8. Results

8.1. Effects of an intracoronary administration of sarpogrelate on coronary hemodynamics

As shown in Fig. 1, both baseline heart rate (HR) and CPP in each group were similar during the experiment. In the sarpo group, baseline HR and CPP averaged 152 ± 10 bpm and 104 ± 8 mmHg, respectively. When CBF was reduced to 33% of baseline, CPP of the LAD coronary artery was decreased to 42 ± 2 mmHg and kept constant thereafter. The intracoronary administration of sarpogrelate increased CBF compared with the control group (33.6 ± 6.1 vs. 44.5 ± 4.4 ml/100 g/min, $P < 0.05$) after 15 min of infusion, and this increase in CBF was completely abrogated by the co-administration of either L-NAME or GR55562 (Fig. 2).

8.2. Cardiac release of NOx in the ischemic canine heart

The infusion of sarpogrelate significantly increased the cardiac release of NOx compared with that of the control group after 20 min of hypoperfusion. Similarly, this increase was abolished by the co-administration of either L-NAME or GR55562 (Fig. 3).

8.3. Cardiac release of serotonin in the ischemic canine heart

Treatment with sarpogrelate for 15 min in a hypoperfused state significantly increased the cardiac release of serotonin (–4.8 ± 3.2 baseline vs. 22.1 ± 1.5 ng/ml, $P < 0.05$), which also reached a significant level compared with the control group after 20 min of hypoperfusion (Fig. 4).

8.4. Serotonin expression in the ischemic canine heart

Immunohistochemical analysis revealed that serotonin was weakly expressed in the non-ischemic heart and potently induced in the ischemic heart after sarpogrelate infusion (Fig. 5).

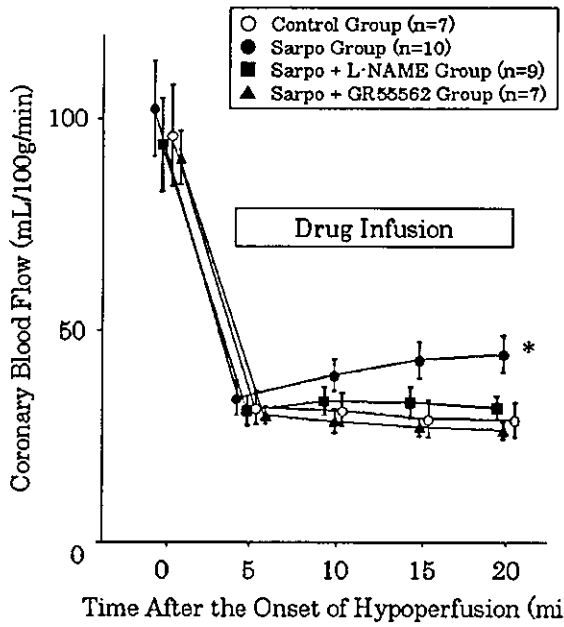

Fig. 2. Changes in CBF after infusion of sarpogrelate with and without L-NAME or GR55562. Although reduced CPP was at a constant low level, sarpogrelate increased CBF, with the effect being blunted by L-NAME or GR55562. * $P < 0.05$ versus the control group.

9. Discussion

We demonstrated here that sarpogrelate, an antagonist of 5-HT2A receptor, increased CBF via a NO-dependent mechanism through 5-HT1B receptor in hypoperfused canine hearts, along with the increase in the cardiac release of serotonin which may be produced in the ischemic myocardium.

