

Full Paper

## Effects of KB-R9032, a new $\text{Na}^+/\text{H}^+$ Exchange Inhibitor, on Canine Coronary Occlusion/Reperfusion-Induced Ventricular Arrhythmias

Chikaomi Yamada<sup>1,\*</sup>, YiXue Xue<sup>1</sup>, Daisuke Chino<sup>1</sup>, and Keitaro Hashimoto<sup>1</sup>

<sup>1</sup>Department of Pharmacology, Interdisciplinary Graduate School of Medicine and Engineering, University of Yamanashi, 1110, Shimogato, Tamaho, Nakakoma, Yamanashi 409-3898, Japan

Received March 15, 2005; Accepted June 9, 2005

**Abstract.** We investigated the effects of KB-R9032 (*N*-(4-isopropyl-2,2-dimethyl-3-oxo-3,4-dihydro-2*H*-benzo[1,4]oxazine-6-carbonyl) guanidine methanesulfonate), a new  $\text{Na}^+/\text{H}^+$  exchange inhibitor, on a coronary artery occlusion/reperfusion-induced arrhythmia model in pentobarbital anesthetized dogs. KB-R9032 reduced the number of ventricular premature contractions seen during the coronary occlusion, while it did not alter the heart rate, mean blood pressure, or electrocardiographic parameters (PR, QRS, or QTc interval). KB-R9032 also decreased the incidence of fatal ventricular fibrillation during coronary artery occlusion and/or after reperfusion. These antiarrhythmic effects were observed not only in the pre-ischemic administration group, but also in the group given KB-R9032 at the 15th min of the 30-min occlusion. These findings support the view that  $\text{Na}^+/\text{H}^+$  exchanger may play an important role in inducing coronary ischemia/reperfusion arrhythmias. This suggests that the use of  $\text{Na}^+/\text{H}^+$  exchange inhibitors, such as KB-R9032, may be an effective clinical approach to suppress sudden cardiac death due to acute myocardial ischemia/reperfusion such as during coronary bypass surgery, cardiac valve surgery, or percutaneous transluminal coronary angioplasty.

**Keywords:** KB-R9032,  $\text{Na}^+/\text{H}^+$  exchange,  $\text{Ca}^{2+}$  overload, arrhythmia ischemia, arrhythmia reperfusion

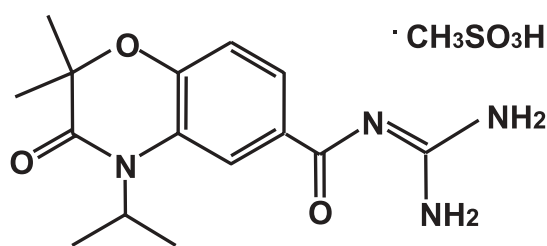
### Introduction

It has been demonstrated that ionic disturbances within the ischemic myocardium may contribute to coronary ischemia/reperfusion injuries, including ventricular arrhythmias (1). Excessive activation of the  $\text{Na}^+/\text{H}^+$  exchanger-1 (NHE-1) may lead to intracellular  $\text{Na}^+$  overload in the ischemic heart, and the elevated  $\text{Na}^+$  may in turn induce  $\text{Ca}^{2+}$  overload via activation of the  $\text{Na}^+/\text{Ca}^{2+}$  exchanger (2, 3). This  $\text{Ca}^{2+}$  overload may be one of the major mechanisms underlying the development of such arrhythmias (4). The NHE-1 is known to be inhibited by amiloride or its analogues, and thus many investigators who have applied these compounds to ischemia/reperfusion models have shown that they significantly reduce ischemic arrhythmias in anesthe-

tized rats (5, 6).

