ATP-sensitive potassium channel opener, myocardial protection, vasodilatory response

Effect of ATP - Sensitive Potassium Channel Opener Nicorandil on Cardioplegic Arrest in Isolated Rabbit Heart

Akihito Mikamo, M.D.

First Department of Surgery, Yamaguchi University of Medicine, Ube, Japan 755
(Received September 27, 1995, Revised December 18, 1995)

Abstract We hypothesized that pretreatment with the adenosine triphosphate - sensitive potassium channel opener nicorandil might enhance myocardial protection and endothelial vasodilatory response to normothermic (37℃) hyperkalemic (20 mmol/L) cardioplegia during myocardial hypoxia. We studied the effect of nicorandil using supplementation of the cardioplegic solution and pretreatment with nicorandil on isovolemic left ventricular function and endothelial function by examining its influence on 5- hydroxytryptamine (5-HT) and nitroglycerin (GTN) induced vasodilatation in the isolated rabbit heart. Twenty one rabbit hearts were perfused using a modified Langendorff preparation. After baseline measurements, hearts were subjected to a 35- minute period of arrest by infusing normothermic crystalloid cardioplegic solution with and without nicorandil (Group B and Group A) and thereafter maintained at 37℃. In a third group, nicorandil (10^{-6}mol/L) was given for 3 minutes prior to the infusion of cardioplegia without nicorandil (Group C). They were reperfused with Krebs - Henseleit bicarbonate buffer at 37℃ for 60 minutes. The isovolemic left ventricular function was not different in the three groups. After reperfusion, the vasodilatory effects of 5- HT and GTN were markedly attenuated in Group A. In Group B, the 5- HT response was reduced but the nitroglycerin effect was unchanged. In Group C, coronary flow was maintained by both drugs. We conclude that cardioplegia with pretreatment of nicorandil preserves endothelial function after 35 minutes of normothermic cardioplegic arrest.

Key Words : ATP - Sensitive Potassium Channel Opener, Myocardial Protection, Vasodilatory Response

Introduction

Noma postulated (1) that adenosine triphosphate-sensitive potassium (K_{ATP}) channels might open during ischemia and therefore be instrumental in reducing the action potential plateau phase, reducing calcium influx, and reducing the calcium - related energy cost of contraction. During ischemia, hypoxia, or metabolic blockade, the K_{ATP} channel is rapidly activated leading to the development of a large outward current, shortening of the action potential, and electrical inexcitability following contractile failure. The net effect of K_{ATP} channel opener appears to delay the progression of ischemic injury and to slow the onset of ischemia - induced contracture and hence enhance postischemic recovery. Drug - induced K_{ATP} channel opening affords myocardial protection in studies of either global and regional ischemia. However, only the hybrid compound 2- nicothinamidoethyl nitrate (nicorandil), a K_{ATP} channel opener developed in Japan, has been approved for clinical use in ischemic heart disease.

The aim of study was to determine the ability of nicorandil to influence global functional recovery and endothelium - dependent

vasodilatory response after normothermic arrest induced by hyperkalemic cardioplegic solution.

Material and Methods

Japanese white rabbits (2Kg body wt.) were used. All animals received humane care in compliance with the *Principales of Laboratory Animal Care and the Guide for the Care and Use of Laboratory Animals* published by the National Institutes of Health (NIH Publication No. 86-23, revised 1985).