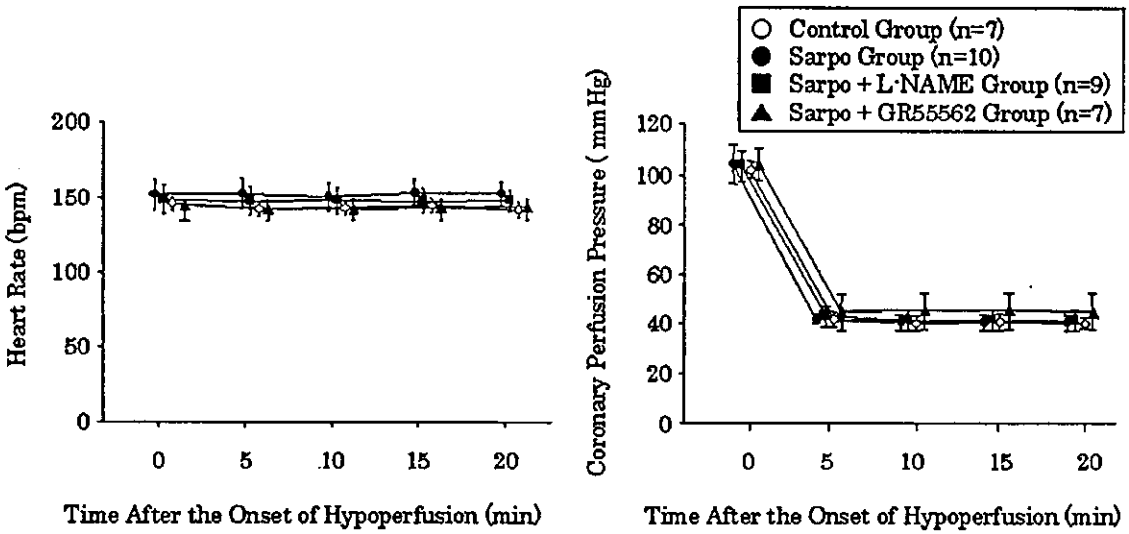

Fig. 1. Changes in HR and CPP among the control, sarpo, sarpo + L-NAME and sarpo + GR55562 groups. Both HR and CPP in each group were similar during the experiment.

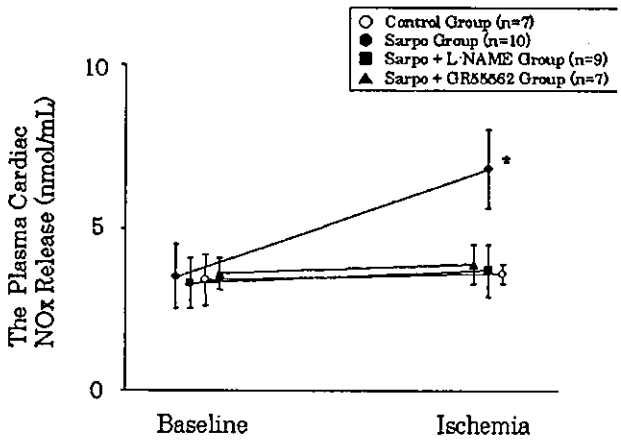

Fig. 3. Changes in the differences in the plasma levels of NOx between coronary arterial and venous blood among the control, sarpo, sarpo + L-NAME and sarpo + GR55562 groups. * $P < 0.05$ versus the control group after 20 min of hypoperfusion (ischemia).

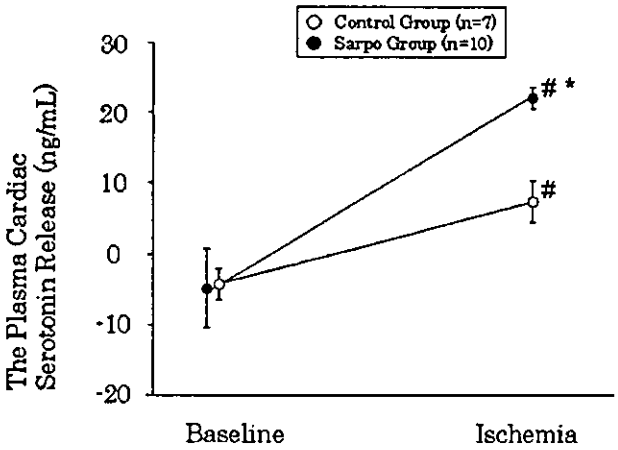

Fig. 4. Changes in the differences in the plasma levels of serotonin between coronary arterial and venous blood among the control and sarpo groups. * $P < 0.05$ versus the control group after 20 min of hypoperfusion (ischemia). # $P < 0.05$ versus before the onset of hypoperfusion (baseline) in each group.

9.1. Mechanisms by which sarpogrelate increased CBF and the cardiac release of NOx in the ischemic myocardium

In this experiment, we clearly demonstrated that sarpogrelate increased CBF in the hypoperfused hearts which may improve myocardial ischemia. Furthermore, the increase in CBF induced by sarpogrelate was abrogated by either the inhibition of NOS by L-NAME or the blockade of 5-HT1B receptor by GR55562. These results suggested that the increase in CBF induced by sarpogrelate was involved in both NOS and 5-HT1B receptors. Coincident with these findings, we confirmed that sarpogrelate increased NOx release in the coronary circulation, which was blunted by co-administration with GR55562. Thus, sarpogrelate increased CBF via a NO-dependent mechanism through 5-HT1B receptors.

