groups can be attributed to the selection of a different population of membrane fragments.

### Alteration in BAR system after ischemia in non-treated rats

The  $B_{max}$  value in I-B was significantly higher than that in I-A, whereas the  $K_d$  value was similar between the two groups (Table 1). Basal, NaF-, and forskolin-stimulated adenylate cyclase activity did not differ between I-A and I-B (Table 1). The concentration of ISP, which elicited a half-maximal response of adenylate cyclase ( $EC_{50}$ ) was similar between I-A (400 nM, mean

(Table 1)

value of five experiments) and I-B (290 nM, mean value of five experiments) (Figure 1). However, maximal adenylate cyclase activity stimulated by 10-100  $\mu$ M of ISP was significantly higher in I-B than I-A, and net adenylate cyclase activity stimulated by ISP was also significantly higher in I-B than I-A (Table 1).

Intrinsic cardiac functions at 80 to 90 min of aerobic perfusion in I-A (n=5) and at 30 to 40 min of reperfusion in I-B (n=5) were as follows. Heart rate before electrical pacing was 262 $\pm$ 23 bpm in I-A and 232 $\pm$ 26 bpm in I-B ( $p < 0.05$ ). LV+dP/dt<sub>max</sub> value before electrical pacing was 829 $\pm$ 59 mmHg/sec in I-A and 659 $\pm$ 48 mmHg/sec in I-B ( $p < 0.05$ ), and that after pacing was 780 $\pm$ 63 mmHg/sec in I-A and 613 $\pm$ 34 mmHg/sec in I-B ( $p < 0.05$ ) (Table 1). The values of LVEDP, with and without electrical pacing, were similar between the two groups.

The inotropic response of hearts to administration of a bolus of ISP was shown in Figure 2. Hearts in I-A showed a greater maximal LV+dP/dt<sub>max</sub> value than those in I-B in response to the lower doses of ISP ( $1 \times 10^{-11}$  to  $3 \times 10^{-11}$  moles). At higher doses of ISP ( $1 \times 10^{-10}$  to  $3 \times 10^{-9}$  moles), there was no difference in the maximal LV+dP/dt<sub>max</sub> achieved by the two groups (Figure 2A). The increase in the absolute value of LV+dP/dt<sub>max</sub> values with a bolus of ISP, calculated as the difference between intrinsic value and the maximal LV+dP/dt<sub>max</sub> values attained ( $\Delta$  LV+dP/dt<sub>max</sub>), was shown in Figure 2B. At higher doses of ISP, hearts in I-B had a significantly greater increase in  $\Delta$  LV+dP/dt<sub>max</sub>. The maximal inotropic response to ISP, expressed as

maximal  $\Delta$  LV+dP/dt<sub>max</sub>, was greater in I-B than in I-A (p<0.05) (Table 1). The values of LVEDP did not alter during the administration of a bolus of ISP. After the administration of a bolus of ISP, hearts rapidly recovered to base-line value in LV+dP/dt<sub>max</sub>.

#### Myocardial BAR system after ISP-pretreatment

B<sub>max</sub> value in II-A was significantly lower than I-A, whereas the K<sub>d</sub> value did not differ between the two groups (Table 1). Basal, NaF-, and forskolin-stimulated adenylate cyclase activity did not differ between I-A and II-A. However, maximal adenylate cyclase activity stimulated by 100  $\mu$ M to 1 mM of ISP in II-A was significantly smaller than that stimulated by 10-100  $\mu$ M of ISP in I-A, and net maximal activity stimulated by ISP was also significantly smaller in II-A than I-A (Table 1, Figure 3).

Whereas intrinsic LV+dP/dt<sub>max</sub> was similar between I-A and II-A, there was a rightward shift in the dose (ISP)-response ( $\Delta$  LV+dP/dt<sub>max</sub>) curve in II-A, in addition to apparently depressed maximum value (Table 1, Figure 4).

#### Alteration in BAR system after ischemia in ISP-pretreated rats

The B<sub>max</sub> value and K<sub>d</sub> value were similar between II-A and II-B, and basal, NaF-, forskolin-, and maximal ISP-stimulated adenylate cyclase activity did not differ between the two groups

(Table 1).