HOE694 (3-methylsulfonyl-4-piperidinobenzoyl guanidine) and HOE642 (4-isopropyl-3-methylsulphonybenzoyl-guanidine methanesulfonate) (cariporide) were developed as selective NHE-1 inhibitors with no cardiovascular side effects (7, 8). These compounds proved to have protective effects against ischemia/reperfusion injuries, including arrhythmias, in rats (7, 8, 9). Similar results have been reported for other NHE-1 inhibitors such as BIIB513 (10, 11), TY-12533 (12), and FR16888 (13). From these reports, common suppressive effects of NHE-1 inhibitors on the arrhythmias based on  $\text{Ca}^{2+}$  overload have been proven. In dogs, we have already reported that cariporide suppressed the occurrence of fatal ventricular fibrillation (VF) (14). However, there may be some differences in the results between rats and dogs. For example, in rats, pre-ischemic injection of cariporide at a dose of 1 mg/kg significantly decreased the total duration and incidence of ventricular tachycardia and VF and the associated mortality (15), while in

\*Corresponding author. Present address for correspondence: Daiichi Suntory Biomedical Research Limited, 1-1-1, Wakayamadai, Shimamoto-cho, Mishima-gun, Osaka 618-8513, Japan.  
FAX: +81-75-962-6448, E-mail: chikaomi\_yamada@dsup.co.jp



**Fig. 1.** Chemical structure of *N*-(4-isopropyl-2,2-dimethyl-3-oxo-3,4-dihydro-2*H*-benzo[1,4]oxazine-6-carbonyl) guanidine methane-sulfonate (KB-R9032).

dogs, cariporide did not suppress the occurrence of ischemia-induced ventricular premature contractions (VPC) (14). Moreover, cariporide (15), TY-12533 (12), and FR 168888 (13) suppressed ischemia and/or reperfusion-induced arrhythmias not only when these were administered before ischemia, but also when it was administered at the onset of reperfusion in rats. However, in dogs, BIIB 513 is the only compound that suppressed the ischemia and/or reperfusion-induced arrhythmias by post-ischemic administration as well as pre-ischemic administration (8). The explanation of these different results in rats and dogs has yet to be elucidated.

KB-R9032 (*N*-(4-isopropyl-2,2-dimethyl-3-oxo-3,4-dihydro-2*H*-benzo[1,4]oxazine-6-carbonyl) guanidine methanesulfonate) is another newly developed selective  $\text{Na}^+/\text{H}^+$  exchange inhibitor (Fig. 1) (16). KB-R9032 had been shown to inhibit NHE-1 selectively ( $\text{IC}_{50} = 120 \text{ nM}$ ) (16) and not to inhibit other ion transport systems (17). It has also been shown to inhibit ischemia/reperfusion-induced arrhythmias and to reduce myocardial infarct size in rats at a dose of 0.3 or 3 mg/kg, i.v. in a dose-dependent manner (18).

The present study was carried out to further elucidate whether NHE-1 inhibitors exert an anti-arrhythmic effect on a canine coronary occlusion/reperfusion model. Namely, it was focused on whether a novel NHE-1 inhibitor, KB-R9032, elicited the anti-arrhythmic effect when it was administered during as well as before the coronary occlusion. We hope this will provide clues to the clinical potential of KB-R9032 in the prevention of those arrhythmias, including fatal VF, that are thought to occur due to intracellular  $\text{Ca}^{2+}$  overload during myocardial ischemia/reperfusion.

## Materials and Methods

The protocols were approved by the University of Yamanashi Animal Experimentation Committee, and animals were obtained through the Animal Laboratory

for Research of University of Yamanashi.