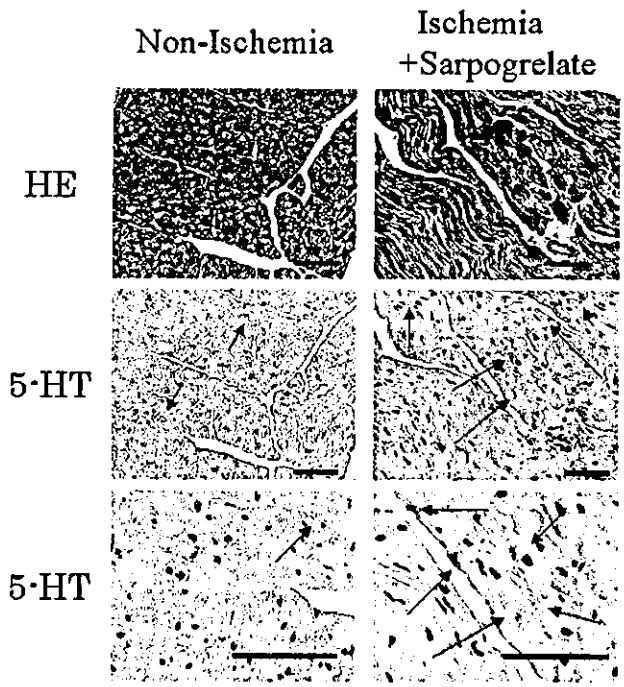

Fig. 5. Immunohistochemical analysis shows staining of serotonin in the non-ischemic myocardium and the ischemic myocardium after 20 min infusion of sarpogrelate. Upper panels show hematoxylin–eosin (HE) staining (×200). Middle (×200) and lower (×400) panels show immunohistochemical staining against 5-HT antibody. Bar indicates 1 μm.

9.2. Mechanisms by which sarpogrelate increased the cardiac release of serotonin in the ischemic myocardium

Vanhoutte [2] reported that serotonin may also enhance NO production via activation of 5-HT1B receptor in the endothelium. Furthermore, there is a report that serotonin evoked NO release in a dose dependent manner in human coronary artery endothelial cells [11]. The resulting production of NO stimulates soluble guanylate cyclase and results in vasodilation [12]. These reports suggest that increased serotonin may increase the cardiac release of NOx via 5-HT1B receptors. Our study confirmed that ischemia induced the cardiac release of serotonin, which was augmented by sarpogrelate. Thus, we suggest that sarpogrelate increased CBF via a NO-dependent pathway through the activation of 5-HT1B receptors induced by enhanced serotonin release. Serotonin is catalyzed by monoamine oxidases (MAO) [13]. A previous report has shown that ischemia decreased MAO activity in an ischemic kidney [14]. Therefore, in ischemic hearts, decreased MAO activity may increase plasma serotonin level by the accumulation of the undegraded serotonin. For the support of this idea, there is a report that the level of serotonin in the coronary effluent was elevated in ischemic isolated rat hearts, suggesting that serotonin was released from the ischemic myocardium [15]. In cardiac tissues, serotonin has been shown to be released from mast cells [16] and ganglia [17]. Although the precise mechanisms by which sarpogrelate augmented an increase in the cardiac release of

serotonin remain unclear, we demonstrated that an infusion of sarpogrelate increased serotonin staining in ischemic cardiomyocytes by immunohistochemistry. This result may partially contribute to an increase in the cardiac release of serotonin in the ischemic myocardium after infusion of sarpogrelate. Importantly, Shimizu et al. [18] showed the contradictory result that interstitial serotonin levels were increased in isolated ischemic rabbit heart that were abrogated by treatment with sarpogrelate. There might be several explanations for this discrepancy. First, we measured the plasma serotonin release in the ischemic canine heart not at the interstitial level as in the infarcted rabbit heart. In our study, we used the dogs with unimpaired endothelium on which serotonin can directly act. Second, as mentioned before, sarpogrelate might have stimulating effects on cardiac myocytes. Although this might be caused by the differences in animal species and models, further investigation is necessary to clarify serotonin released and metabolic mechanisms in the ischemic heart.

10. Clinical implications

In clinical settings, sarpogrelate is a selective 5-HT2A receptor blocker used for patients with arteriosclerotic obliterance because of its vasodilating and antiplatelet action [4]. Furthermore, sarpogrelate has been reported to be protective effects against human angina pectoris through an increase of collateral circulation [19]. We previously reported that an increase of CBF by benidipine attenuated the severity of ischemia gauged by lactate extraction rate and fractional shortening in the hypoperfused canine hearts [20]. Thus, the findings of this study may suggest another cardioprotective effect of sarpogrelate in ischemic heart disease.

11. Study limitation

Since coronary arteries in canine heart are covered with unimpaired endothelium, the beneficial effects of sarpogrelate in patients with arteriosclerosis may differ from our results. Further investigation will be needed to clarify these issues.

Acknowledgements

We appreciate Ms. Hiroko Okuda, Tomi Fukushima, Yoko Nagamachi for their excellent technical assistance.