(Table 1)

Intrinsic cardiac functions at 80 to 90 min of aerobic perfusion in II-A (n=5) and at 30 to 40 min of reperfusion in II-B (n=5) were as follows. Intrinsic heart rate was  $257 \pm 18$  bpm in II-A and  $229 \pm 13$  bpm in II-B ( $p < 0.05$ ).  $LV+dP/dt_{max}$  value before electrical pacing was  $847 \pm 53$  mmHg/sec in II-A and  $650 \pm 56$  mmHg/sec in II-B ( $p < 0.05$ ). After pacing,  $LV+dP/dt_{max}$  was also greater in II-A than II-B ( $p < 0.05$ ) (Table 1). Intrinsic  $LV+dP/dt_{max}$  value was similar between I-B and II-B (Table 1). The values of LVEDP, with and without electrical pacing, were similar among these groups.

(Table 1)

(Table 1)

Hearts in II-A showed a greater maximal  $LV+dP/dt_{max}$  value than those in II-B at all doses of ISP ( $3 \times 10^{-11}$  to  $1 \times 10^{-8}$  moles) (Figure 5A). The value of  $\Delta LV+dP/dt_{max}$  was also greater in II-A than II-B at all doses of ISP (Figure 5B). Also, the maximal inotropic response to ISP, expressed as maximal  $\Delta LV+dP/dt_{max}$ , was greater in II-A than II-B ( $p < 0.05$ ) (Table 1). After the administration of a bolus of ISP, hearts rapidly recovered to base-line value in  $LV+dP/dt_{max}$ .

(Fig 5)

(Table 1)

(Table 1)

... of ...  
 ... 257 ± 18 bpm in ...  
 ... value before ...

## DISCUSSION

## Alterations in BAR system after ischemia in normal hearts

The present study demonstrated that during early reperfusion (30 to 40 min) after 40 min of hypothermic (10°C) global ischemia in normal rat hearts, myocardial BAR system showed an increase in BAR density with enhanced BAR-mediated responses in maximal ISP-stimulated adenylate cyclase activity and inotropic response to ISP. However, these findings might pertain only to early reperfusion, since subsequent changes in BAR system could be expected to occur later. Mukherjee et al. [15, 16] demonstrated that myocardial BAR density increased after 1 hr of coronary artery ligation in normal dog. Unlike previous investigations with normothermic ischemia, we used hypothermic ischemia as a model of ischemia. In spite of the difference of temperature, BAR density increased after ischemia in our experiment using normal hearts. The mechanisms of these increase in BAR density after ischemia remains obscure. The change in turnover [15] or the augmentation of externalization [13] of receptors from light vesicle to sarcolemmal membrane might be related to this phenomenon.

Regarding the mechanisms of increased maximal ISP-stimulated adenylate cyclase activity, this increase may mainly relate to the increase in BAR density. Because the basal adenylate activity and activities after stimulation by NaF and forskolin

did not alter in this situation, it was suggested that there was less contribution of GTP-binding stimulatory protein (Gs), catalytic unit of adenylate cyclase, and interaction between Gs and adenylate cyclase in this phenomenon after hypothermic ischemia. Additionally, as we have found that the receptor affinity for beta-agonist enhanced under the same condition [23], this increased affinity also might in part relate to the increase in maximal ISP-stimulated adenylate cyclase activity.

In 30 to 40 min of reperfusion after hypothermic ischemia, intrinsic myocardial contractile function, i.e.  $LV+dP/dt_{max}$ , decreased to 74 % of the nonischemic value. However, the maximal inotropic response to Isp ( $\Delta LV+dP/dt_{max}$ ) was enhanced reaching to a similar level as in nonischemic hearts. This enhancement of inotropic response to ISP in reperfused hearts may relate to a possible increase in intracellular cAMP level mediated by enhanced BAR density and maximal ISP-stimulated adenylate cyclase activity.