Animals were anesthetized with halothane. The chest was opened and 10 mg heparin was administered intravenously. The heart was excised and promptly placed in a cold saline bath. The aortic root was cannulated with a polyethylene cannula, and 20mL cold (4℃) cardioplegic solution was infused (Table 1). The left atrial wall was removed and a balloon catheter was introduced through the mitral valve into the left ventricle to measure left ventricular pressure under isovolemic conditions. The isolated heart was mounted on a perfused circuit and perfused via the aortic root at a pressure of 100 cm H_2O by the Langendorff technique using Krebs-Henseleit bicarbonate buffer (KHBB, consisting of the following [in mM] : NaCl 119.0, $NaHCO_3$ 25.0, KCl 4.6, KH_2PO_4 1.2, $MgSO_4$ 1.2, $CaCl_2$ 1.3, and glucose 11.0 gassed with 95% O_2 and 5% CO_2 [pH 7.4 at 37℃]. After the initial 15 min. stabilization period, a baseline measurement was obtained. The heart rate (HR), left ventricular developed pressure (LVDP), and left ventricular end-diastolic pressure (LVEDP) were recorded as the balloon volume was increased in 0.1-mL increments from 0 to 0.5 mL. Left ventricular pressure was measured using a Uniflow Dispo Transducer (Baxter) and recorded on a strip chart (Polygraph 362-2, Nihon Denki Sanei Inc., Tokyo, Japan). Coronary flow was measured at the baseline balloon volume for 2 minutes.

Table 1 Composition of Cardioplegic Solution

Na^+ (mmol/L)	85
K^+ (mmol/L)	20
Ca^{++} (mmol/L)	0.5
Mg^{++} (mmol/L)	10
Cl^- (mmol/L)	85
pH	7.4
Glucose (g/L)	25
Osmolarity (mOsm/L)	350

The hearts were perfused with 10^{-7} mol/L 5-hydroxytryptamine (5-HT, Sigma Chemical Co., St. Louis, MO) (2,3) for 3 minutes in order to determine endothelium-dependent flow changes. Coronary flow was recorded for the last 2 minutes of perfusion of 5-HT. The coronary circulation was perfused with drug-free buffer for the next 12 minutes in order to reestablish basal coronary flow. The hearts were subsequently perfused with 10μg/mL nitroglycerin (GTN, The Green Cross Co., Tokyo, Japan) for 3 minutes in order to determine smooth muscle-dependent flow changes. Coronary flow was measured for the last 2 minutes of perfusion. The mean increases in coronary flow following 5-HT and GTN perfusion were calculated and expressed as percentages of the control value.

Then buffer with nicorandil (10^{-6}mol/L, Chugai, Tokyo, Japan) was perfused for 3 minutes prior to induction of normothermic cardioplegic arrest in Group C. The temperature was controlled by a circulating water bath. Three perfusion groups were studied: Group A, cardioplegia without nicorandil; Group B, cardioplegia with nicorandil (10^{-3} mol/L) ; Group C, pretreatment of buffer containing nicorandil (10^{-6}mol/L) and cardioplegia without nicorandil (Fig.1).

At the end of the 35 minute period of normothermic cardioplegic arrest hearts were reperfused for 60 minutes. After reperfusion, HR, LVDP, and LVEDP were measured, and coronary flow was measured under the same conditions of the baseline measurement. Perfusions with 5-HT (10^{-7} mol/L) and GTN (10μg/mL) were repeated for 3 minutes with a 12-minute washout interval as described above. The percentage increase in coronary flow induced by 5-HT and GTN