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Original Article

Opening of Ca^{2+}-activated K^+ channels is involved in ischemic preconditioning in canine hearts

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Abstract

Brief periods of ischemia that precede sustained ischemia can markedly reduce infarct size (IS), a phenomenon that is known as ischemic preconditioning (IP). Several investigators have shown that elevation of the intracellular Ca^{2+} level ($[Ca^{2+}]_i$) during the antecedent brief periods of ischemia triggers the cardioprotective mechanism of IP. Since opening of Ca^{2+} activated K^+ (K_{Ca}) channels is reported to be cardioprotective, we hypothesized that these channels may be involved in the cardioprotective mechanism of IP. In anesthetized open-chest dogs, myocardial ischemia/reperfusion injury was created by occlusion of the left anterior descending coronary artery (LAD) for 90 min followed by 6 h of reperfusion. First, we showed that the treatment with NS1619, a K_{Ca} channel opener, reduced IS (IS in NS1619 group and control group, 19.8 ± 5.5% vs. 45.4 ± 3.5% of the area at risk, $P < 0.05$). Next, four cycles coronary occlusion for 5 min and reperfusion (IP) were performed before the 90-min occlusion with or without the infusion of potent K_{Ca} channel inhibitors, iberiotoxin (IbTX) and charybdotoxin (ChTX). IP markedly reduced IS (IS in the IP group was 8.2 ± 1.8%, $P < 0.01$ vs. control group). Infusion of either of K_{Ca} channel blockers during IP blunted the IS-limiting effect of IP (IS in the IP + IbTX and IP + ChTX groups was 30.7 ± 7.0% and 35.5 ± 3.7%, respectively, $P < 0.05$, vs. IP group). However, the cardioprotective effect of IP was not blunted by the treatment with ChTX when treated only during reperfusion (14.0 ± 4.1%). Thus, we conclude that the opening of K_{Ca} channel is involved in early trigger phase of the molecular mechanism of IP.

Keywords: Ischemic preconditioning; Ischemia; Reperfusion; Myocardial infarction; Ca^{2+}-activated K^+ channel

1. Introduction

Brief periods of ischemia that precede sustained ischemia can markedly decrease infarct size (IS), a phenomenon which is known as ischemic preconditioning (IP) [1–3]. This endogenous self-defense mechanism is one of the most powerful cardioprotective defenses against ischemia/reperfusion injury that has been demonstrated so far. Several investigators have previously revealed that an increase of the intracellular Ca^{2+} level ($[Ca^{2+}]_i$) during the antecedent brief periods of ischemia triggers or mediates the cardioprotective mechanism of IP [4,5]. Among the intracellular sequelae of Ca^{2+} overload, opening of Ca^{2+}-activated K^+ channels (K_{Ca} channels) is known to occur. Indeed, we have reported that opening of K_{Ca} channels is involved in the limitation of IS by 17β-estradiol or raloxifene [6,7]. A recent report also suggested that K_{Ca} channel opening mediates cardioprotection [8], but it has not been elucidated whether cardioprotection

Abbreviations: $[Ca^{2+}]_i$, intracellular Ca^{2+} level; ChTX, charybdotoxin; IbTX, iberiotoxin; IP, ischemic preconditioning; K_{Ca} channel, Ca^{2+}-activated K^+ channel; LAD, left anterior descending coronary artery.

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due to IP is mediated through the K_{Ca} channels as well as ATP-sensitive K^+ channels.

We hypothesized that opening of the K_{Ca} channel in response to elevation of $[Ca^{2+}]_i$ may be involved in the cardioprotective mechanism of IP. We found that potent K_{Ca} channel inhibitors, charybdotoxin (ChTX) and iberiotoxin (IbTX), could abolish IP-induced cardioprotection in a canine ischemia/reperfusion model. We also found that this channel contributed to early phase of IP-induced cardioprotective machinery rather than late phase.

2. Materials and methods

All procedures were performed in accordance with the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH publication No. 85-23, revised 1996).

2.1. Instrumentation

Beagle dogs weighing 9–14 kg were anesthetized with intravenous sodium pentobarbital (30 mg/kg), intubated with a cuffed endotracheal tube, and ventilated using room air mixed with oxygen (1.5 l/min), as described previously [9]. Thoracotomy was performed through the left fifth intercostal space, and the heart was suspended in a pericardial cradle. After an intravenous dose of heparin (500 U/kg), the proximal left anterior descending coronary artery (LAD) was cannulated and perfused with blood via an extracorporeal tube from the left carotid artery. Further heparin (100 U/kg) was administered intravenously every 3 h throughout the protocol. An occluder was attached to the bypass tube of the carotid-to-LAD shunt, and manual clamping of the tube was performed to produce myocardial ischemia. The pressure-resistant tube from the proximal portion of the cannula was connected to a multichannel recorder (Rm-6000; Nihon Kohden) to monitor arterial pressure. In addition, the left atrium was cannulated for the injection of microspheres.

