Original Articles

COMPARATIVE STUDY FOR THE CARDIOPROTECTIVE EFFECTS OF NICORANDIL AND KRN 4884(ATP- SENSITIVE POTASSIUM CHANNEL OPENERS) IN ISCHEMIC REPERFUSED ISOLATED RABBIT'S HEART

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تلعب قنوات البوتاسيوم الحساسة للأدينوسين تراى فوسفات الموجودة في أنسجة القلب دوراً مهماً في اعتلال عضلة القلب. وقد تم دراسة التأثير الوقائي والحمائي لبعض فاتحات قنوات البوتاسيوم على القلب الفاقي المعزول. وكذلك دراسة الميكانيكية المفترضة لهذا التأثير. وقد وجد أن الفاقة الدموية أدت إلى نقص إحصائي في ضغط البطين الأيسر وزيادة في نشاط الكرياتين كينيز في سائل التروية. كذلك وجدت زيادة إحصائية في أيض أدينوسين تراى فوسفات في سائل التروية. بالإضافة إلى انخفاض معدل استهلاك الأكسجين في الميتوكندريا وذلك في القلب الفاقي بالمقارنة بالقلب الطبيعي. وقد أثبتت هذه الراسة أن العلاج بالنيكوراندیل أو (ك ر ن 4884) لمدة خمس عشرة دقيقة قبل حدوث الفاقة الدموية في القلب أدى إلى ضعف حدوث زيادة في ضغط البطين الأيسر كما ثبط من خروج مركبات الأيض خاصة للأدينوسين تراى فوسفات أثناء التروية. بالإضافة إلى الحفاظ على معدل استهلاك الميتوكندريا للأكسجين أثناء فترات النقص. نستنتج من هذا البحث أن فاتحات قنوات البوتاسيوم الحساسة للأدينوسين تراى فوسفات لها القدرة على تقليل حدوث الضرر الذي يحدث في القلب الفاقي المعزول.

Adenosine Triphosphate (ATP) sensitive potassium channels (KATP) exist in cardiac tissue and have a potential role in pathogenesis of myocardial ischemia. Studies in vitro models of myocardial ischemia and reperfusion have indicated that KATP openers exert protective effects. The present study examined the effect of (KATP) channel opener, nicorandil and the long acting potassium channel opener KRN 4884 in ischemic/reperfused isolated rabbit's heart and the possible mechanism (s) of action for the improvement of energy metabolism in hearts subjected to 35 min ischemia and 60 min reperfusion. Ischemia induced a significant decrease in left ventricular pressure (LVDP), a rise in left ventricular end diastolic pressure (LVEDP) ,and an increase in creatine kinase activity (CK) in the perfusate. ATP metabolites in the perfusate increased significantly during ischemia and reperfusion while myocardial energy metabolites (ATP and creatine phosphate, CP) are decreased during ischemia and are little restored during reperfusion. Mitochondrial oxygen consumption rate was lower in ischemic hearts than that in normoxic hearts. The treatment with nicorandil (1 mM) or KRN4884 (1m M) for the final 15 min of preischemia induced recovery of LVDP, attenuated the postischemic rise in LVEDP and suppressed the release of CK and ATP metabolites during reperfusion. The treatment also restored myocardial ATP and CP contents and preserved the mitochondrial oxygen consumption rate at the end of ischemia and reperfusion.In another set of experiments, myocardial skinned bundles were incubated for 30 min under hypoxic conditions in the presence and absence of nicorandil or KRN 4884 and then mitochondrial oxygen consumption rate was determined. Hypoxia decreased the mitochondrial oxygen consumption rate of skinned bundles to approximately 36.5 and 35.6% of the prehypoxic value. Treatment with nicorandil or KRN 4884 preserved the mitochondrial oxygen consumption rate during hypoxia. In conclusion, the present

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attenuating ischemia/reperfusion injury of isolated perfused hearts due to preservation of mitochondrial function during ischemia, probably through opening of mitochondrial (K_{ATP}) channels.

Key words: Ischemia-reperfusion, nicorandil,KRN4884

Introduction

A high degree of interest is presently focused on the physiological and pathophysiological role of KATP channels which are found in a variety of tissue types including smooth muscle, heart, skeletal muscles, pancreas, brain and kidney. K_{ATP} channels are metabolically gated channels that are predominantly in a closed state at normal physiologic concentrations of intracellular ATP. They are believed to relax smooth muscles by inhibiting calcium influx secondary to an enhanced outward potassium current and consequent hyperpolarization (1).