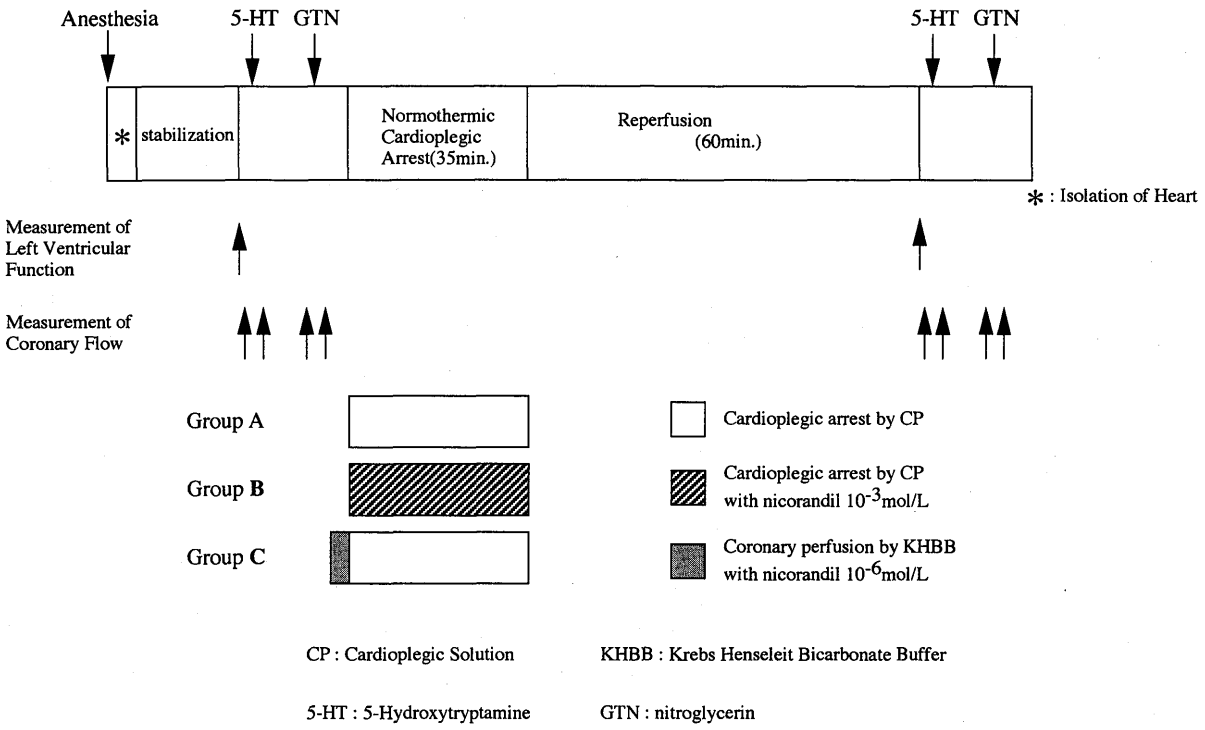

Fig. 1 Protocol for comparing the effects of nicorandil, an ATP-sensitive potassium channel opener, on cardioplegic arrest in an experimental model of ischemia and reperfusion in isolated rabbit hearts.

was calculated as follows :
% increase in coronary flow
= [(After - Before) infusion of drug/Before infusion of drug] × 100

Statistical Analysis

Statistical analysis was performed using analysis of variance (ANOVA) or student's *t* - test. All values are expressed as the mean±standard error of the mean with a difference among three groups and in each group being considered significant if the p value was less than 0.05.

Results

Left ventricular function (Table 2)
i) LVDP. The pressure developed by the left ventricule following 60 minutes of reperfusion was determined at the balloon volume of up to 0.5mL. There was no significant difference in LVDP before cardioplegic arrest among the three groups. After reperfusion, LVDP showed significant decrease compared to that before cardioplegic arrest at each balloon volume in each group ($p<0.001$). However, LVDP after reperfusion was not significantly different among the three groups.
ii) LVEDP. The LVEDP incresed with increases in balloon volume to 0.5 mL. No significant differences were noted before cardioplegic arrest among the three groups. After reperfusion, the LVEDP increased in the similar pattern to that before cardioplegic arrest, and was significantly higher than the baseline value at each balloon volume in each group ($p<0.01$). However, LVEDP after reperfusion showed no significant differences among the three groups.
iii) Response of coronary arteries to 5- HT and GTN.