2.2. Experimental protocols

2.2.1. Protocol 1: Effect of an intracoronary K_{Ca} channel opener (NS1619) on infarct size

Fig. 1 shows the details of this protocol. After hemodynamic stabilization, we injected NS1619 (11 μg/kg per min, Sigma, St. Louis, MO, USA: NS1619 group; $n = 6$) or NS1619 plus ChTX (0.3 μg/kg per min, Peptide Institute, Minoh, Osaka, Japan: NS1619 + ChTX group; $n = 6$) into the LAD through the bypass tube from 10 min before coronary occlusion until 1 h after reperfusion without a period of occlusion. We chose these doses of NS1619 and ChTX because these doses are maximal each that does not alter either systemic blood pressure or heart rate (HR), and sufficient to open and block the K_{Ca} channels, respectively. The ED_{50} of NS1619 is about 10 μM [10] and the calculated concentration

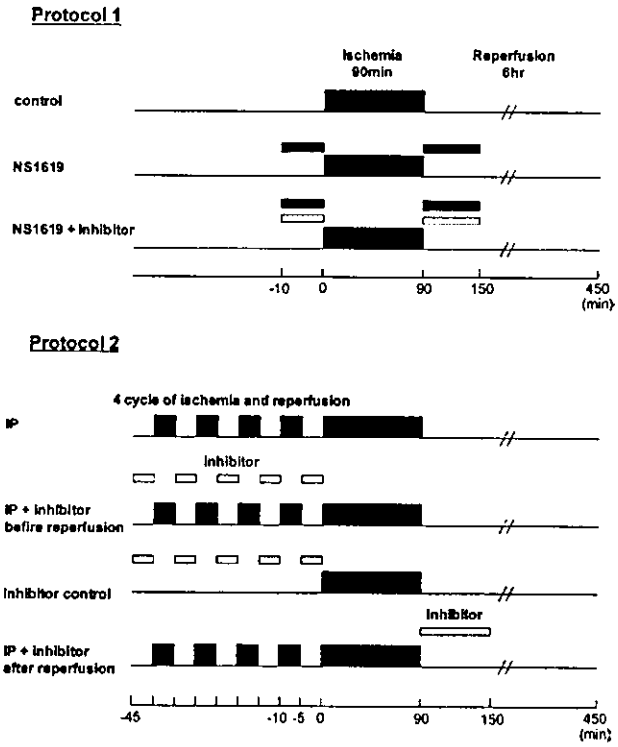

Fig. 1. All experimental protocols in this study are shown. IP: ischemic preconditioning.

of NS1619 used in this model was 40 μM. On the other hand, since K_d of ChTX is 2.1 nM [11], we need to use 10–100 times higher dose of K_d to fully block the channels. The calculated concentration of ChTX used in the present study was 100 nM, indicating that the dose of ChTX used in this protocol are sufficient to block the K_{Ca} channels.

Hemodynamic parameters were measured before the initiation of each protocol, 60 min after the onset of ischemia, and 1, 3 and 6 h after the onset of reperfusion.

2.2.2. Protocol 2: Effect of K_{Ca} channel inhibitors on IP

Fig. 1 displays the details of this protocol. After hemodynamic stabilization, four cycles of coronary occlusion for 5 min and subsequent reperfusion for 5 min (IP) were performed using the occluder with or without infusion of a K_{Ca} channel inhibitor throughout the IP procedure except during coronary occlusion. We used two different K_{Ca} channel inhibitors, IbTX (0.6 μg/kg per min, Peptide Institute) and ChTX. The following six groups were studied: Control group ($n = 6$), IP group ($n = 6$), IP + IbTX before reperfusion group ($n = 6$), IP + ChTX before reperfusion group ($n = 6$), IbTX group ($n = 6$), and ChTX group ($n = 6$). We chose the dose of IbTX because this dose is maximal and does not alter either systemic blood pressure or HR, and is sufficient to block the K_{Ca} channels. Since K_d of IbTX is 1 nM [12], we need to use 10–100 times higher dose of K_d to fully block the channels. The calculated concentrations of IbTX in this study is 200 nM, indicating that the dose of IbTX was also sufficient to block the K_{Ca} channels.

To distinguish the role of K_{Ca} channel in IP during ischemia or reperfusion period, we infused ChTX for 60 min after reperfusion in a group subjected to IP (IP + ChTX after reperfusion group, ($n = 8$)).

Hemodynamic parameters were measured at the same five times as in protocol 1.

2.3. Exclusion criteria

To ensure that all of the animals included in the analysis of IS were healthy and exposed to a similar extent of ischemia, we adopted the following criteria for exclusion of unsatisfactory dogs: (1) subendocardial collateral flow >15 ml/100 g per min, and (2) more than two consecutive attempts required to correct ventricular fibrillation with a low-energy counter pulse applied directly to the heart.

2.4. Measurement of infarct size and regional myocardial blood flow

We measured IS and regional myocardial blood flow as described previously [9]. For randomization, all measurements were done at completion of the protocol by persons without the knowledge of the treatment given to each heart.

2.5. Statistical analysis

Data are expressed as the mean ± S.E. Statistical significance was assessed with ANOVA, and if differences were found among groups, they were evaluated by Bonferroni's post-hoc test with $P < 0.05$ being considered as significant. The effect of collateral blood flow on IS was analyzed by ANCOVA, with regional collateral flow in the inner half of the left ventricular wall as covariant.