The physiological role of (K_{ATP}) channels in myocardial tissue is of great interest. At present, it is known that they have a role in pathologic states such as acute myocardial ischemia (2). Ischemic myocytes have been shown to leak potassium into the extracellular space in a number of models. This potassium release occurs rapidly and then reaches a plateau within the first few minutes of ischemia. As time during ischemia progress, a large upsurge in potassium release occurs which is probably related to cell death. This outward potassium current observed during ischemia is through K_{ATP} channels and is responsible for early repolarization observed in the ischemic zone and participates in the ST-segment shifts observed in early ischemia (2).

In recent years, the role of cardiac ATP-sensitive (K_{ATP}) channels, a metabolism-sensitive ion conductance, has emerged as a promising therapeutic strategy against ischemic injury in the myocardium (3). In particular, potassium channel openers, which promote opening of (K_{ATP}) channels, have been found to decrease infarct size, mimic ischemic pre-conditioning, and improve functional and energetic recovery of cardiac muscle after ischemic and hypoxic insults (4). Moreover, in the majority of studies, antagonists of K_{ATP} channels, such as the sulfonyl urea drugs, abolished the beneficial effect of potassium channel openers, further implying a cardioprotective role for K_{ATP} channels (5). However, in the absence of more direct evidence that channel proteins themselves are responsible for cardioprotection, this concept has been continuously contested (6), partly because of the complexity of regulation of K_{ATP} channel behavior under ischemic conditions, as well as the difficulty in separating K_{ATP} channel-dependent from K_{ATP} channel-independent protective mechanisms that coexist within a cardiac cell (7).

Ischemia/reperfusion injury, remains a major limitation to the survival of the heart during clinical transplantation (8). It involves two distinct but interdependent processes. During ischemia, high-energy substrates are lost, eventually leading to ischemic contracture when cardiac ATP levels fall below a critical level (9). Reperfusion of the myocardium leads to the formation of oxygen free radicals, which are involved in a range of pathological changes at the cellular and tissue levels (10).

Nicorandil, which is a hybrid of adenosine triphosphate (ATP)-sensitive K^+ channel opener and nitrates, can enhance the recovery of post ischemic contractile dysfunction and reduce infarct size (15). The mechanism involved in the protective action of nicorandil is not actually known. Contribution of reducing preload and afterload, anti-free radical and neutrophil modulating properties, the opening of K_{ATP} channels and the vasodilatation of the small coronary arteries have been postulated (16,17).

KRN4884 is a newly synthesized 3-pyridine derivative that was discovered in a screening program (18). KRN4884 is believed to have an ATP-sensitive K channel-opening (K_{ATP}) action (19).

The goal of this study was to assess the functional improvement of nicorandil and KRN4884 on ischemic/reperfused heart and to elucidate their possible mechanism (s) of actions as cardio-protectives. The effects of K_{ATP} channel openers on the mitochondria might indirectly link to improvement of energy production of reperfused hearts. However, it is unclear whether nicorandil and KRN4884 affects mitochondrial K_{ATP} channels in the myocardium. In the present study, the possible actions of K_{ATP} channel openers nicorandil and KRN4884 on the mitochondrial function to produce energy in the ischemic/reperfused isolated rabbit's heart have been examined.

Materials

KRN4884 was kindly provided by pharmaceutical Development Laboratory, Kirin Brewery Co., Ltd. (Gunma, Japan).Nicorandil and all other chemicals were purchased from Sigma chemical Co (St Louis, MO).

Animals

This study was conducted on 96 male albino rabbits of the blanc du Bouscal strain, weighing (2.5-3kg). The animals were conditioned at 23 ± 1°C with a constant humidity of 55 ± 5% and a 12h light/dark cycle and given free access to food and tap water according to the "Guide for the Care and Use of Laboratory Animals" as promulgated by the National Research Council. The Protocol of this study was approved by the Committee of Animal Use and Welfare of our University.