Coronary flows before cardioplegic arrest and after reperfusion are shown in Table 3. In Group A, after reperfusion at 37℃, the percentage increase in coronary flow caused by 5- HT was markedly attenuated compared

Table2. Left

Group	Variables		Balloon volume (ml) 0	0.1	0.2	0.3	0.4	0.5
A (n = 7)	Heart rate (/min.)	Before	187±12					
		After	165±13					
	Developed Pressure (mmHg)	Before	69.8±2.7	83.9±5.2	90.5±5.6	95.4±5.9	102.2±7.7	104±7.3
		After	50.1±3.6	57.5±5.4	63.9±5.9	70.3±6.6	71.9±6.2	72.6±6.6
	LVEDP (mmHg)	Before	0	3.5±0.7	5.1±0.8	6.8±1.2	9.9±2.1	11.5±2.4
		After	0	7.4±1.4	11.5±2.6	15.9±3.2	20±3.5	24.1±4.0
B (n = 7)	Heart rate (/min.)	Before	181±11					
		After	155±8					
	Developed Pressure (mmHg)	Before	73.6±10.2	79.5±10.3	85.8±9.7	91.5±9.4	95±7.9	100.4±7.6
		After	60.9±7.2	67.1±6.9	73.8±6.5	80.4±6.7	84.2±5.8	85.1±5.3
	LVEDP (mmHg)	Before	0	1.6±0.3	4.2±0.9	6.2±1.6	7.6±2.2	9.7±2.7
		After	0	4.2±0.7	8.2±1.8	11.1±2.1	14.1±2.6	18.1±3.1
C (n = 7)	Heart rate (/min.)	Before	181±5.4					
		After	164±6					
	Developed Pressure (mmHg)	Before	71.5±4.9	74.8±5.4	76.5±6.5	79.5±6.5	80.6±8.1	81.5±8.8
		After	51.8±8.3	55.3±8.6	57.8±8.4	60.7±8.5	63.8±10	69.1±6.8
	LVEDP (mmHg)	Before	0	3.8±1.0	7.0±1.9	9.8±3.1	13.8±4.5	18.6±6.1
		After	0	4.6±0.8	9.4±1.6	14±2.5	18.1±3.6	24.5±4.4

Table 2 Left ventricular function before cardioplegic arrest and after reperfusion. No significant differences were found in left ventricular function before cardioplegic arrest and after reperfusion among the three groups. After reperfusion, LVDP showed significant decrease at each balloon volume in each group ($p<0.001$ vs.before cardioplegic arrest), and LVEDP significantly increase at each balloon volume in each group ($p<0.01$ vs. before cardioplegic arrest). LVDP : Left Ventricular Developed Pressure, LVEDP : Left Ventricular End-Diastolic Pressure, Before : Before Cardioplegic Arrest, After : After Reperfusion. Data are presented as the mean ±standard error of the mean.

Table 3 Coronary Flow before Cardioplegic Arrest and after Reperfusion

Group	Coronary Flow (ml/min.) Before	After
A	42.8±2.3	33.4±2.9
B	40.0±2.8	36.4±0.9
C	38.5±0.9	35.1±2.3

Before : Before Cardioplegic Arrest, After : After Reperfusion

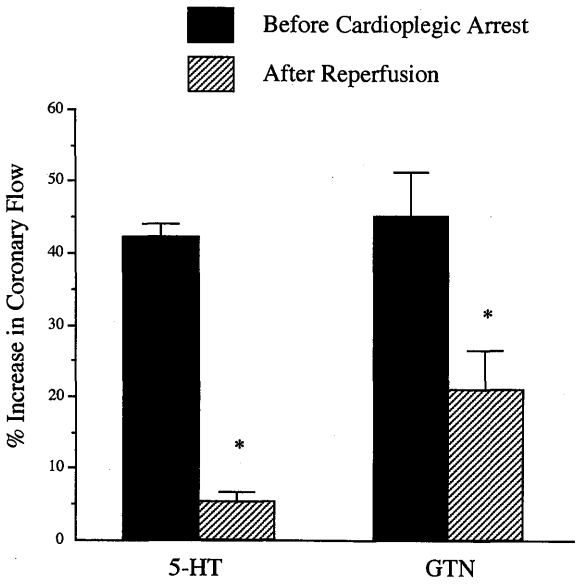

Fig. 2 Percentage increase (% increase) in coronary flow caused by 5-HT and GTN infusion in Group A. Percentage increase in coronary flow decreases significantly after reperfusion. 5-HT : 5-hydroxytryptamine, GTN : nitroglycerin. Data are presented as the mean±standard error of the mean. * $p<0.05$ vs. before cardioplegic arrest in GTN, * * $p<0.01$ vs. before cardioplegic arrest in 5-HT.

