Korean J Physiol Pharmacol
Vol 3: 587–595, December, 1999

Effects of Calcium Channel Blockers on Porcine Cardiac and Coronary Arterial Function in Ischemia-Reperfusion

Yung Hong Baik, Hyun Kook, Sun Hee Park, Seong Joo Jeong, and Dong Yoon Lim[1]

Department of Pharmacology, Research Institute of Medical Sciences, Chonnam University Medical School; [1]College of Medicine Chosun University, Kwangju 501–191, Korea

This study was designed to investigate effects of calcium antagonists on endothelial and neuronal dysfunction of right coronary artery (RCA) induced by ischemia- reperfusion in anesthetized, open-chest pigs. After reperfusion, pigs were sacrificed and the RCA was rapidly dissected for in vitro experiments. Experimental groups were divided into 4 groups: control (C-RCA), ischemia-reperfusion only (I-RCA), verapamil infusion (VI-RCA) and nifedipine infusion (NI-RCA) group, respectively. The ischemia did not affect hemodynamics, mean arterial pressure, heart rate, LV*dP/dt*max, and decreased RCA flow. Arterial pressure and heart rate during ischemia-reperfusion were decreased in VI-RCA and NI-RCA, and RCA flow during reperfusion was increased in NI-RCA. 5-Hydroxytryptamine (5-HT) produced concentration-dependent contractions in C-RCA. The 5-HT-induced contractions were potentiated in I-RCA and VI-RCA, but not in NI-RCA. Endothelium-dependent relaxation by calcium ionophore A23187 was inhibited in I-RCA and VI-RCA, and recovered in NI-RCA. Cyclic GMP contents were decreased in I-RCA group alone. Electrical field stimulation in C-RCA produced transient and frequency-dependent contractions and at 50 Hz caused biphasic contractions. The transient contractions were not affected by pretreatment with phentolamine and atropine, but the biphasic contraction was altered by the pretreatment. Both contractions were inhibited in I-RCA, and were partially recovered in VI-RCA and NI-RCA. Ischemia-reperfusion of RCA in pigs causes endothelial and neuronal dysfunctions, and calcium antagonists partially prevent both.

Key Words: Calcium antagonist, Porcine coronary artery, Ischemia, Reperfusion, Endothelial dysfunction

INTRODUCTION

The neuroeffector-endothelial interaction between perivascular nerves and vascular endothelium serves to maintain normal blood vessel tone (Tesfamariam et al, 1987; Matsuda et al, 1997). The presence of a network of perivascular adrenergic and nonadrenergic, noncholinergic nerve fibers has been identified in several vessels (Opgard & Edvinsson, 1998; Racchi et al, 1999). These fibers release a variety of secretory products. The vasoconstrictors norepinephrine (NE), adenosine 5'-triphosphate (ATP) and neuropeptide Y (NPY) seem to coexist in sympathetic nerve fibers and parasympathetic nerve fibers seem to release vasoactive intestinal polypeptide (VIP) with acetylcholine (ACh). Nonadrenergic, noncholinergic nerve fibers release a variety of neurotransmitters, including ATP, substance P, and calcitonin gene-related peptide (CGRP) (Burnstock, 1987; Opgard & Edvinsson, 1998).

The endothelial cells are local regulators of vascular function and in response to many humoral and physical stimuli, release a number of vasoactive substances including EDRF, endothelium-derived hyperpolarizing factor, prostacyclin (PGI_2), endothelium-derived contracting factors (EDCFs) and endothelin (Yanagisawa et al, 1988; Vane et al, 1990; Kovitz et al, 1993).

Recently, the myocardial injury after ischemia-reperfusion has been well established through a num-

Corresponding to: Yung Hong Baik, Department of Pharmacology, Chonnam University Medical School, Kwangju 501-191, Korea. (Tel) 062-220-4232, (Fax) 062-232-6974, (E-mail) yhbaik@chonnam.ac.kr

ber of studies (Chen et al, 1993; Klein et al, 1995). However, endothelial injury and dysfunction induced by ischemia-reperfusion remains to be defined. Some studies have proposed that ischemia-reperfusion attenuates endothelium-dependent vasodilation of coronary arteries (Nichols et al, 1988; Mehta et al, 1989); whereas, others have proposed that endothelium-dependent relaxation in large coronary arteries is relatively refractory to ischemia-reperfusion (Quillen et al, 1990).

Cytosolic calcium ions modulate a number of functions in neuronal cells (Hofmeier & Lux, 1981; Isenberg, 1986), myocardium and vascular smooth muscles (Schwartz & Triggle, 1984). Many studies proposed that calcium antagonists could protect the myocardium from injury after ischemia-reperfusion and that the protective effects may involve energy-sparing mechanisms (Kida et al, 1990; Yaghi & Watts, 1993) or other subcellular effects without inducing energy-sparing mechanisms during ischemia (Kida et al, 1990; Watts et al, 1991). Calcium antagonists have been proposed to improve myocardial recovery after ischemia-reperfusion (van Zwieten & Pfaffendorf, 1993; Noll & Luscher, 1998), however, their effects on coronary flow autoregulation remain to be defined. In addition, the understanding of the underlying mechanism of the endothelial dysfunction by hypoxia or ischemia-reperfusion in coronary artery is of particular interest because the endothelial dysfunction may play a role in the pathogenesis of coronary artery spasm (Shepherd et al, 1990). We investigated the effects of two calcium antagonists and their ability to protect from impairment of endothelial and perivascular nerve function induced by ischemia-reperfusion in the isolated porcine right coronary artery.