### *Production of coronary occlusion/reperfusion induced arrhythmias in beagle dogs*

Beagle dogs of either sex, weighing 8–12 kg, were anesthetized with an intravenous injection of sodium pentobarbital (30 mg/kg), followed by an infusion at a rate of 0.1 mg/kg per min. After cannulation of the trachea, the animals were ventilated with room air at a stroke volume of 20 ml/kg and at a rate of 12 strokes/min. Body temperature was maintained at  $37.0 \pm 0.5^\circ\text{C}$ . Using the method described earlier (19), a thoracotomy for coronary occlusion was performed at the fifth intercostal space. The pericardium was opened and the heart was suspended in the pericardiac cradle. The left anterior descending coronary artery (LAD) was isolated just proximal to the first diagonal branch. Since the incidence of coronary occlusion/reperfusion arrhythmia is known to be quite variable (20), experiments were randomized (by coin-flip) using a pair of beagle dogs, one of each pair receiving KB-R9032 and the other, vehicle. A catheter was inserted into the right femoral artery and connected to a pressure transducer (DX-100; Nihon Kohden Co., Tokyo) to record blood pressure (BP) on a polygraph system (Nihon Denki San-ei, Tokyo). A double lumen catheter was inserted into the right femoral vein for administration of anesthetics and the test compound. Leads I and II of the electrocardiogram (ECG) were recorded by means of needle electrodes inserted subcutaneously in the limbs. A pair of epicardial electrodes was sutured onto the border zone of the ischemic area in the left ventricle for continuous recording of the ventricular surface electrogram. The PR, QRS, RR, and QT interval were determined from the lead II ECG. QTc-interval was calculated using Bazett's formula:  $\text{QTc} = \text{QT}/\sqrt{\text{RR}}$ . The heart rate (HR) was measured from the lead II ECG.

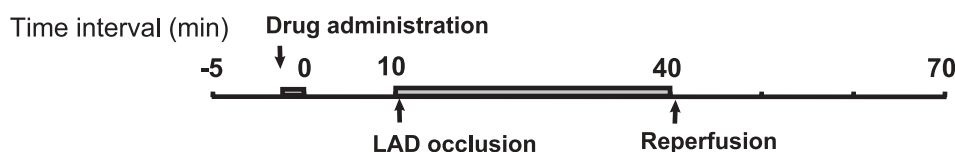
### *Evaluation of antiarrhythmic effects*

VPC were judged to occur when the QRS complexes had a different shape from the normal one. Ventricular tachycardia was defined as a tachycardia consisting of more than three consecutive VPC. VF was defined as a tachycardia without a regular occurrence of normal QRS complexes or a loss of normal pulsatile blood pressure record, which continued for more than 2 min.

### *Experimental protocol*

The protocols used in this study are shown in Fig. 2. The two experiments were performed with separate vehicle control groups. In protocol I, after a stabilization period of more than 20 min, KB-R9032 (3 mg/kg body weight) or the vehicle was administered 10 min before

### Protocol I (Pre-treatment group)



### Protocol II (Post-treatment group)

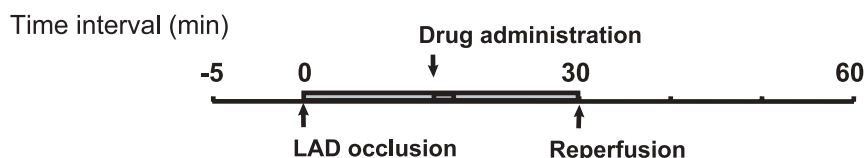


Fig. 2. Experimental protocols.

LAD occlusion. In protocol II, KB-R9032 (3 mg/kg body weight) or vehicle was administered 15 min after the start of LAD occlusion. In both experiments, after 30 min of LAD occlusion, reperfusion was allowed by releasing the ligature. If VF did not occur, observations were continued for 30 min. The dogs in the control groups received the vehicle (5% glucose) instead of KB-R9032.

#### Drugs

The following drugs were used: KB-R9032 (kindly supplied by Nippon Organon Ltd., Osaka), sodium pentobarbital (Tokyo Kasei Kogyo, Tokyo), and 5% glucose in distilled water (Otsuka Pharmaceuticals, Tokushima). KB-R9032 was dissolved in 5% glucose solution at a concentration of 10 mg/ml.