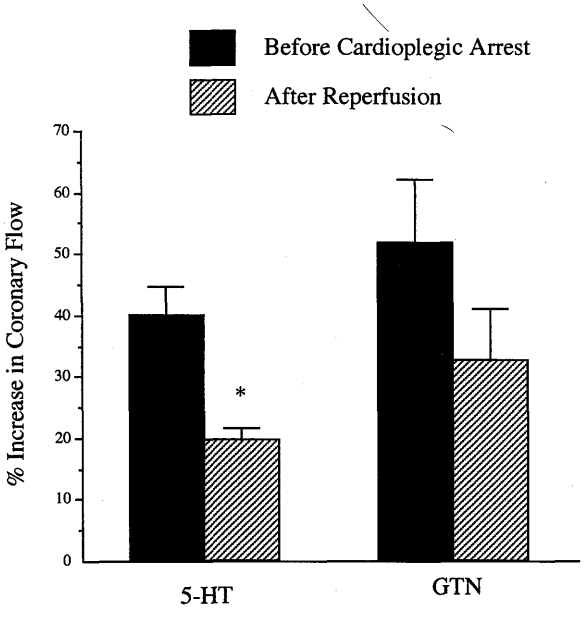

Fig. 3 Percentage increase (% increase) in coronary flow by 5-HT and GTN infusion in Group B. Percentage increase in coronary flow of 5-HT infusion decreases significantly after reperfusion, but GTN infusion does not reduce % increase in coronary flow significantly after reperfusion. 5-HT : 5-hydroxytryptamine, GTN : nitroglycerin. Data are presented as the mean±standard error of the mean. * $p<0.01$ vs. before cardioplegic arrest in 5-HT, N.S., not significant.

to the percentage increase in coronary flow before cardioplegic arrest (42.2±1.9% before cardioplegic arrest vs. 5.3±1.5% increase after reperfusion, $p<0.001$) and percentage increase in coronary flow by GTN-induced vasodilation was also reduced (45.1±6.1% increase before cardioplegic arrest vs. 20.9±5.4% increase after reperfusion, $p<0.05$) (Fig. 2). In Group B, the ischemia induced by cardioplegia with nicorandil had a similar effect on the response to 5-HT, but the vasodilatory response to GTN was preserved. Specially, 5-HT and GTN caused a 40.0±4.5% and 51.8±10.3% increase in preischemic coronary flow, respectively. After reperfusion, 5-HT caused a poor vasodilatory response (19.6±1.9% increase in flow, $p<0.05$); however, the vasodilation induced by GTN was only mildly reduced (32.6±8.3% increase in flow, N.S. ; Fig. 3). In Group C, there were no significant differences in 5-HT and GTN-induced vasodilatory responses before cardioplegic arrest and after reperfusion (before cardioplegic arrest, 5-HT, 30.0±10.9%, GTN, 28.4±3.1% : after reperfusion, 5-HT, 14.1±7.4%, GTN, 34.6±4.1%; Fig. 4). These data indicate that the vasodilatory responses to either 5-HT or GTN were preserved after reperfusion in Group C.