METHODS

Surgical preparation

Healthy farm pigs of both sexes weighing 19~35 kg were anesthetized with ketamine (10 mg/kg, intramuscular) plus sodium pentobarbital (~30 mg/kg intravenously, i.v.). The animal was ventilated with oxygen, and pancuronium (10 mg/h i.v.) was injected as a muscle relaxant. After median thoracotomy, the right coronary artery (RCA) was dissected free near its origin, and a coronary occluder (polyethylene tube and clip) was placed at the free portion. The reason using RCA instead of left anterior descending coronary artery (LAD) in this study was due to be able to obtain much longer segment of ischemic coronary artery than LAD. An electromagnetic flow probe (Nihon Kohden) was placed at the beginning of the distal third for continuous measurement of RCA flow. Left ventricular pressure was measured by a pressure transducer-tipped catheter (Miller Inst.) inserted into the left ventricle via an apical incision. Left ventricular pressure was electronically differentiated to obtain LV*dP/dt*max. Mean arterial pressure (MAP) was measured with a pressure transducer (Grass) in the right femoral artery, and heart rate (HR) was counted from the pulse. All signals were recorded on a polygraph (Grass).

Experimental protocols

After 30~60 min following surgical preparation, baseline values of hemodynamic parameters were obtained. The pigs were divided into 4 groups. In the nonischemic control group (C-RCA, n=3), the RCA was not occluded and received no treatments. In the ischemia-reperfusion group (I-RCA, n=4), the RCA was occluded for 40 min and then reperfused for 60 min and saline (2 ml/min, i.v.) was infused from 20 min before the occlusion of RCA and throughout the reperfusion period. In the verapamil-ischemia-reperfusion group (VI-RCA, n=4) and nifedipine-ischemia-reperfusion group (NI-RCA, n=4), verapamil (5 μg/kg/min, i.v.) and nifedipine (0.2 μg/kg/min, i.v.), respectively, were infused as described for I-RCA. After the reperfusion period, the heart was rapidly excised for in vitro studies on changes of vascular functions and placed in ice cold saline.

Tension experiments

The right coronary artery (RCA) was carefully removed from the heart and trimmed clean of connective tissues. The cleaned artery was then cut into rings (width 5~6 mm) or spiral strips (~1.5 mm long, ~2 mm wide). The ring preparations were used to investigate changes of tension induced by drugs, and the spiral strips were used to investigate effects of electrical field stimulation (EFS) on the RCA. The rings and the strips were mounted under 5 g and 2 g force resting tension in tissue baths, respectively. The changes in tension were recorded on a polygraph

(Grass) through the isometric force transducer. The bath solution was saturated with 95% O_2/5% CO_2 at 37°C (pH=7.35). The composition of physiological salt solution (PSS) was as follows (in mM): NaCl, 126.9; KCl, 4.7; $CaCl_2$, 1.6; $MgSO_4$, 1.17; KH_2PO_4, 1.18; $NaHCO_3$, 18.0; and dextrose, 5.5. The preparations were equilibrated in PSS for 2 hr and tested for viability by challenging with 35 mM KCl.

Cyclic GMP studies

The experimental protocols for assaying cyclic GMP were designed to parallel the conditions used in the tension experiment. After 2 hr of equilibration, each ring was exposed to 2 times of 35 mM KCl and washed out the KCl with fresh PSS. The preparations were rapidly frozen between clamps precooled in liquid nitrozen and kept at −80°C. Frozen tissues were homogenized in 0.5 ml of 10% trichloroacetic acid. The homogenate was centrifuged at 2500×g for 30 min at 4°C. Supernatant fractions were extracted with water-saturated ether, and a portion of aqueous solution was acetylated and tested by radioimmunoassay (RIA) for cyclic GMP.

Electrical field stimulation

After challenging with KCl, a pair of platinum electrodes connected with the electrical stimulater (Grass) was inserted into the tissue baths. The electrical field stimulation was delivered to the strips via the electrodes, and square wave 5 msec pulses of supramaximal voltage (50 V) were applied at selected frequencies (5, 10, 20, 50 Hz) for 20 seconds.

Materials and statistics

Calcium ionophore A23187 was obtained from Calbiochem; 5-hydroxytryptamine creatinine sulfate, prostaglandin $F_{2\alpha}$, verapamil HCl, and atropine sulfate were obtained from Sigma, nifedipine and phentolamine mesylate were obtained from Miles Lab. and RBI, respectively, and cyclic GMP RIA kit was obtained from NEM/DuPont.

Values are expressed as means±SE. Significance was determined by Student's unpaired *t* test, $p<0.05$.

RESULTS

Hemodynamic changes in ischemia-reperfusion-induced hearts

During 40 min of RCA occlusion and 60 min of reperfusion (ischemia-reperfusion) in 3 groups, hemodynamic changes are summarized in Table 1. RCA occlusion for 40 min and 60 min reperfusion did not cause any significant changes of mean arterial blood pressure, heart rate and LV*dP/dt*max. RCA flow was abolished during the occlusion and slightly increased during the reperfusion, but this increase was not significant. Verapamil (5 μg/kg/min) infusion decreased significantly the blood pressure during ischemia-reperfusion and heart rate and LVdP/*dt*max were reduced during the reperfusion, and RCA flow showed a tendency to slightly increase during reperfusion. The blood pressure was decreased from 20 min after infusion of nifedipine (0.2 μg/kg/min) to the end of the reperfusion. The nifedipine infusion increased the heart rate and RCA flow during the reperfusion, but did not affect LV*dP/dt*max. The pigs were sacrificed at the end of 60 min of the reperfusion, and the hearts were dissected for in vitro experiments.