Methods

Perfusion of Hearts

The rabbits were anaesthetized with diethyl ether. After thoracotomy, the hearts were rapidly isolated, transferred to a Langendorff apparatus, and perfused at 37°C with a constant flow of 9.0ml/min with the Krebs-Henseleit bicarbonate buffer composed of 118.5 mM NaCl, 3.2 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 1.25 mM $CaCl_2$, 25 mM $NaHCO_3$ and 5.5 mM glucose. The perfusion buffer is equilibrated with a gas mixture of 95% O_2 and 5% CO_2, pH 7.4. A latex balloon, with an uninflated diameter of 3.7 mm and connected to a pressure transducer (TP-200; Nihonkohden, Tokyo, Japan), was inserted into the left ventricular cavity through the mitral opening and secured with a ligature that included the left atrial remnants.

An initial left ventricular end-diastolic pressure (LVEDP), 5 mmHg was loaded to the perfused heart. After equilibration for 15 minutes, the heart was paced at 300 beats/min and further equilibrated for 15 minutes. Left ventricular developed pressure (LVDP), a convenient marker of cardiac contractile function, was monitored by a pressure transducer connected to a carrier amplifier (AP-621G; Nihonkohden) throughout the experiment (20).

After a 30 minutes equilibration, an additional 15 minutes perfusion was carried out, and then the perfusion was stopped (ischemia). The heart was submerged in an organ bath filled with the Krebs-Henseleit bicarbonate buffer in which 11 mM glucose was replaced with 11 Mm Tris-HCl (ischemic buffer). The ischemic buffer was previously equilibrated with a gas mixture of 95% N_2 and 5% CO_2, pH 7.4, and maintained at 37°C during the experiment to avoid hypothermia induced cardioprotection. After 35 minutes of ischemia, the buffer in the organ bath was drained, and the hearts were reperfused for 60 minutes at 37°C with the Krebs-Henseleit bicarbonate buffer equilibrated with a gas mixture of 95% O_2 and 5% CO_2 reperfusion. The perfused hearts were paced throughout the experiment except for the first 15 minutes of reperfusion to prevent contractile irregularities that may frequently occur during this period. For the purpose of comparison, hearts were perfused for 35 minutes under normoxic conditions, followed by 60 minutes of normoxic perfusion (normoxic group) (21).

Treatment of the perfused hearts with drugs was carried out by infusing the appropriate concentration of the agent into Krebs-Henseleit bicarbonate buffer for the last 15 minutes of pre-ischemia. The agents were dissolved as1mM for nicorandil (22) and KRN4884 (19) in the perfusion buffer and infused through an injection port of a cannula positioned just proximal to the aorta at a flow rate of 100 µl/min by means of an infusion pump (STC-523; Terumo, Tokyo, Japan) (22).

Examination of Perfusate

The perfusate eluted from the heart was collected to determine creatine kinase (CK) activity using a commercially available kit (CK-NAC; Boehringer Mannheim, Mannheim, Germany) according to the method of Bergmeyer et al. (23). The release of the enzyme was estimated as the total CK activity in the perfusate.

The perfusate was also used for determination of nucleosides and purine contents (as total ATP metabolites), by means of the HPLC method described by Takeo et al. (24).

Determination of Myocardial Energy Metabolites

After 60 minutes perfusion, hearts were quickly frozen by clamping with aluminum tongs pre-cooled with liquid nitrogen. The frozen ventricles were isolated and pulverized by a mortar-driven homogenizer with a pestle and mixed with 0.3 N $HCLO_4$ and 0.25 mM EDTA under liquid nitrogen cooling. After having been left for 10 min at room temperature, the extract was centrifuged at 8000 g

for 15 min at 4 C°. The supernatant fluid was neutralized with 2.5 M K2C03 and centrifuged again at 8000 g for 15 min at 4 C°. The resulting supernatant fluid was sampled for determination of myocardial ATP and creatine phosphate (CP) contents. Myocardial ATP was measured by the HPLC method described by Takeo et al. (25). Myocardial CP was converted to ATP according to the enzymatic method of Lowry and Passonneau (26) and then determined by the same HPLC method as used for ATP.