Discussion

Noma (1) reported that ATP-sensitive potassium channels may provide an intrinsic

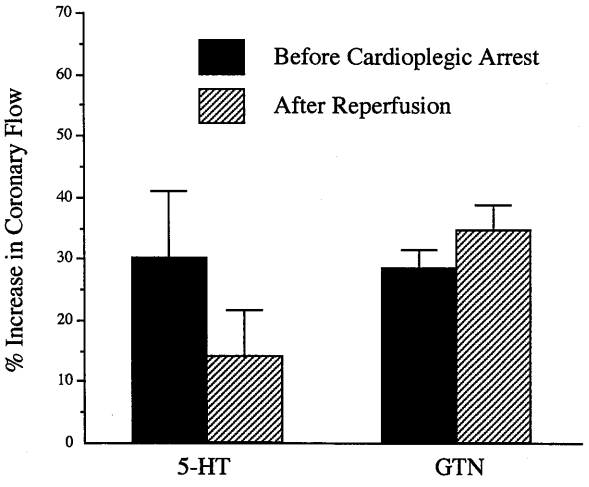

Fig. 4 Percentage increase (% increase) in coronary flow induced by 5-HT and GTN infusion in Group C. Percentage increase in coronary flow does not decrease significantly after reperfusion. 5-HT : 5-hydroxytryptamine, GTN : nitroglycerin. Data are presented as the mean±standard error of the mean.

energy sparing mechanism. During ischemia, the ATP-sensitive potassium channel is rapidly activated leading to the development of a large outward current, resulting in shortening of the action potential and therefore bringing about inexcitability and contractile failure (4,5). The anti-ischemic properties of potassium channel openers have been reported previously (6,7,8,9,10). Mitani et al. (9) reported that activation of the K_{ATP} channel and the subsequent increase in outward current and decrease in action potential duration causes rapid reduction in the contractile force of the ischemic myocardium, limits intracellular calcium accumulation during ischemia, and reduces cellular energy damage. The opening of the K_{ATP} channel and subsequent potassium efflux may thus play an important role in causing rapid mechanical arrest of ischemic myocardium and thus limit further ischemic injury. Early electrical arrest will limit calcium influx through the voltage-dependent calcium channel, which will reduce calcium overload.

The present study showed that pretreatment with nicorandil had more of a protective effect during cardioplegic arrest than cardioplegia containing nicorandil. Sugimoto et al. (11) reported that nicorandil pretreatment enhances myocardial protection during normothermic reoxygenation following 120 minutes of cold cardioplegic hypoxia in guinea pig right ventriclar papillary muscles. In addition, Mitani et al. (9) reported beneficial pretreatment effects. On the other hand, lemakalim, the pure isomer of the K_{ATP} channel opener cromakalim, was investigated by Galiñanes et al. (12) in the isolated Langendorff-perfused rat heart during normothermic global ischemia with cardioplegia with and without high potassium concentrations. Although significant anti-ischemic effects and acceleration of contractile arrest were observed with lemakalime, these effects were lost when it was used in combination with high-potassium cardioplegia. Their findings support the results of the present study.

Changes in both myocardial and vascular function after ischemia and reperfusion may limit the reflow pattern as is seen in the no reflow phenomenon. The cause of no reflow in hearts may include both myocardial and vascular components. Endothelium-dependent vasodilatory responses are reduced following ischemia and reperfusion and could be mediated by free radicals produced by neutrophils (13) or non-neutrophil sources (14). In addition, the loss of vasodilator tone could contribute to the development of no reflow (15). The present study demonstrated that nicorandil-pretreatment prior to cardioplegic arrest preserved both endothelium-dependent and endothelium-independent coronary vasodilation.