5-Hydroxytryptamine-induced contractions

5-HT contracted the ring preparations in the C-RCA group obtained from the control hearts in a concentration-dependent fashion (Fig. 1). The 5-HT-induced contractions were markedly potentiated in the rings in the I-RCA group. This supersensitivity of 5-HT contraction was not affected in the rings in the VI-RCA group but was significantly inhibited in the rings in the NI-RCA group.

Calcium ionophore A23187-induced relaxation

A23187 (10^{-6} M) markedly relaxed the rings of the C-RCA group precontracted with 10^{-6} M prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) (Fig. 2). The endothelium-dependent relaxation by A23187 was significantly reduced in the rings of the I-RCA group, partially recovered in the rings of the VI-RCA group, and almost completely recovered in the rings of the NI-RCA group.

Table 1. Hemodynamic variables in anesthetized open-chest pigs at 40 min of ischemia and 60 min of reperfusion in right coronary artery

Treatments	MAP (mmHg)	HR (beats/min)	LV*dP/dt*max (mmHg/s)	RCAflow (ml/min)
Saline				
Baseline	101±6	89±6	2650±140	18±4
Post-saline	98±6	92±5	2490±160	19±5
Ischemia	92±7	86±7	2430±210	2±4
Reperfusion	87±7	79±8	2180±170	27±6
Verapamil				
Baseline	103±7	92±5	2560±180	17±4
Post-V	84±6	81±6	2310±160	20±5
Ischemia	75±8*	74±7	1980±190	3±5
Reperfusion	68±7*	66±7*	1760±210*	30±6
Nifedipine				
Baseline	97±5	88±6	2560±170	19±5
Post-N	74±6*	96±8	2480±150	26±6
Ischemia	65±7*	102±7	2160±180	3±4
Reperfusion	63±6*	115±8*	2350±210	41±6*

Values are means±SE; n=4 for each group; MAP=mean arterial pressure; HR=heart rate; LV*dP/dt*max=maximum rise in left ventricular pressure; RCA flow=right coronary artery blood flow. * $p<0.05$ versus baseline. In each group, the infusion of the indicated agents was started 20 min before the RCA occlusion and was continued to 60 min of reperfusion. Post-saline, Post-V and Post-N indicate measurements at 20 min after the start of the infusion of saline (2 ml/min), verapamil (5 μg/kg/min) and nifedipine (0.2 μg/kg/min), respectively.

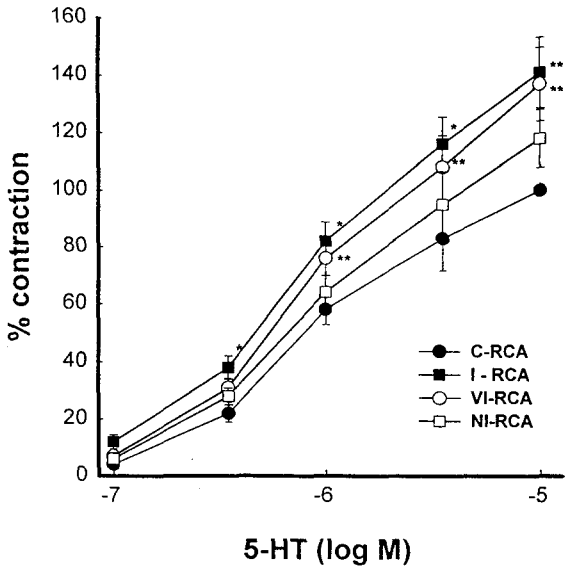

Fig. 1. Effects of verapamil- and nifedipine-infusion on the potentiation of 5-hydroxytryptamine-induced contractions by ischemia-reperfusion in porcine right coronary artery. Each curve shows means±SE obtained from 6~10 rings. * $p<0.05$ and ** $p<0.01$ vs C-RCA. See text for other details.

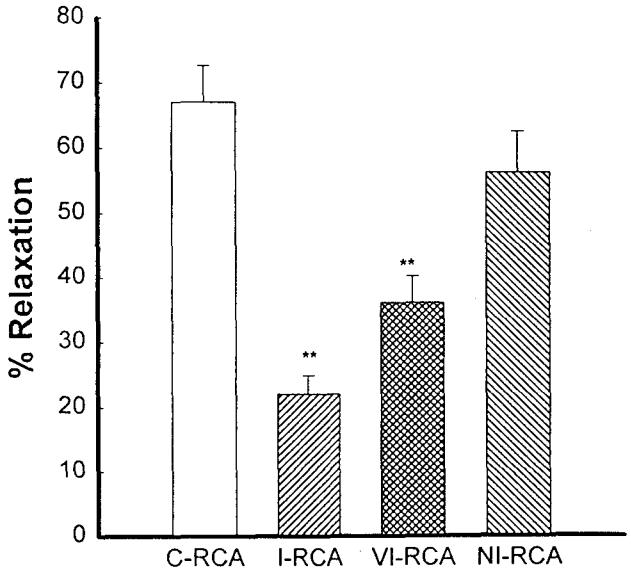

Fig. 2. Effects of verapamil- and nifedipine-infusion on the inhibition of calcium ionophore A23187-induced relaxation by ischemia-reperfusion in porcine right coronary artery. Each column shows means±SE obtained from 8-10 rings. ** $p<0.01$ vs C-RCA. See text for other details.