3. Results

3.1. Mortality and exclusions

We excluded 11 dogs from analysis because subendocardial collateral blood flow was greater than 15 ml/100 g per min. Ventricular fibrillation that matched the exclusion criterion occurred in seven animals during the 6-h reperfusion period and six of the seven died of ventricular fibrillation (Table 1). There were no significant differences in the number of exclusions among the groups.

3.2. Hemodynamic parameters, area at risk, and collateral blood flow

During protocols 1 and 2, the HR and mean arterial blood pressure (MAP) remained stable throughout the study (Table 2). The area at risk and the collateral blood flow were also similar among all of the groups, and there was no statistical difference (Table 3).

3.3. Infarct size

Fig. 2 shows IS in the nine groups from protocols 1 and 2. Treatment with NS1619 before and after ischemia reduced IS (19.8 ± 5.5% vs. 45.4 ± 3.5% of the area at risk, compared with the control group), while ChTX completely blocked this IS-limiting effect (42.2 ± 5.8%). IP markedly reduced IS compared with the control group (8.2 ± 1.8%). Treatment with either K_{Ca} channel inhibitor during IP blunted the IS-limiting effect (IP + IbTX group and IP + ChTX group: 30.7 ± 7.0%* and 35.5 ± 3.7%*, respectively, * $P < 0.05$ vs. the IP group). Either inhibitor alone, had no influence on IS (IbTX group and ChTX group: 37.1 ± 4.5% and 40.7 ± 6.1%, respectively). However, cardioprotective effect of IP was not influenced by treatment after reperfusion with ChTX (14.0 ± 4.1%).

ANCOVA test showed that these effects of K_{Ca} channel opener and inhibitor were independent from that of collateral blood flow (Fig. 3, and Table 4).

4. Discussion

We showed that an intracoronary administration of a K_{Ca} channel opener (NS1619) mimicked the IS-limiting effect in

Table 1
Number of dogs assigned to and excluded from each group for measurement of IS

Group	Number of dogs originally assigned	Number of dogs used for data analysis	Reason for exclusion		
			Vf (>2) during 6 h of reperfusion	Death due to Vf	High collateral flow (>15 ml/100 g per min)
Protocols 1, 2					
Control	7	6	0	1	0
NS1619	10	6	0	1	3
NS1619 + ChTX	8	6	0	0	2
IP	7	6	0	0	1
IP + IbTX before reperfusion	11	6	0	2	3
IP + ChTX before reperfusion	7	6	1	0	0
IbTX	7	6	0	1	0
ChTX	6	6	0	0	0
IP + ChTX after reperfusion	11	8	0	1	2

Vf: ventricular fibrillation, IP: ischemic preconditioning, IbTX: iberiotoxin, ChTX: charybdotoxin.

Table 2
Hemodynamic parameters during protocol

Group	Baseline		60 min of ischemia		1 h after reperfusion		3 h after reperfusion		6 h after reperfusion	
	MAP (mmHg)	HR (bpm)	MAP (mmHg)	HR (bpm)	MAP (mmHg)	HR (bpm)	MAP (mmHg)	HR (bpm)	MAP (mmHg)	HR (bpm)
Protocols 1, 2										
Control	99 ± 5	141 ± 7	95 ± 5	139 ± 12	98 ± 5	139 ± 9	95 ± 4	132 ± 12	92 ± 4	133 ± 9
NS1619	104 ± 4	135 ± 7	92 ± 7	134 ± 6	92 ± 5	138 ± 7	91 ± 4	146 ± 7	92 ± 4	141 ± 6
NS1619 + ChTX	106 ± 5	142 ± 3	103 ± 6	134 ± 8	100 ± 9	137 ± 9	97 ± 6	136 ± 8	93 ± 6	142 ± 8
IP	106 ± 6	134 ± 7	100 ± 7	133 ± 7	104 ± 7	134 ± 7	102 ± 6	134 ± 7	101 ± 8	132 ± 7
IP + IbTX before reperfusion	104 ± 4	135 ± 9	96 ± 7	134 ± 5	91 ± 3	134 ± 9	92 ± 7	128 ± 14	90 ± 7	133 ± 14
IP + ChTX before reperfusion	105 ± 9	143 ± 8	90 ± 10	146 ± 8	92 ± 9	129 ± 8	96 ± 7	136 ± 9	94 ± 9	138 ± 9
IbTX	101 ± 8	139 ± 8	102 ± 8	137 ± 6	104 ± 7	134 ± 7	98 ± 6	130 ± 6	92 ± 6	133 ± 6
ChTX	99 ± 3	133 ± 9	98 ± 8	136 ± 8	100 ± 7	133 ± 7	104 ± 7	130 ± 7	102 ± 8	134 ± 8
IP + ChTX after reperfusion	104 ± 2	143 ± 6	101 ± 3	135 ± 8	101 ± 3	151 ± 7	99 ± 4	142 ± 9	106 ± 4	137 ± 6

MAP: mean arterial pressure, IP: ischemic preconditioning, IbTX: iberiotoxin, ChTX: charybdotoxin. Data were shown by mean ± S.E. There was no significant difference among each protocol.