Activation of ATP-sensitive potassium channel produces hyperpolarization of membranes of both vascular smooth muscle cells and endothelium, and inhibits calcium influx via voltage sensitive channels. This reduction in calcium influx causes vascular relaxation (16). Endothelial cells regulate coronary tone through the release of constrictors and dilator substances (i.e., endothelium-dependent relaxing factor, EDRF) (17). Release of EDRF from the coronary endothelium has

been shown to be impaired after myocardial ischemia and reperfusion (18,19). Several studies have examined the effects of calcium antagonists on endothelial cell function and damage. Verapamil reduces damage to endothelium-dependent vasodilatory responses associated with myocardial ischemia and reperfusion in dogs (20). Benidipine also reduces the injury to endothelial function of mesenteric artery following transient arterial occlusion (21). These calcium channel antagonists inhibit calcium influx, as do K_{ATP} channel opening agents. Although calcium is required for the release of EDRF, both calcium channel antagonists and hyperpolarization by K_{ATP} channel opening agents do not appear to block EDRF synthesis or release (22). This suggests that the entry of calcium for the release of EDRF is not via the classic voltage operated calcium channels.

Other types of calcium channels have been observed in endothelial cells (23). Busse et al. (24) reported that one of crucial factors which modulates calcium entry into endothelial cells mediating nitric oxide release is the membrane potential. In these studies, hyperpolarization caused by opening K^+ channels enhanced nitric oxide formation in stimulated endothelial cells. In our study, we assume nicorandil works during reperfusion and that endothelial cells remain hyperpolarized. This hyperpolarization may play an important role in maintaining adequate coronary reflow. There have been few reports about the effects of ATP-sensitive potassium channel opening agents on coronary flow response. The present study demonstrated that ATP-sensitive potassium channel opening agents preserved the coronary flow response in rabbit hearts subjected to cardioplegic ischemia and reperfusion, suggesting a role for agents in myocardial protection.

References

1) Noma, A. : ATP-regulated K^+ channels in cardiac muscle. : *Nature*, **305** : 147-148,1983.
2) Pearl, J.M., Laks, H., Drinkwater, D. C., et al : Loss of endothelium-dependent vasodilatation and nitric oxide release after myocardial protection with University of Wisconsin solution. *J. Thorac. Cardiovasc. Surg.*, **107** : 257-264, 1994.
3) Mankad, P.S., Chester, A.H., Yacoub, M.H. : Role of potassium concentration in cardioplegic solutions in mediating endothelial damage. *Ann. Thorac. Surg.*, **51** : 89-93, 1991.
4) Wilde, A.A.M., Escade, D., Schumacher, C.A., et al : Potassium accumulation in the globally ischemic mammalian heart : a role of the ATP-sensitive potassium channel. *Circ. Res.*, **67** : 835-843, 1990
5) Venkatesh, N., Lamp, S.T., Weiss, J. N. : Sulfonylureas, ATP-sensitive K^+ channels, and cellular K^+ loss during hypoxia, ischemia, and metabolic inhibition in mammalian ventricle. *Circ. Res.*, **69** : 623-637, 1991.
6) Cole, W.C., McPherson, C.D., Sontag, D. : ATP-regulated K^+ channels Protect the Myocardium Against Ischemia/Reperfusion Damage. *Circ. Res.*, **69** : 571-581, 1991.
7) Hiraoka, M., Fan, Z. : Activation of ATP Sensitive-Outward K^+ Current by Nicorandil (2-Nicotinamidoethyl Nitrate) in Isolated Ventricular Myocytes. *J. Pharmacol. Exp. Ther.*, **250** : 278-285, 1989.
8) Auchampach, J. A., Maruyama, M., Cavero, I. and Gross, G.J. : Pharmacological Evidence for a Role of ATP-Dependent Potassium Channels in Myocardial Stunning. *Circulation*, **86** : 311-319, 1992.
9) Mitani, A., Kinoshita, K., Fukamachi, K., et al : Effects of glybenclamide and nicorandil on cardiac function during ischemia and reperfusion in isolated perfused rat hearts. *Am. J. Physiol.*, **261** : H1864-1871, 1991.
10) Gross, G. J., Auchanpach, J. A., Maruyama, M., Wartlier, D. C. and Pieper, G. M. : Cardioprotective effcts of nicorandil. *J. Cardiovasc. Pharmacol.*, **20** (Suppl. 3) : S22-28, 1992.
11) Sugimoto, S., Puddu, P.E., Monti, F. M. and Schiariti, M. : Pretreatment with the adenosine triphosphate-sensitive potassium channel opener nicorandil and improved myocardial protection during