Changes of cyclic GMP contents

In the rings of the C-RCA group, cyclic GMP content was 25.7±3.7 pmole/g wet weight (mean± SE, n=12). The cyclic GMP content decreased in the rings of the I-RCA group, and the content in the rings of VI-RCA and NI-RCA groups was comparable to the C-RCA group (Fig. 3).

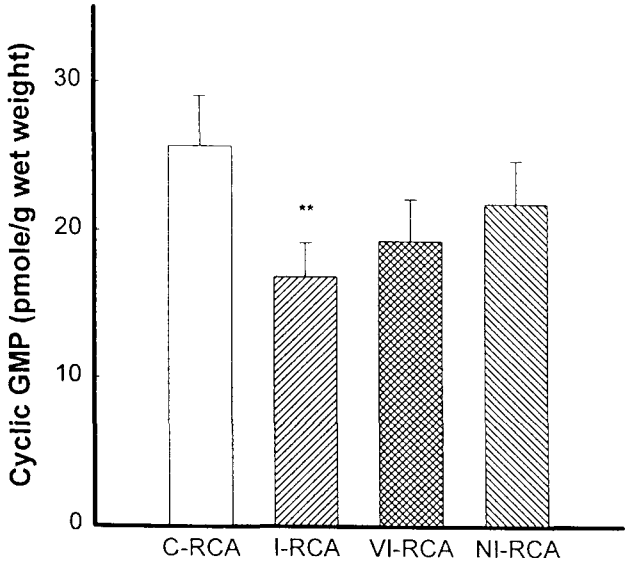

Fig. 3. Effects of verapamil- and nifedipine-infusion on the decrease of cyclic GMP contents induced by ischemia-reperfusion in porcine right coronary artery. Each column shows means±SE obtained from 8~10 rings. ** $p<0.01$ vs C-RCA. See text for other details.

Electrical field stimulation-induced contractions

The EFS produced transient and frequency-dependent contractions in the rings of C-RCA group. High frequency (50 Hz) stimulation elicited a biphasic contraction, a transient contraction followed by a slow and prolonged contraction (Fig. 4). The prolonged contraction was abolished with exposure to 10^{-6} M phentolamine and 10^{-6} M atropine, but the transient contractions were not affected by the pretreatment with both drugs. Compared with the rings of C-RCA group, both contractions (transient and biphasic) in the rings of I-RCA group were significantly inhibited. Both inhibited contractions of the I-RCA group demonstrated a tendency to recover in the rings of VI-RCA and of NI-RCA groups (Fig. 4 and 5).

DISCUSSION

The aim of the present study was to determine whether calcium antagonists have a beneficial effect on endothelial and perivascular nerve dysfunction of

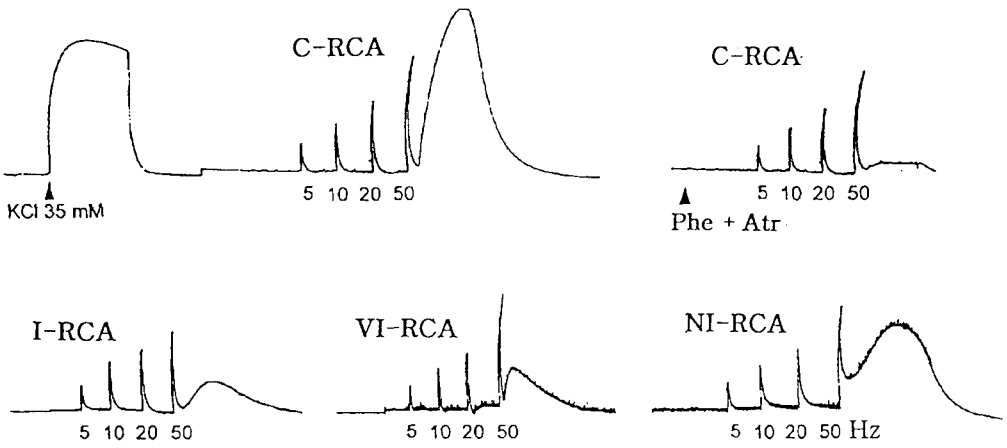

Fig. 4. Typical traces showing effects of verapamil- and nifedipine-infusion on the inhibition of transient and frequency-dependent contractions and the biphasic contractions by ischemia-reperfusion in porcine right coronary artery. Numerals indicate the stimulation frequency (Hz). Phe+Atr indicates the addition of 10^{-6} M phentolamine and 10^{-6} M atropine to the bath. Electrical field stimulations were delivered via two parallel platinum electrodes placed along both sides of the spiral strips. Pulses were fixed at 50 Volts and 5 msec durations and were delivered over a 20 sec period at frequencies ranging from 5 to 50 Hz. See text for other details.