Estimation of mitochondrial Oxygen Consumption Rate

The mitochondrial oxygen consumption rate was determined according to the method of Sanbe et al. (27), which is a modification of the method of Saks et al. (28). It is defined as a measure of the maximal mitochondrial oxygen consumption capacity in cardiac tissue. After perfusion, the hearts were quickly removed from the perfusion apparatus. Myocardial bundles, 0.3 to 0.4 mm in diameter and 3 to 4 mm in length, were prepared from the left ventricular free wall by use of a McIIwain Tissue Chopper (Mickle Lab Engineering Co., Westbury, NY) and transferred into relaxing solution (A) composed of 10 mM EGTA, 3 mM $MgSO_4$, 20 mM taurine, 0.5 mM dithiothreitol, 20 mM imidazole, 160 mM potassium 2-(N-morpholino)-ethanesulfonate, 5 mM ATP and 15 mM CP, pH 7.0. Eight to ten bundles were incubated for 20 minutes in 1ml of solution (A) containing 75µg/ml saponin. After incubation, the bundles (skinned bundles) were washed for 10 minutes in fresh solution (A) to remove the saponin. All procedures were carried out at 4°C. The oxygen consumption rate of skinned bundles was determined by means of a Clark-type electrode connected to an Oxygraph (Central Kagaku, Tokyo, Japan) containing 7 to 10 skinned bundles in 1.0 ml of solution (B) (a solution (A) without ATP and CP but supplemented with 0.5% bovine serum albumine (BSA) at 30°C with continuous and gentle stirring). The basal oxygen consumption rate was measured by the addition of 5 mM glutamate, 3 mM malate, and 3 mM KH_2PO_4. Total oxygen consumption rate was measured after the further addition of 1 mM ADP and 7.5 mM creatine. The maximal velocity of oxygen consumption rate (V_{max}) of skinned bundles was taken as the differences between total and basal oxygen consumption rates. After determination of oxygen consumption rate, the skinned bundles were transferred to a test tube and washed with saline to remove BSA and dithiothreitol. The skinned bundles were solubilized with 0.5 ml of 2N NaOH for 30 minutes at 60°C, and then the protein concentration was determined according to the method of Lowry et al. (29). The mitochondrial oxygen consumption rate was expressed as nano-atoms of oxygen consumed/minute/mg protein.

Estimation of the effect of nicorandile or KRN 4884 on skinned bundles

In another set of experiments, skinned bundles were prepared from the left ventricular free wall of normal rabbits to determine whether nicorandil or KRN4884 can directly affect mitochondria. Hypoxia was induced by incubating the skinned bundles in the solution (B) for 30 minutes in an atmosphere of 100% nitrogen gas in a tightly sealed chamber at 30°C. The skinned bundles were exposed to the hypoxic conditions as mentioned in the absence and presence of nicorandil or KRN4884. After 30 minutes, hypoxic or normoxic incubation, the skinned bundles were quickly transferred to the glass cell, and then their oxygen consumption rates were determined as mentioned above

Statistical Analysis

The results were expressed as the mean ± SE. One way comparison of variances (ANOVA test), together with Tukey- Kramer multiple comparisons post ANOVA test were used for comparison between the groups treated with nicorandil or KRN4884 and untreated ones. A difference of $p<0.05$ was considered as the level of significance.

Results

Cardiac function of perfused hearts

Changes in LVDP and LVEDP of ischemic /reperfused hearts untreated or treated with either (1 mM) nicorandil or (10^{-3}M KRN 4884 are shown in table I. Ischemia induced a significant decline in LVDP.By the end of the reperfusion period LVDPs of the heart recovered significantly to approximately 43.8%(KRN 4884) and 48.46% (nicorandil) of the normal pre-ischemic value .when the hearts were treated with nicorandil or KRN 4884 for the last 15 minutes of pre -ischemia period their LVDPs were recovered significantly to approximately 83.7% (KRN 4884)) and 72.57% (nicorandil) the

preischemic value. The recovery value was higher in KRN 4884 group than in nicorandil group. (Table I)

The LVEDP of the untreated hearts raised significantly during ischemia. This increase was sustained throughout the reperfusion period and was significantly attenuated with nicorandil or KRN 4884. (Table I).

Table 1. Effects of KRN4884 and nicorandil on LVDP and LVEDP in isolated ischemic / reperfused rabbit's hearts.