high - potassium cardioplegic hypoxia. *J. Thorac. Cardiovasc. Surg.*, **108** : 455-466, 1994.
12) Galiñanes, M., Shattock, M. J. and Hearse, D.J. : Effects of potassium channel modulation during global ischemia in isolated rat heart with and without cardioplegia. *Cardiovasc. Res.*, **26** : 1063-1068, 1992.
13) Ma, X.L., Tsao, P.S., Viehman, G. E. and Lefer, A.M. : Neutrophil-Mediated vasoconstriction and endothelial dysfunction in low - flow perfusion - reperfused cat coronary artery. *Circ. Res.* **69** : 95-106, 1991.
14) Tsao, P.S. and Lefer, A.M. : Time course and mechanism of endothelial dysfunction in isolated ischemic - and hypoxic - perfused rat hearts. *Am. J. Physiol.* **256** : H1660-1666, 1990.
15) Gavin, J.B., Humphrey, S.M. and Herdson, P.B. : The no - reflow phenomenon in ischemic myocardium. *Int. Rev. Exp. Patol.* **25** : 361-383, 1983.
16) Chiu, P.J.S., Tetzloff, G., Ahn, H-S. and Syberts, E.J. : Effects of BRL 34915, a putative K channel opener, on transmembrane ^{45}Ca movements in rabbit aortic smooth muscle. *Eur. J. Pharmacol.*, **155** : 229-237, 1988.
17) Rubanyi, G.M. : Endothelium - Drived Relaxing and Constricting Factors. *J. Cell. Biochem.*, **46** : 27-36, 1991.
18) Perason, P.J., Schaff, H.V. and Vanhoutte, P.M. : Acute Impairment of Endothelium - Dependent Relaxations to Aggregating Platelets Following Reperfusion Injury in Canine Coronary Arteries. *Circ. Res.*, **67** : 385-393, 1990.
19) Perason, P.J., Schaff, H.V. and Vanhoutte, P.M. : Long - term Impairment of Endothelium - Dependent Relaxations to Aggregating Platelets after Reperfusion Injury in Canine Coronary Arteries. *Circulation*, **81** : 1921-1927, 1990.
20) Vanbenthuysen, K.M., Mcmurtry, I.F. and Horwitz, L.D. : Reperfusion after Acute Coronary Occlusion in Dogs Impairs Endothelium - dependent Relaxation to Acetylcholine and Augments Contractile Reactivity In Vitro. *J. Clin. Invest.*, **79** : 265-274, 1987.
21) Karasawa, A., Rochester, J.A., Ma,X. L. and Lefer, A.M. : Protection of Endothelial Damage and Systemic Shock by Benidipine, a Calcium Antagonist, in Rats Subjected to Splanchnic Ischemia and Reperfusion. *Circ. Shock.*, **33** : 133-141, 1991.
22) Mügge, A., Peterson, T. and Harrison, D. G. : Release of Nitrogen Oxides From Cultured Bovine Aortic Endothelial Cells Is Not Impaired by Calcium Channel Antagonists. *Circulation*, **83** : 1404-1409, 1991.
23) Bossu, J.L., Elhandani, A., Feltz, A., Tanzi,F., Aunis, D. and Thierse, D. : Voltage - gated Ca entry in isolated bovine capillary endothelial cells. evidence of a new type of BAY K 8644-sensitive channel. *Pflügers Arch.*, **420** : 200-207, 1992.
24) Busse, R., Müllsch, A., Flemming, I. and Hecker, M. : Mechanisms of Nitric Oxide Release from the Vascular Endothelium. *Circulation*, **87**(Suppl. V) : V15-V25, 1993.