#### Alterations in BAR system after ischemia in BAR-desensitized hearts

Because desensitization of BAR system can be induced by exposure of ISP [10, 14, 24, 25], ISP-pretreatment was performed in the present study to make desensitized hearts using the method of Chang et al. [3]. After a 24 hr in vivo infusion of ISP, BAR density and maximal ISP-stimulated adenylate cyclase activity

decreased in spite of no alteration in basal, NaF-, and forskolin-stimulated adenylate cyclase activity. Additionally, there was a rightward shift in the dose (ISP)-response (contractile function) curve with depressed maximal value after ISP-pretreatment. These results confirmed desensitization of myocardial BAR system occurred in our experimental model.

The present study demonstrated that in preischemic BAR-desensitized hearts, myocardial BAR density and BAR mediated responses did not increase during early reperfusion after hypothermic ischemia in contrast to normal hearts. BAR desensitization induced by beta-adrenergic agonist consists of two steps in its process: the internalization of cell surface BAR in the early phase [4, 5] and the absolute decrease in total receptor number involving internalized receptor in the late phase [6, 25]. Whereas it was not examined which type of desensitization was induced in this experiment, the receptor traffic between intracellular pool and cell surface might be restricted in either case, and this might be related to the different profile of alteration in BAR after ischemia in BAR-desensitized hearts.

In BAR-desensitized hearts, BAR-mediated adenylate cyclase activity were not enhanced after hypothermic ischemia. In these hearts, basal as well as NaF- and forskolin-stimulated adenylate cyclase activities did not change after ischemia, and it was suggested that alterations of Gs protein and catalytic unit of adenylate cyclase did not occur. Therefore, the lack of

enhancement of maximal ISP-stimulated adenylate cyclase activity in desensitized hearts can be explained as the result of no increase in BAR density.

Whereas BAR density and maximal ISP-stimulated adenylate cyclase activity remained unchanged, the maximal inotropic response to ISP rather decreased after ischemia in BAR-desensitized hearts. Although a large dose of ISP is capable of producing myocardial damage [22], it was reported that the basal contractile force of atrial appendage or isolated myocyte did not fall even after 1 week of ISP-pretreatment particularly in the model of Chang et al. [3, 26], which we have utilized. Intrinsic ventricular contractile function without beta-stimulation was similar between nonischemic normal hearts and nonischemic BAR-desensitized hearts in this study, suggesting non-significant damage to the myocardial contractile system from 24 hr of ISP-pretreatment. In addition, this was also confirmed in reperfused hearts. Therefore, to account for the decrease in maximal ISP-stimulated inotropic response in this situation, myocardial contractile reserve might be reduced in BAR-desensitized hearts. Regarding this contractile reserve, various factors including myocardial structural damage as well as biochemical derangement may be involved. Further studies are required to elucidate the phenomenon described above.

**CONCLUSION**

The present study demonstrated that myocardial BAR density and BAR-mediated physiological responses increased during early reperfusion after hypothermic ischemia in normal hearts, while BAR density and maximal ISP-stimulated adenylate cyclase activity did not increase after ischemia in BAR-desensitized hearts induced by ISP-pretreatment. We also demonstrated that maximal inotropic response to ISP was depressed after ischemia in BAR-desensitized hearts.

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Table 1. BAR density, affinity, adenylate cyclase activity, and positive inotropic response to ISP.

Group	I-A nontreated nonischemic	I-B nontreated reperfused	II-A ISP-pretreated nonischemic	II-B ISP-pretreated reperfused
Number	5	5	5	5
Binding study ([ <sup>3</sup> H]CGP-12177)				
B <sub>max</sub> (fmol/mg protein)	35.4±2.4	41.8±3.1 *	23.9±2.8 *\$	23.6±2.9 *\$
K <sub>d</sub> (nM)	0.86±0.13	0.97±0.11	0.89±0.14	0.81±0.15
Adenylate cyclase activity (pmol cAMP/min/mg protein)				
basal	28.5±2.4	28.0±1.3	24.7±2.0	24.9±2.9
NaF (10 mM)	190.0±29.8	208.8±28.2	188.7±16.9	194.0±12.7
forskolin (100 μM)	280.2±30.7	296.8±31.5	280.7±33.1	277.9±46.7
max ISP (10-100 μM)	40.3±4.7	51.2±3.1 *	30.2±4.1 *\$	31.2±5.3 *\$
max ISP - basal	11.8±3.1	23.2±2.4 *	5.5±2.9 *\$	6.3±3.5 *\$
LV+dP/dt <sub>max</sub> (mmHg/sec)				
basal	780±63	613±34 *	804±68 \$	626±65 *#
max ISP	2270±111	2263±83	1896±161 *\$	1352±179 *\$#
max ISP - basal	1490±83	1650±59 *	1092±105 *\$	726±123 *\$#