Treatment	Parameter	Normal	Ischemia	Reperfusion	Treated- group
KRN4884	LVDP (mm Hg)	64.67 ± 2.79	17.17 ±2.13*	28.33 ±2.52#	54.17±3.07#
	LVEDP (mm Hg)	15.67 ± 0.68	32.5 ± 3.90*	29.17 ±2.63	17.33±0.73#
Nicorandil	LVDP (mm Hg)	65 ± 2.84	23.17 ± 3.97*	31.5 ± 1.76#	47.17± 2.13#
	LVEDP (mm Hg)	15 ± 0.59	35 ± 1.86*	28.5 ± 1.14#	19.17 ±0.85#

Each value represents the mean ± S.E of 6 expeiments. * P< 0.05 compared with the control group. # P< 0.05 compared with the ischemic group.

Table 2. Effects of KRN4884 and nicorandil on perfusate CK activity and ATP metabolites of isolated ischemic / reperfused rabbit's hearts.

Treatment	Parameter	Normal	Ischemia	Reperfusion	Treated- group
KRN4884	CK (nmol NADPH/ min/g wet tissue)	1.65 ± 0.25	19.82±0.94*	14.90 ±0.72#	7.85 ± 0.81#
	Perfusate ATP Metabolites (µmol / g wet tissue)	0.32 ± 0.06	2.15 ± 0.26*	2.53 ± 0.18	0.93 ± 0.09#
Nicorandil	Perfusate CK (nmol NADPH/ min/g wet tissue)	1.18 ± 0.18	19.32 ± 1.39*	15.95 ± 0.89	8.35 ± 0.95#
	Perfusate ATP Metabolites (µmol / g wet tissue)	0.31 ± 0.75	2.65 ± 0.20*	2.2 ± 0.14	0.95 ± 0.08#

Each value represents the mean ± S.E. of 6 expeiments. * P< 0.05 compared with the control group. # P< 0.05 compared with the ischemic group.

Table 3. Effects of KRN4884 and nicorandil on myocardial ATP and CP contents in isolated ischemic / reperfused rabbit's hearts.

Treatment	Parameter	Normal	Ischemia	Reperfusion	Treated- group
KRN4884	Myocardial ATP (μmol /g dry tissue)	26.01 ± 0.61	12.21 ± 0.6*	16.68 ± 0.42#	22.08±0.57#
	Myocardial CP (μmol /g dry tissue)	35.53 ± 0.59	17.55 ± 0.70*	20.62 ± 0.45#	22.32 ± 0.85#
Nicorandil	Myocardial ATP (μmol /g dry tissue)	26.19 ± 0.89	12.18 ± 0.62*	17.1 ± 0.57#	20.74 ± 0.77#
	Myocardial CP (μmol /g dry tissue)	34.93 ± 0.67	16.85 ± 0.69*	20.57 ± 0.57#	25.38 ± 1.53#

Each value represents the mean ± S.E. of 6 expeiments. * P< 0.05 compared with the control group. # P< 0.05 compared with the ischemic group.

Table 4. Effects of KRN4884 and nicorandil on mitochondrial oxygen consumption rate of myocardial skinned bundles subjected to 30 min hypoxia.

MITOCHONDRIAL OXYGEN CONSUMPTION RATE (nano atoms O / min / mg protein)			
Normoxia	Hypoxia	KRN4884	Nicorandil
57.02 ± 2.27	25.70 ± 1.987*	52 ± 1.6#	48 ± 1.53#

Each value represents the mean ± S.E. of 6 expeiments. * P< 0.05 compared with the control group. # P< 0.05 compared with the ischemic group.

Table 5. Effects of KRN4884 and nicorandil on mitochondrial oxygen consumption rate of left ventricular skinned bundles isolated from perfused hearts.

MITOCHONDRIAL OXYGEN CONSUMPTION RATE (nano atoms O / min / mg protein)				
Treatment	Normal	Ischemia	Reperfusion	Treated- group
KRN4884	58.29 ± 2.06	21.28 ± 0.81*	31.57 ± 0.85*	57.04 ± 1.55#
Nicorandil	57.57 ± 0.84	20.5 ± 0.57*	31.5 ± 0.57*	50.37 ± 1.46#

Each value represents the mean ± S.E. of 6 expeiments. * P< 0.05 compared with the control group . # P< 0.05 compared with the ischemic group.