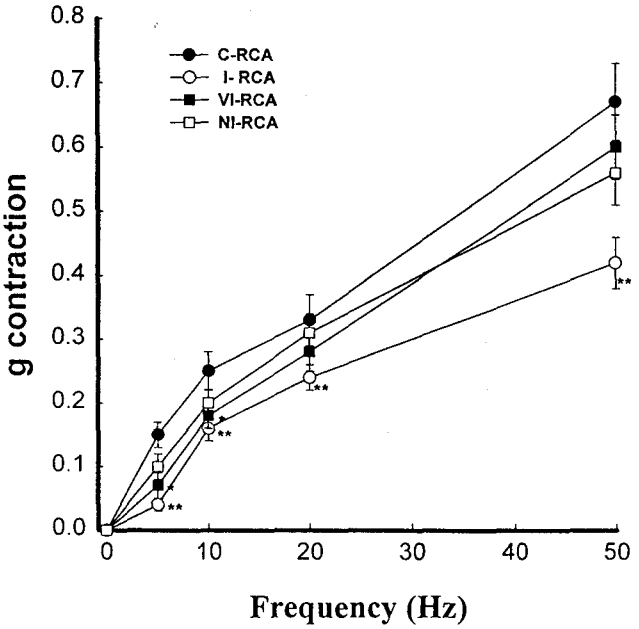

Fig. 5. Effects of verapamil- and nifedipine-infusion on the inhibition of transient and frequency- dependent contractions by ischemia-reperfusion in porcine right coronary artery. Each curve shows means ± SE obtained from 6~8 rings pretreated with 10^{-6} M phentolamine and 10^{-6} M atropine. * $p<0.05$ and ** $p<0.01$ vs C-RCA. See text for other details.

RCA induced by ischemia-reperfusion in open-chest pigs. Ischemia-reperfusion did not change cardiac functions including blood pressure, heart rate, and LV*dP/dt*max, with the exception of a decrease in RCA flow during ischemia. However, after ischemia-reperfusion, the endothelium-dependent relaxation by calcium ionophore A23187 (Horwitz et al, 1990) was inhibited, and the 5-HT-induced contraction was potentiated in comparison to the corresponding controls. Cyclic GMP contents were decreased by ischemia-reperfusion. The results suggest that ischemia-reperfusion caused an endothelial dysfunction and a supersensitivity to 5-HT contraction. It has been known that hypoxia or ischemia-reperfusion attenuates endothelium-dependent relaxations in various arteries including coronary arteries both in vitro and in vivo (Nichols et al, 1988; Kovitz et al, 1993; Dagenais et al, 1997). However, the precise mechanism of endothelial dysfunction induced by hypoxia or ischemia-reperfusion has been unsolved. Recently, proposed mechanisms of endothelial dysfunction include (1) an increase in release of EDCFs (Holden & McCall, 1984; Johns et al, 1989); (2) a decrease in EDRF release (Tsao et al, 1990; Graser & Vanhoutte, 1991); and (3) disruption of the balance of constrictor and dilator tone (VanBenthuysen et al, 1987; Watts et al, 1992).

5-HT produces contractile responses in quiescent preparations of isolated blood vessels, but 5-HT induces endothelium-dependent relaxations in the preparations precontracted with a vasoconstrictor agent (Moldering et al, 1989; Schoeffter & Hoyer, 1990). The potentiation of 5-HT contractions in the I-RCA group suggests that the supersensitivity results from a decrease of EDRF release from the endothelium impaired by ischemia-reperfusion. This hypothesis is supported by other reports that the response of coronary arteries to 5-HT changes from dilation to constriction after balloon denudation (Shimokawa & Vanhoutte, 1991; Shibano & Vanhoutte, 1994) and that 5-HT releases EDRF from endothelium and increases cyclic GMP content in arteries (Glusa & Richter, 1993; Dagenais et al, 1997).

Infusion with verapamil decreased blood pressure, heart rate, and LV*dP/dt*max except RCA flow during the reperfusion period, while nifedipine-infusion decreased blood pressure but increased heart rate and RCA flow during the reperfusion period. The differing hemodynamic changes result from differences in their selectivities. Verapamil is more selective for cardiac muscles than vascular smooth muscles; whereas, nifedipine exhibitis the reverse sequence of selectivities (van Zwieten & Pfaffendorf, 1993; Noll & Luscher, 1998). Verapamil inhibited cardiac contractility and heart rate as well as vasodilation. However, nifedipine caused a prominent vasodilation in peripheral and coronary arteries with subsequent hypotension and increase in RCA flow with a reflex tachycardia. These hemodynamic changes by nifedipine were similar to the changes by the treatment with nisoldipine, another derivative of 1,4-dihydropyridine calcium antagonist (Sassen et al, 1991). In addition, calcium antagonists can act on both vascular and myocardial cells, increase coronary blood supply, and decrease energy demand of cardiac cells during hypoxia or ischemia-reperfusion (Nayler, 1987; Dagenais et al, 1997).