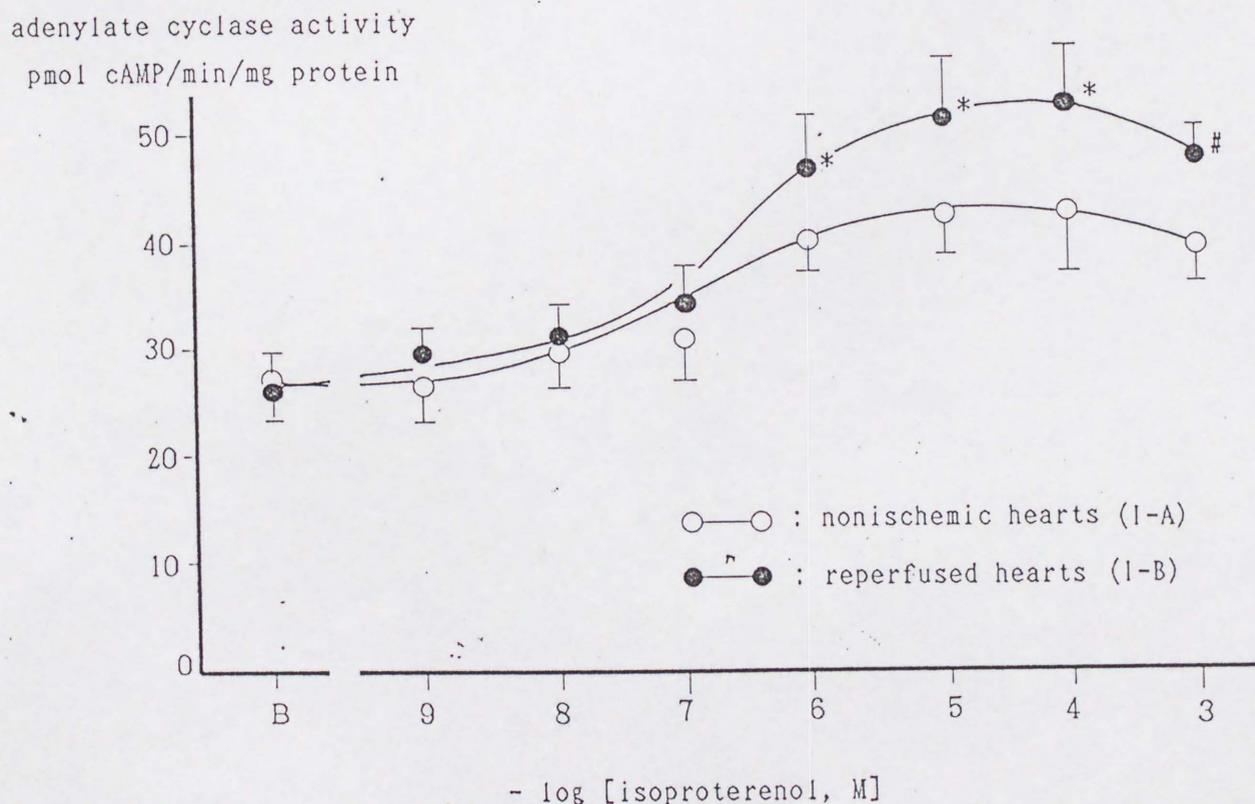
Values are means±SD.

\*: Statistically different from values in I-A (p<0.05).