Table 3
Collateral blood flow and area at risk among experimental groups

Group	Collateral blood flow (ml/100 g per min)	Area at risk (%)
Protocols 1, 2		
Control	7.8 ± 1.6	46 ± 4
NS1619	6.5 ± 1.3	49 ± 6
NS1619 + ChTX	7.3 ± 1.5	39 ± 2
IP	9.0 ± 1.4	41 ± 3
IP + IbTX before reperfusion	6.8 ± 1.3	51 ± 5
IP + ChTX before reperfusion	7.9 ± 1.1	42 ± 2
IbTX	7.3 ± 1.4	44 ± 4
ChTX	8.5 ± 1.1	39 ± 2
IP + ChTX after reperfusion	8.7 ± 1.0	41 ± 2

IP: ischemic preconditioning, IbTX: iberiotoxin, ChTX: charybdotoxin. Data were shown by mean ± S.E. There was no significant difference among each protocol.

Table 4
Linear regression model test in each group

Group	Formula
Control	$y = 50.032 - 0.586x$
NS1619	$y = 30.649 - 1.667x$ *
NS1619 + ChTX	$y = 61.806 - 2.694x$
IP	$y = 15.228 - 0.786x$ *
IP + IbTX before reperfusion	$y = 49.482 - 2.792x$ *, †
IP + ChTX before reperfusion	$y = 42.162 - 0.852x$ †
IbTX	$y = 47.653 - 1.439x$
ChTX	$y = 56.948 - 1.921x$
IP + ChTX after reperfusion	$y = 37.367 - 2.703x$ *

IP: ischemic preconditioning, IbTX: iberiotoxin, ChTX: charybdotoxin. * $P < 0.05$ vs. control group, † $P < 0.05$ vs. IP group.

a canine ischemia/reperfusion model potent, and K_{Ca} channel inhibitors (ChTX and IbTX) blocked the cardioprotective effect of IP. We also showed that the cardioprotective effect of IP was not blunted by the treatment with ChTX only during the reperfusion period. These data suggest that opening of K_{Ca} channel is involved in early phase of the molecular mechanism of IP.

Since Murry et al. [1] first demonstrated the intriguing phenomenon known as IP, numerous studies have been done to elucidate the cellular mechanisms responsible. There is considerable evidence that $[Ca^{2+}]_i$ increases transiently during ischemic episodes that produce IP and it may be the key factor in IP [4,5,13–15]. Direct measurement of $[Ca^{2+}]_i$ has shown that a brief period of ischemia increases it two to fourfold [16,17]. On the other hand, exogenous calcium triggers the IS-limiting effect, and our previous study showed that a calcium chelator abolished the cardioprotective effect of IP [5]. While $[Ca^{2+}]_i$ is increased during antecedent ischemia, it is paradoxically reduced during subsequent sustained ischemia [18,19]. Indeed, it is well recognized that ischemia/reperfusion causes intracellular Ca^{2+} overload and thus leads to the death of cardiomyocytes [20]. Generally, the K_{Ca} channel opens after elevation of $[Ca^{2+}]_i$ and causes membrane hyperpolarization, which reduces voltage-dependent Ca^{2+} influx by increasing K^+ efflux and thus prevents Ca^{2+} overload. This sequence suggests that K_{Ca} channel opening is a candidate for mediating IP.

The outward K^+ channels comprise the voltage-dependent channel (K_V channel) and the calcium-dependent channel (K_{Ca} channel). The K_{Ca} channel is separated into three subclasses (BK, IK, and SK) according to its conductance. It has been demonstrated that the K_{Ca} channel (generally BK) is present in various muscular and non-muscular tissues. In addition to its distribution in a variety of cell types, Kawakubo et al. [21] demonstrated the existence of BK channels on ventricular cardiomyocytes by using the patch-clamp technique. In addition, we have previously shown that opening of the K_{Ca} channels has a cardioprotective effect without affecting coronary blood flow. Indeed, it has been reported that the K_{Ca} channel is located on vascular smooth muscle cells (SMC), and that endothelium-derived hyperpolarizing factor causes vasodilation by activating this channel [22,23]. To exclude the possibility of an effect on coronary and collateral flow, we determined the dose of NS1619 (11 μg/kg per min) that did not increase coronary flow. In fact, there was no significant difference of collateral flow (Table 3), which implies that opening of K_{Ca} channels in cardiac tissues other than SMC may also be important.