In the present studiy, endothelial dysfunction was evaluated by inhibition of calcium ionophore A23187-mediated relaxation, supersensitivity to 5-HT contraction, and decrease in cyclic GMP contents. Infusion with verapamil showed a tendency only to ameliorate endothelial dysfunction after ischemia-reperfusion,

while infusion with nifedipine improved endothelial function. These findings agree in part with other reports that the endothelium-dependent relaxation is suppressed after ischemia-reperfusion (Tsao et al, 1990; Ma et al, 1991) and that verapamil and nisoldipine reduced the damage to endothelium-dependent relaxation associated with myocardial ischemia-reperfusion in perfused hearts (VanBebthuysen et al, 1987; Yaghi & Watts, 1993; Dagenias et al, 1997). The mechanisms of the beneficial effect of calcium blockers on the endothelial dysfunction after ischemia-reperfusion observed in these studies are not well understood. There appear to be three plausible explanations for the beneficial effect. First, calcium channel blockers inhibit Ca^{2+} overload in endothelial and smooth muscle cells via an influx through L-type channels. The Ca^{2+} overload, which occurs upon ischemia-reperfusion, can trigger a chain of destructive events in the endothelial cells (Opie, 1989; Sassen et al, 1991). Second, calcium blockers prevent the ATP depletion associated with ischemia-reperfusion. This effect could be accomplished by the negative inotropy and reduction of preload and afterload, which may explain the energy-sparing effects of calcium channel blockers (Kida et al, 1990; van Amsterdam et al, 1990; Levy et al, 1997). Finally, Calcium blockers have antioxidant properties, which can reduce the endothelial impairment after ischemia-reperfusion (Herbette et al, 1989; Mak et al, 1992; Yaghi & Watts, 1993). It is well established that endothelial injury was more rapidly induced by ischemia-reperfusion than by ischemia alone, because the endothelial damage results from interactions between oxygen free radicals induced by reperfusion, the endothelial cells, neutrophils, and other factors (Shirai et al, 1998; Hayward & Lefer, 1999). We observed that the beneficial effect of nifedipine on the endothelial dysfunction was higher than the effect of verapamil. The difference result from that the potency of incorporation into biological membranes and antioxidant activity may be higher in nifedipine than in verapamil (Herbette et al, 1989; Mak et al, 1992; Yaghi & Watts, 1993).

EFS of low frequencies (<50 Hz) produced transient and frequency-dependent contractions, but at a higher frequency (50 Hz) caused a biphasic contraction, a transient relaxation followed by a prolonged contraction. The transient contractions were resistant to pretreatment with phentolamine and atropine, but the prolonged component of the biphasic contraction was abolished by the pretreatment. The perivascular cholinergic nerves release VIP with ACh, and both neurotransmitters mediate vasodilation, while the sympathetic fibers release NE with ATP, both of which mediate the vasoconstriction (Burnstock, 1987; Claing et al, 1992; Opgard & Edvinsson, 1998). These results suggest that the prolonged contraction is mediated by perivascular adrenergic nerves, and the transient contractions are mediated by noncholinergic, nonadrenergic fibers.

Both transient and biphasic contractions were inhibited after ischemia-reperfusion, and the prolonged component was more sensitive. Okamura et al (1997) has reported that hypoxia reduced the activity of perivascular nerve in canine cerebral artery. This suggests that ischemia-reperfusion can attenuate the perivascular nerve activities and that the adrenergic nerves are more sensitive to ischemia-reperfusion than the noncholinergic, nonadrenergic fibers. The inhibitory effect of both contractions by ischemia-reperfusion was recovered in part by the pretreatment with verapamil and nifedipine, with the latter demonstrating greater efficacy. It has been reported in various neural tissues that hypoxia or ischemia-reperfusion induces elevation of intracellular Ca^{2+}, and the elevated cytosolic Ca^{2+} results in neuronal dysfunction (Ohta et al, 1991; Xie et al, 1992; Kaplin et al, 1996). In addition, there were reports that calcium antagonists improved or protected the neuronal dysfunction via a blockade of Ca^{2+} influx or via an improvement of other Ca^{2+} metabolisms (Mattson & Cheng, 1993; Okamura et al, 1997). It appears that calcium antagonists have some beneficial effects on perivascular nerve dysfunction after ischemia-reperfusion and that, in this respect, nifedipine is more effective than verapamil. Although our findings do not completely elucidate the mechanism of the protective action, they do suggest a role for nifedipine. That is, nifedipine is more selective to vascular tissues, more lipophylic and a more potent antioxidant than verapamil (Herbette et al, 1989; Yaghi & Watts, 1993).

In conclusion, ischemia-reperfusion in RCA of open-chest pigs induces endothelial and perivascular nerve dysfunction, and calcium antagonists have a beneficial effect.

ACKNOWLEGEMENT

This study was supported by '95 SPECIAL FUND

(F0903) for UNIVERSITY RESEARCH INSTITUTE, Korea Research Foundation.

REFERENCES

Burnstock G. Mechanisms of interaction of peptide and nonpeptide vascular neurotransmitter systems. *J Cardiovasc Pharmacol* 12: S74−S81, 1987

Chen CC, Morishige N, Masuda M, et al. R56865, an Na^+-and Ca^{2+}-overload inhibitor, reduces myocardial ischemia-reperfusion injury in blood perfused rabbit hearts. *J Mol Cell Cardiol* 25: 1445−1459, 1993

Claing A, Telemaque S, Cadieux A, Fournier A, Regoli D, D'Orleans Juste P. Nonadrenergic and noncholinergic arterial dilatation and venoconstriction are mediated by calcitonin gene-related peptidel and neurokinin-1 receotprs, respectively, in the mesenteric vasculature of the rat after perivascular nerve stimulation. *J Pharmacol Exp Ther* 263: 1226−1232, 1992

Dagenais F, Cartier R, Hollmann C, Buluran J. Calcium-channel blockers preserve coronary endothelial reactivity after ischemia-reperfusion. *Ann Thorac Surg* 63: 1050−1056, 1997