Release of CK and ATP Metabolites

Total CK activity in the perfusate of the heart perfused for 95 min under normoxic conditions was 1.65 ± 0.25 NADPH /min /g wet tissue for KRN 4884 and 1.18 ± 0.18 NADPH /min /g wet tissue for nicorandil .CK activity increased markedly during ischemia and reperfusion. Treatment with nicorandil or KRN 4884 attenuated the release of CK from the perfused heart (Table 2).

The amount of ATP metabolites released during ischemia and reperfusion was also determinted (Table 2). The release of ATP metabolites from hearts perfused for 95 min under normoxic conditions was about 0.32 ± 0.06 umol/ g wet tissue for KRN 4884 and 0.31± 0.75 /g wet tissue for nicorandil . The release of ATP metabolites was markedly increased during ischemia and continue during reperfusion. Treatment with nicorandil or KRN 4884 suppressed the release of ATP metabolites during reperfusion ($P < 0.05$).

Myocardial Energy Metabolites

Myocardial energy metabolites such as ATP and CP were determined in the heart untreated or treated with either nicorandil or KRN 4884 to examine the myocardial energy profile (Table 3). Myocardial ATP and CP contents at the end of ischemia were approximately 46.9% and 49.39% and 46.5 and 48.2 % of the preischemic values respectively.

Reperfusion of the ischemic heart resulted in little restoration of myocardial ATP and CP contents (approximately 64.1% and 65.29 % for ATP and 58.03 and 58.88% for CP of the preischemic values respectively).

Treatment with nicorandil or KRN 4884 restored myocardial ATP and CP contents during reperfusion to approximately 79.19% and 72.65% for nicorandil and 84.89% and 62.82% for KRN 4884 of the preischemic values respectively.

Mitochondrial Oxygen Consumption
rate of Hypoxic Heart

After 30 min hypoxic incubation, the mitochondrial oxygen consumption rate was decreased to approximately 45.07 % of the value for normoxic skinned bundles .When the skinned bundles were incubated in presence of nicorandil or KRN 4884 under hypoxic conditions, the hypoxia induced decrease in the mitochondrial oxygen consumption rate was attenuated.(Table 4)

Mitochondrial Oxygen Consumption
rate of perfused Heart

The mitochondrial oxygen consumption rate of the untreated left ventricular muscle and of that treated with either nicorandil or KRN 4884 were also determined (Table 5). The mitochondrial oxygen consumption rate of the untreated heart under ischemic conditions was significantly lower than that of normoxic heart ($P < 0.05$). This decline in The mitochondrial oxygen consumption rate persisted during reperfusion. In contrast treatment with nicorandil or KRN 4884 preserved the mitochondrial oxygen consumption rate at the end of ischemia- reperfusion.

Discussion

Several studies have demonstrated that an adaptive response can be elicited in myocardium in which brief episodes of reversible ischemia preceed a prolonged interval of ischemia (30). This myocardial preconditioning has been demonstrated to provide protective effects in the setting of both global and regional myocardial ischemia (31). It has been proposed that a contributory factor for the protective effects of myocardial preconditioning is the activation of the ATP-sensitive potassium channels(K_{ATP})(32). Although the activation of these K_{ATP} channels is through a reduction in intracellular ATP, pharmacological activation of these channels can be achieved through the potassium channel openers (PCOs) (30).

In the present study, treatment with nicorandil or KRN 4884 during preischemia markedly enhanced the postischemic contractile recovery of ischemic/reperfused hearts especially in KRN 4884 groups. This improvement was associated with restoration of myocardial high-energy phosphates during reperfusion, appreciable levels of high-energy phosphates in the nicorandil or KRN 4884 treated reperfused heart may be substantially beneficial for the recovery of myocardial contractility of the reperfused heart (33,34).

Treatment with nicorandil or KRN 4884 suppressed the release of CK and ATP metabolites from the reperfused heart in. This implies that an ischemia-induced increase in membrane permeability of macromolecules such as CK protein across cell membranes and / or induction of cardiac cell necrosis in the reperfused heart was suppressed by treatment with nicorandil or KRN 4884 (35). The

finding also suggests that restoration of myocardial ATP by treatment may be due to reduction in the the loss of ATP metabolites during reperfusion (36). In addition, the myocardial CP content of the hearts treated with nicorandil or KRN 4884 was restored to the preischemic value during reperfusion, this suggests that the ability to produce energy in mitochondria during reperfusion is retained by nicorandil or KRN 4884 pretreatment.