#### Statistical analyses

All values were expressed as the mean  $\pm$  S.E.M. A one-way analysis of variance (ANOVA) was used to compare the QTc, HR, and MAP values between the drug-treated and control groups. When a statistically significant difference was detected, Student's *t*-test was performed. Since the occurrence of ischemia-induced VPC varied over time, the numbers of VPC in the treated and control groups were compared using the two-factor analysis of variance (repeated measures ANOVA). When a statistically significant difference was detected, Mann-Whitney's U test was performed for each time-point. Differences in the incidence of VF between the treated and control groups were analyzed using a chi-square test or Fisher's exact probability test. A *P* value of less than 0.05 was considered to be significant.

## Results

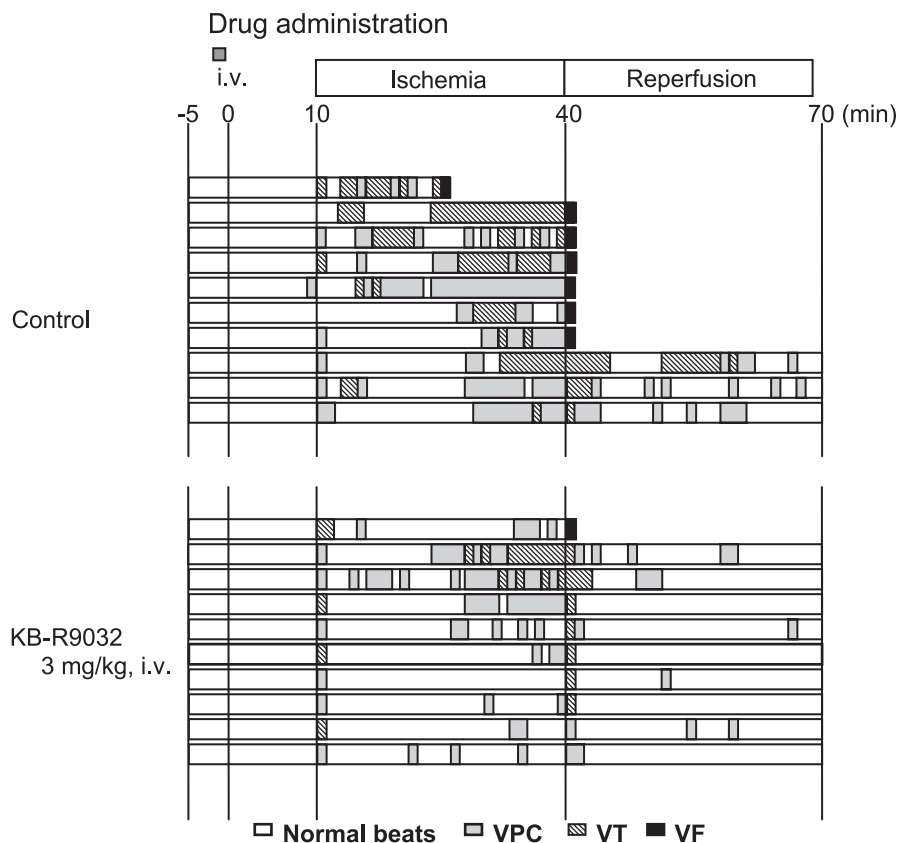
### Effects of KB-R9032 on ECG and hemodynamic parameters

Hemodynamic and ECG data are summarized in Table 1. Before the administration of KB-R9032 or the vehicle, there were no significant differences among all the values obtained for HR, mean BP, or ECG parameters (PR, QRS, and QTc interval). Nine minutes after administration of KB-R9032 (3 mg/kg, i.v.) or the vehicle (before coronary occlusion), there were no significant alterations in any of the measured parameters.