Glusa E, Richter M. Endothelium-dependent relaxation of porcine pulmonary arteries via 5-HT_{1C}-like receptors. *Naunyn Schmiedebergs Arch Pharmacol* 347: 471−477, 1993

Graser T, Vanhoutte PM. Hypoxic contraction of canine coronary arteries: role of endothelium and cGMP. *AM J Physiol* 261: H1769−H1777, 1991

Hayward R, Lefer AM. Time course of endothelial-neutrophil interaction in splanchnic artery ischemia-reperfusion. *Am J Physiol* 275: H2080−H2086, 1999

Herbette LG, VantErve YMH, Rhodes DG. Interaction of 1,4 dihydropyridine calcium channel antagonists with biological membranes: lipid bilayer partitioning could occur before drug binding to receptors. *J Mol Cell Cardiol* 21: 187−201, 1989

Hofmeier G, Lux HD. The time courses of intracellular free calcium and related electrical effects after injection of $CaCl_2$ into neurons of the snail, Helix pomatia. *Pflueg Arch* 391: 242−251, 1981

Holden WE, McCall E. Hypoxia-induced contractions of porcine pulmonary artery strips depend on intact endothelium. *Exp Lung Res* 7: 101−112, 1984

Horwitz LD, VanBenthuysen KM, Sheridan FM, Lesnefsky EJ, Dauber IM, McMurtry IF. Coronary endothelial dysfunction from ischemia and reperfusion: effect of reactive oxygen metabolite scavengers. *Free Radic Biol Med* 8: 381−386, 1990

Isenberg G. Ca-dependent and Ca-independent F-actin capping proteins determine microfilament assembly in neuronal cells. In: Calcium Electrogenesis and Neuronal Functioning, edited by Heinemann U, Klee, M, Neher E and Singer W, *Springer* pp 271−278, 1986

Johns RA, Linden JM, Peach MJ. ENdothelium-dependent relaxation and cyclic GMP accumulation in rabbit pulmonary artery are selectively impaired by moderate hypoxia. *Circ Res* 65: 1508−1515, 1989

Kaplin AI, Snyder SH Linden DJ. Reduced nicotinamide adenine dinucleotide-selective stimulation of inositol 1,4,5-trisphosphate receptors mediates hypoxic mobilization of calcium. *J Neurosci* 16: 2002−2011, 1996

Kida M, Fujiwara H, Ischida M, Kawai C, Fujiki H, Ohura M, Miura I, Yabuuchi Y. Protective effect of verapamil in ischemic porcine hearts: analysis of ultrastructural and metabolic changes using in vivo ^{31}P-NMR spectroscopy. *J Mol Cell Cardiol* 22: 759−769, 1990

Klein HH, Pich S, Lindert-Heimberg S, Maisch B, Nebendahl K. Effects of R56865, an Na^+-and Ca^{2+}-overload inhibitor, on myocardial injury in ischemic, reperfused porcine hearts. *J Cardiovasc Pharmacol* 25: 163−167, 1995

Kovitz KL, Aleskowitch TD, Sylvester JT, Flavahan NA. Endothelium-derived contracting and relaxing factors contribute to hypoxic responses of pulmonary arteries. *Am J Physiol* 265: H1139−H1148, 1993

Levy E, Hasin Y, Navon G, Horowitz M. Chronic heat improves mechanical and metabolic response of trained rat heart on ischemia and reperfusion. *Am J Physiol* 272: H2085−H2094, 1997

Ma XL, Tsao PS, Viehman GE, Lefer AM. Neutrophil-mediated vasoconstriction and endothelial dysfunction in low-flow perfusion-reperfused cat coronary artery. *Circ Res* 69: 95−106, 1991

Mak T, Boehme P, Weglicki WB. Antioxidant effects of calcium channel blockers against free radical injury in endothelial cells. Correlation of protection with preservation of glutathione levels. *Circ Res* 70: 1099−1103, 1992

Matsuda H, Dawaguchi A, Tamai J, Uematsu M, Nagata S, Miyatake K. Attenuated inhibition of adrenergic contraction by nitric oxide in injured guinea pig femoral artery. *Heart Vessels* 12: 10−18, 1997

Mattson MP, Cheng B. Growth factors protect neurons against excitotoxic/ischemic damage by stabilizing calcium homeostasis. *Stroke* 24: I136−I140, 1993

Mehta JL, Nichols WW, Donnelly WH, Lawson DL, Saldeen TGP. Impaired canine coronary vasodilator response to acetylcholine and bradykinin after occlusion-reperfusion. *Circulation* 64: 43−54, 1989

Molderings GJ, Engel G, Roth E, Gothert M. Characterization of an endothelial 5-hydroxytryptamine (5-HT) receptor mediating relaxation of the porcine coronary artery. *Naunyn-Schmiedeberg's Arch Pharmacol* 340: 300−308, 1989

Nayler WG. Calcium antagonists and the ischemic myocardium. *Int J Cardiol* 15: 267−285, 1987

Nichols WW, Mehta JL, Donnelly WH, Lawson D, Thompson L, Ter Riet M. Reduction in coronary vasodilator reserve following coronary occlusion and reperfusion in anesthetized dog: role of endothelium-derived relaxing factor, myocardial neutrophil infiltration and prostaglandins. *J Mol Cell Cardiol* 20: 943−954, 1988