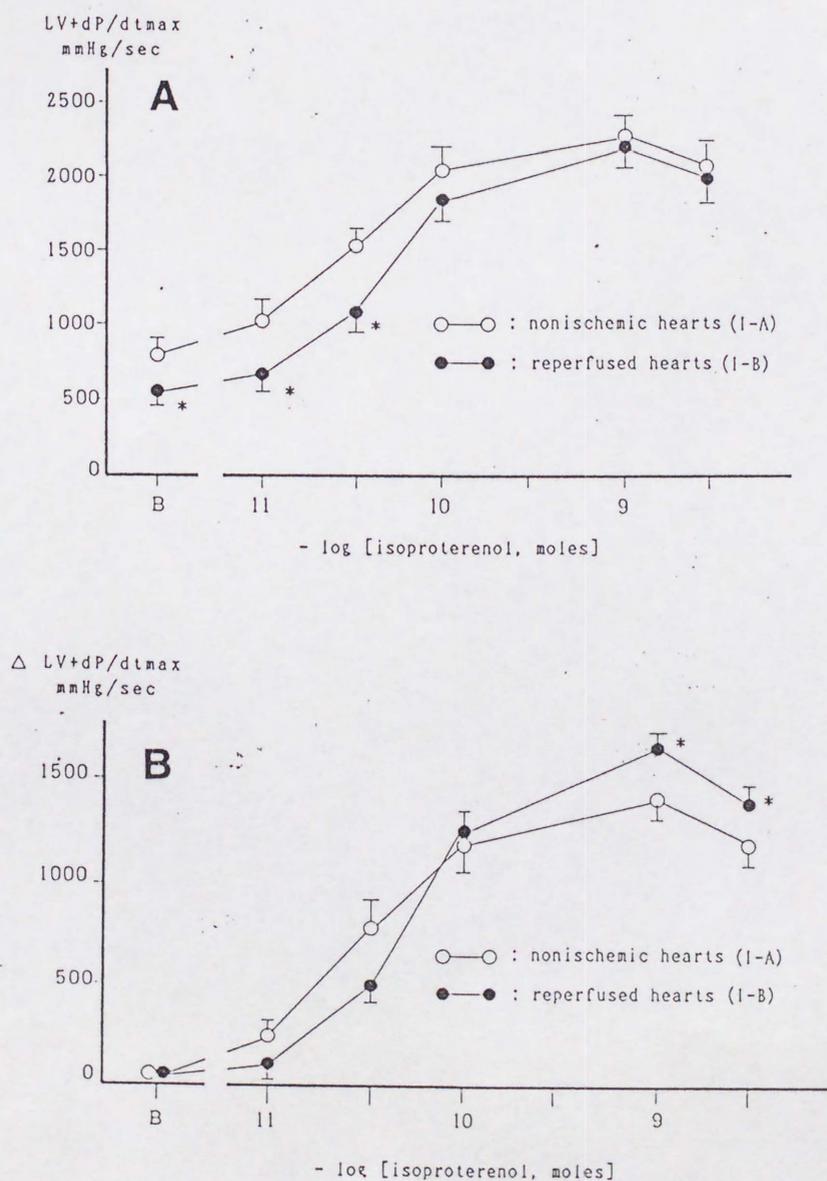
#: Statistically different from values in I-B (p<0.05).

\$: Statistically different from values in II-A (p<0.05).