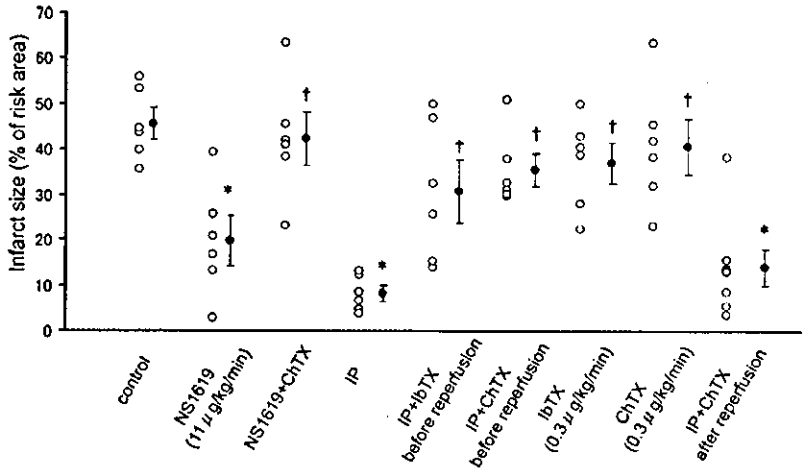

Fig. 2. IS as a percentage of the area at risk in all nine experimental groups in protocols 1 and 2. Data from individual animals and mean ± S.E. are shown. * P <0.05 vs. control group, † P <0.05 vs. IP group. IP: ischemic preconditioning, IbTX: iberiotoxin, ChTX: charybdotoxin.

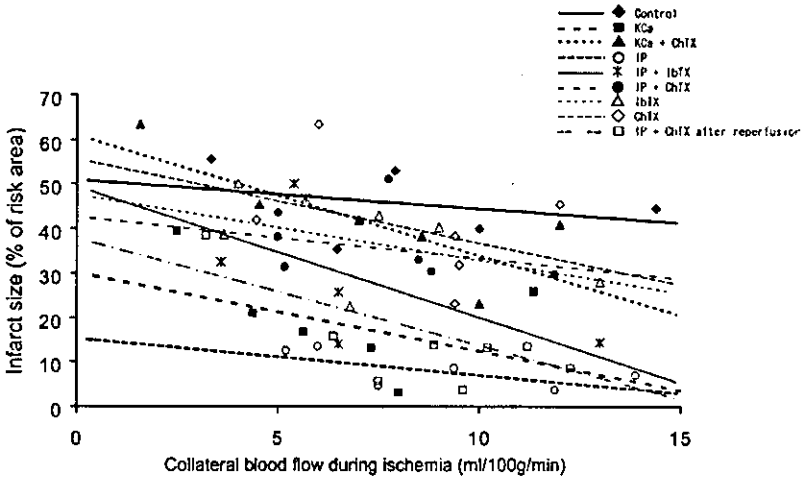

Fig. 3. IS in protocols 1 and 2 as a percentage of the risk area vs. regional collateral blood flow during ischemia. See Fig. 2 legend for explanation of each group. The results of ANCOVA test is indicated in Table 4.

Recently, it was demonstrated that infusion of NS1619 5–10 min before sustained ischemia could reduce IS through mitochondrial K_{Ca} channels in an isolated rabbit heart model [8]. It was suggested that opening of this channel improves mitochondrial ATP production and decreases both the production of reactive oxygen species and Ca^{2+} overload in the mitochondria. This mechanism may also contribute to the cardioprotective effect of the K_{Ca} channel during IP in addition to reduction of voltage-dependent Ca^{2+} influx by promotion of K^+ efflux.

Generally, some care is needed when interpreting experiments that employ pharmacological agents. ChTX mainly acts on BK channels, but it also interacts with IK1 and K_V 1.3 type channels [24]. We tested the influence of IbTX on IP as well as ChTX, since IbTX is a selective blocker of large-conductance BK type K_{Ca} channels. Our results showed that both K_{Ca} channel inhibitors abolished IP-induced cardioprotection, making it likely that this channel is involved in the mechanism of IP rather than the possibility of a nonspecific effect or the contaminating effect of other channels.

Protein kinase C (PKC) is believed to play an important role in triggering IP, and elevation of $[Ca^{2+}]_i$ activates PKC [5,25]. However, activation of PKC was reported to inhibit the K_{Ca} channel [26]. Since we have previously reported that PKA is involved in IP [27] and many authors have shown that PKA activates the K_{Ca} channel, [28–32] it seems that PKA activation following brief ischemia also has a role in opening the K_{Ca} channel as well as the elevation of $[Ca^{2+}]_i$. Adenosine has also been reported to open K_{Ca} channels, and it may be involved in the mechanism of IP [33].

In the present study, we found evidence that K_{Ca} channel opening contributes to IP, as well as the ATP-sensitive K^+ channel which is believed to play a central role in IP. Further investigations will be required to clarify the importance of the K_{Ca} channel to IP.

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