Table 1. Effects of KB-R9032 on canine hemodynamic and electrocardiogram parameters

	Control	KB-R9032 (3 mg/kg, i.v.)
Body weight (kg)	10.1 $\pm$ 0.2	9.8 $\pm$ 0.2
Before administration		
HR (bpm)	149 $\pm$ 5	146 $\pm$ 6
MBP (mmHg)	120 $\pm$ 5	118 $\pm$ 4
PR (ms)	80 $\pm$ 2	77 $\pm$ 1
QRS (ms)	56 $\pm$ 1	58 $\pm$ 3
QTc (ms/s <sup>1/2</sup> )	368 $\pm$ 8	376 $\pm$ 6
Nine minutes after administration		
HR (bpm)	144 $\pm$ 5	145 $\pm$ 5
MBP (mmHg)	118 $\pm$ 4	111 $\pm$ 3
PR (ms)	81 $\pm$ 3	77 $\pm$ 2
QRS (ms)	57 $\pm$ 1	57 $\pm$ 2
QTc (ms/s <sup>1/2</sup> )	372 $\pm$ 10	378 $\pm$ 5

HR: heart rate, MBP: mean arterial blood pressure. Values are means  $\pm$  S.E.M.



**Fig. 3.** Summary of the effects of KB-R9032 on the incidence of coronary occlusion/reperfusion-induced ventricular premature contractions in protocol I. VPC: ventricular premature contractions. VT: ventricular tachycardia. VF: ventricular fibrillation. Each column indicates the response of one group of dogs ( $n = 10$ ).

#### *Effects of KB-R9032 on ischemia-induced arrhythmias in protocol I (pre-treatment group)*

During the 30 min of complete LAD occlusion, ventricular arrhythmias, including VPC and the ventricular tachycardia, occurred in the vehicle control group. However the incidence of ischemia-induced arrhythmias tended to be lower in the KB-R9032-treated groups (Fig. 3), that is, the reduction of the average number of VPC occurring during coronary occlusion was significant both in terms of the total number ( $40 \pm 20$  beats/30 min compared to  $273 \pm 61$  beats/30 min in the control group,  $P < 0.01$ ) (Fig. 4B) and in terms of the time-related occurrence of VPC (Fig. 4A).

With regard to the occurrence of fatal VF, KB-R9032 reduced the incidence of VF during LAD occlusion and/or after reperfusion in a dose-dependent manner, the reduction seen in the KB-R9032-treated group being significant (1 out of 10 dogs in the treated group compared to 7 out of 10 dogs in the control group,  $P < 0.05$ ) (Fig. 4C).

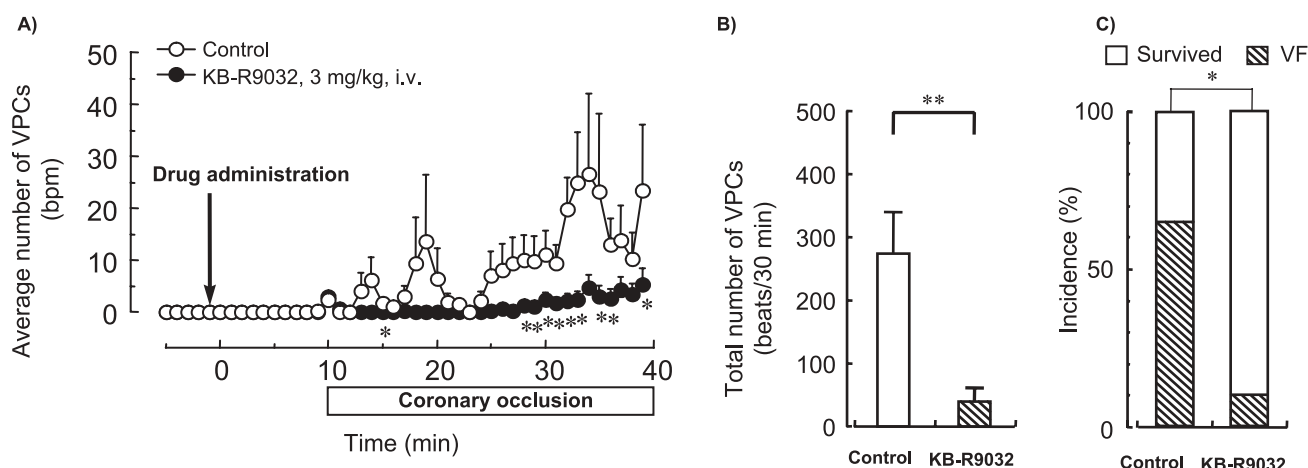
#### *Effects of KB-R9032 on ischemia-induced arrhythmias in the protocol II (post-ischemic treatment group)*

As for the incidence of ischemia-induced VPC, there was no difference between the control and KB-R9032-treated groups before drug administration. When KB-R9032 was administered 15 min before reperfusion (15 min after coronary occlusion), there was a significant reduction in the average number of VPC (Fig. 5A).