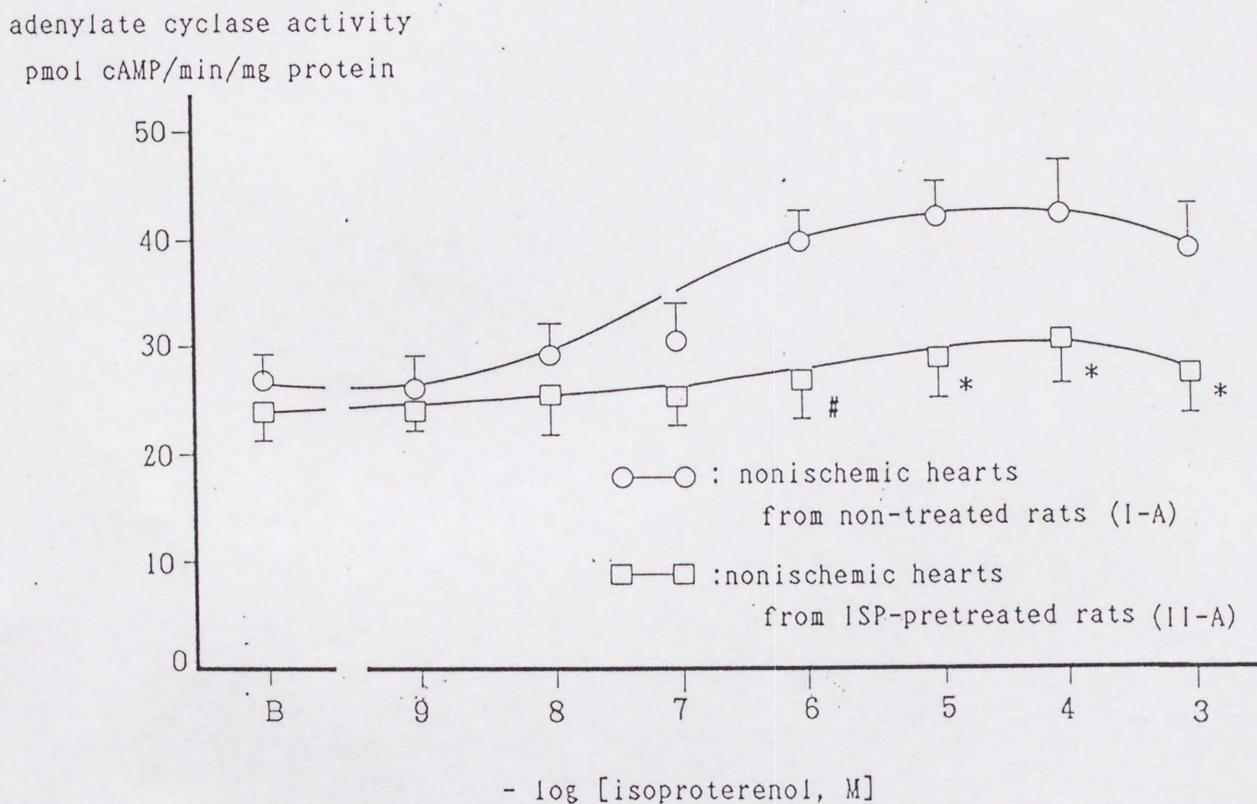
**Figure 1.** Isoproterenol (ISP)-stimulated adenylate cyclase activity in crude membrane in nonischemic hearts from non-treated rats (I-A, O), which perfused aerobically for 90 min, or from reperfused hearts from non-treated rats (I-B, ●), which reperfused for 40 min after 40 min of hypothermic (10°C) global ischemia. Data are the mean of five experiments. Bars indicate SD. \*: p<0.05 vs nonischemic hearts. #: p<0.01 vs nonischemic hearts.