The sarcolemmal KATP channel may be involved in cardioprotection against ischemia/ reperfusion-induced injury(37,38). KATP channel opening has been shown to reduce infarct size, and to enhance post ischemic recovery of cardiac contractile force (39,40). Numerous studies have suggested that KATP channel blockers abolish the cardioprotective effects of KATP channel openers.(41) However, the degree of action potential shortening was divorced from the extent of protection.(42,43) . In addition, the treatment of non beating cardiomyocytes with KATP channel opener can exert a cardioprotective effect against ischemia / reperfusion-induced damage, in which action potential abbreviation can not be a factor (44). These findings suggest that the opening of sarcolemmal KATP channels appears not to be a major mechanism for cardioprotection against ischemia / reperfusion injury.

Recently, it has been shown that there is another isoform of (K_{ATP})channel on the mitochondrial inner membrane.(45) which differs from the sarcolemmal (K_{ATP}) channel,(46) this regulates electron transport in mitochondria and is blocked by K_{ATP} channel blocker glyburide.(41) We measured the oxygen consumption rate of saponin-skinned myocardial bundles to determine whether nicorandil or KRN 4884 may protect the cardiac mitochondria . At the end of the ischemia, the oxygen consumption rate of myocardial skinned bundle decreased, whereas this decreases was restored by reperfusion. Nicorandil and mainly KRN 4884 preserved this mitochondrial function of the heart during ischemia and reperfusion.(47,48,49,50) Thus, the results suggest that both nicorandil and KRN 4884 are capable of preserving mitochondrial oxidative phosphorylation activity during ischemia/ reperfusion.

Although we observed that nicorandil or KRN 4884 attenuated the ischemia-induced decrease in mitochondrial activity, it remained to be elucidated which agent may directly affect the mitochondria. Skinned myocardial bundles were prepared from the left ventricular free wall and exposed to 30-min hypoxia in presence of nicorandil or KRN 4884,this resulted in a decrease in mitochondrial oxygen consumption rate. When hypoxic skinned bundles were incubated in the presence of nicorandil or KRN 4884, the decrease in the oxygen consumption rate was attenuated. Therefore, sarcolemmal (K_{ATP}) channels of the skinned bundles appear not to functioning in our study. Accordingly, the agents used may act preferentially on mitochondrial rather than on sarcolemmal channels.(51) Thus, the observed preservation of mitochondrial consumption capacity by nicorandil or KRN 4884 may be exerted via mitochondrial (K_{ATP}) channel opening.

In a preliminary study, when perfused hearts were treated with nicorandil or KRN 4884 only during reperfusion (data not shown) ,the postischemic recovery of LVDP was not enhanced. The mitochondrial oxygen consumption rate of the untreated heart was also decreased at the end of ischemia before reperfusion injury and showed that presence nicorandil or KRN 4884 during ischemia is necessary to elicit improvement in recovery of contractility of the reperfused heart. If so, ischemia / reperfusion -induced damage to cardiac function might be initiated by impairment in mitochondrial function during ischemia but not during reperfusion. The results suggest that the reduction in mitochondrial oxygen consumption capacity in the ischemic heart may be one of the causes rather than effects of ischemia / reperfusion injury.

Ozcan et al (52) showed that oxidation of endogenous flavoprotein of cardiac cells, a marker of mitochondrial redox state, was increased by a mitochondrial K_{ATP} channel opener diazoxide, and suggested that mitochondrial (K_{ATP}) channels may mediate the proctection of (K_{ATP}) channel openers. So nicorandil and KRN 4884 might exert their cardioprotective effects in the ischemic / reperfused heart by enhancement of the mitochondrial oxidation and reduction.

In conclusion, the present study has shown that both nicorandil and KRN 4884 K_{ATP} channels openers are capable of protecting the myocardium against ischemia / reperfusion injury and enhancing the recovery of postischemic myocardial contractile function associated with restoration of myocardial high-energy phosphate content. The mechanism underlying the cardioprotective effect of nicorandil or KRN 4884 may be attributed to preservation of mitochondrial function during ischemia/ reperfusion

probably via activation of mitochondrial KATP channel opening.

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