Noll G, Luscher TF. Comparative pharmacological properties among calcium channel blockers: T-channel versus L-channel blockade. *Cardiology* 89 Suppl 1: 10−15, 1998

Ohta S, Smith ML, Siesjo BK. The effect of a dihydropyridine calcium antagonist (isradipine) on selective neuronal necrosis. *J Neurol Sci* 103: 109−115, 1991

Okamura T, Ayajiki K, Toda N. Hypoxia-induced inhibition of the response to nitroxidergic nerve stimulation in canine cerebral arteries. *J Cereb Blood Flow Metab* 17: 807−813, 1997

Opgard OS, Edvinsson L. Vasoactive substances affect vascular tonus. New perspectives for prevention and treatment. *Lakartidningen* 95: 160−163, 1998

Opie LH. Reperfusion injury and its pharmacologic modification. *Circulation* 80: 1049−1063, 1989

Quillen JE, Sellke FW, Brooks LA, Harrison DG. Ischemia-reperfusion impairs endothelium-dependent relaxation of coronary microvessels but does not affect large arteries. *Circulation* 82: 586−594, 1990

Racchi H, Irarrazabal MJ, Howard M, Moran S, Zalaquett R, Huidobro-Toro JP. Adenosine 5'-triphosphate and neuropeptide Y are co-transmitters in conjunction with noradrenaline in the human saphenous vein. *Br J Pharmacol* 126: 1175−1185, 1999

Sassen LMA, Bezstarosti K, Verdouw PD, Lamers JMJ. Effects of nisoldipine on recovery of coronary blood flow, sarcoplasmic reticulum function and other biochemical parameters in post-ischaemic porcine myocardium. *Biochem Pharmacol* 41: 43−51, 1991

Schoeffter P, Hoyer D. 5-Hydroxytryptamine (5-HT)-induced endothelium-dependent relaxation of pig coronary arteries is mediated by 5-HT receptors similar to the 5-HT_{1D} receptor subtype. *J Pharmacol Exp Ther* 252: 387−395, 1990

Schwartz A, Triggle DJ. Cellular action of calcium blocking drugs. *Ann Rev Med* 35: 325−339, 1984

Shepherd JT, Katusic ZS, Vedernikov YP, Vanhoutte PM. Mechanisms of coronary vasospasm: role of endothelium. *J Mol Cell Cardiol* 23: 125−131, 1990

Shibano T, Vanhoutte PM. Involvement of 5-HT_2 receptors in chronic endothelial dysfunction after balloon injury of porcine coronary arteries. *Circulation* 89: 1776−1785, 1994

Shimokawa H, Vanhoutte PM. Angiographic demonstration of hyperconstriction induced by serotonin and aggregating platelets in porcine coronary arteries with regenerated endothelium. *J Am Coll Cardiol* 17: 1197−1202, 1991

Shirai T, Rao V, Weisel RD, Ikonomidis JS, Li RK, Tumiati LC, Merante F, Mickle DA. Preconditioning human cardiomyocytes and endothelial cells. *J Thorac Cardiovasc Surg* 115: 210−219, 1998

Tesfamariam B, Weisbrod RM, Cohen RA. Endothelium inhibits responses of rabbit carotid artery to adrenergic nerve stimulation. *Am J Physiol* 253: H792−H798, 1987

Tsao PS, Aoki N, Lefer DJ, Johnson G 3d, Lefer AM. Time course of endothelial dysfunction and myocardial injury during myocardial ischemia and reperfusion in the cat. *Circulation* 82: 1402−1412, 1990

van Amsterdam FTM, Punt NC, Haas M, Zaagsma J. Calcium antagonists show two modes of protection in ischemic heart failure. *J Pharmacol Exp Ther* 253: 277−283, 1990

Van Zwieten PA, Pfaffendorf M. Similarities and differences between calcium antagonists: pharmacological aspects. *J Hypertens Suppl* 11: S3−S11, 1993

VanBenthuysen KM, McMurtry IF, Horwitz LD. Reperfusion fater acute coronary occlusion in dogs impairs endothelium-dependent relaxation to acetylcholine and augments contractile reactivity in vitro. *J Clin Invest* 79: 265−274, 1987

Vane JR, ANggard EE, Botting RM. Regulatory functions of the vascular endothelium. *N Engl J Med* 323: 27−36, 1990

Watts JA, Chapat S, Johnson DE, Janis RA. Effects of nisoldipine upon vasoconstrictor responses and binding of endothelin-1 in ischemic and reperfused rat hearts. *J Cardiovasc Pharmacol* 19: 929−936, 1992

Watts JA, Hawes EM, Jenkins SH, Williams TC. Effects of nisoldipine on the no-reflow phenomenon in globally ischemic rat hearts. *J Cardiovasc Pharmacol* 16: 487−494, 1991

Xie Y, Seo K, Ishimaru K, Hossmann KA. Effect of calcium antagonists on postischemic protein biosynthesis in gerbil brain. *Stroke* 23: 87−92, 1992

Yaghi MM, Watts JA. Effects of nisoldipine upon endothelial dysfunction following ischaemic and peroxidative injury in the perfused rat heart. *Cardiovasc Res* 27: 990−996, 1993

Yanagisawa M, Kurihara H, Kimura S, Tomobe Y, Kobayashi M, Mitsui Y, Yazaki Y, Goto K, Masaki T. A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature Lond* 332: 411−415, 1988