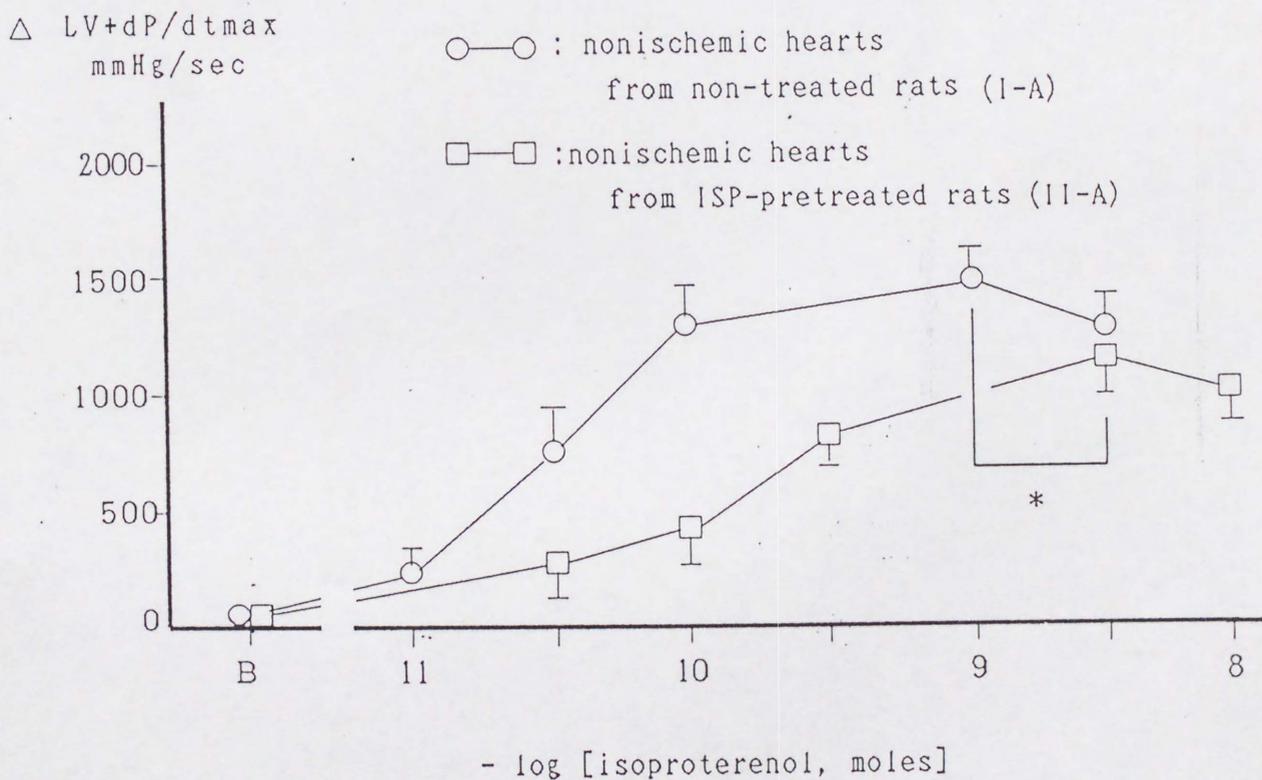
**Figure 2.** Inotropic responses to isoproterenol (ISP) administration in Langendorff-perfused hearts obtained from non-treated rats: maximal rate of left ventricular pressure development (LV+dP/dt<sub>max</sub>) (A), and the change in LV+dP/dt<sub>max</sub> ( $\Delta$  LV+dP/dt<sub>max</sub>) (B) in nonischemic hearts (I-A, O) and reperfused hearts (I-B, ●). Basal values are shown at 0 dose of ISP. Data are the mean of five experiments. Bars indicate SD. \*: p<0.05 vs nonischemic hearts.



**Figure 3.** Isoproterenol (ISP)-stimulated adenylate cyclase activity in crude membrane of nonischemic hearts from non-treated rats (I-A, O ) and nonischemic hearts from ISP-pretreated rats (II-A, □ ). Data are the mean of five experiments. Bars indicate SD. \*:  $p < 0.05$  vs hearts from non-treated rats. #:  $p < 0.01$  vs hearts from non-treated rats.



**Figure 4.** Inotropic response to isoproterenol (ISP) administration in nonischemic hearts obtained from non-treated rats (I-A,  $\circ$ ) and from ISP-pretreated rats (II-A,  $\square$ ): the change in maximal rates of left ventricular pressure development ( $\Delta$  LV+dP/dt<sub>max</sub>) Basal values are shown at 0 dose of ISP. Data are the mean of five experiments. Bars indicate SD. \*:  $p < 0.05$  vs hearts from non-treated rats.



**Figure 5.** Inotropic responses to isoproterenol (ISP) administration in Langendorff-perfused hearts obtained from ISP-pretreated rats: maximal rate of left ventricular pressure development ( $LV+dP/dt_{max}$ ) (A), and the change in  $LV+dP/dt_{max}$  ( $\Delta LV+dP/dt_{max}$ ) (B) in nonischemic hearts (II-A,  $\square$ ) and reperfused hearts (II-B,  $\blacksquare$ ). Basal values are shown at 0 dose of ISP. Data are the mean of five experiments. Bars indicate SD. \*:  $p < 0.05$  vs nonischemic hearts.