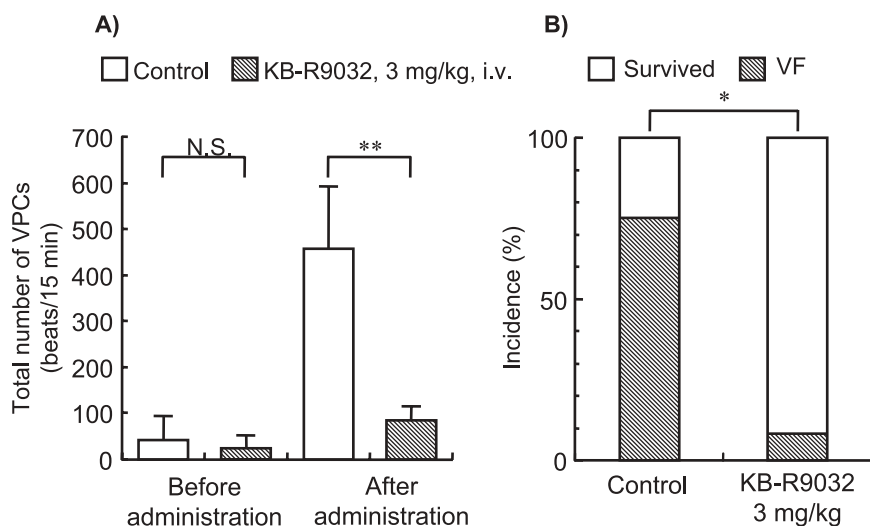
In protocol II, as in protocol I, KB-R9032 significantly reduced the incidence of VF (Fig. 5B). However, a pilot study using dogs in which KB-R9032 was administered 5 min before the start of reperfusion (25 min after coronary occlusion), suppression of ischemia/reperfusion-induced arrhythmias was not observed (data not shown).

### **Discussion**

This study examined the effects of KB-R9032, a selective NHE-1 inhibitor, on ischemia/reperfusion-induced arrhythmias in canine heart in vivo. KB-R9032 (3 mg/kg, i.v.) as well as many of the other NHE-1



**Fig. 4.** Effects of pre-ischemic administration of KB-R9032 (protocol I) on the incidence of canine ischemia/reperfusion-induced arrhythmias: A) time-dependent inhibition, B) total number of ischemia-induced VPC, C) reperfusion-induced VF. VPC: ventricular premature contractions. VF: ventricular fibrillation. Values are means  $\pm$  S.E.M. ( $n = 10$ ). \* $P < 0.05$ , \*\* $P < 0.01$ .



**Fig. 5.** Effects of post-ischemic administration of KB-R9032 (protocol II) on the incidence of canine ischemia/reperfusion-induced arrhythmias: A) total number of ischemia-induced VPC, B) reperfusion-induced VF. VPC: ventricular premature contractions. VF: ventricular fibrillation. Values are means  $\pm$  S.E.M. ( $n = 12$ ). \* $P < 0.05$ , \*\* $P < 0.01$ . N.S.: Not significant.

inhibitors suppressed ischemia/reperfusion-induced lethal VF without significantly altering HR or BP in the pre-ischemic treatment group. We have already reported that cariporide (HOE642), a selective NHE-1 inhibitor, significantly suppressed the occurrence of ischemia-induced fatal VF in dogs, but it did not decrease the number of VPC at a dose of 1 mg/kg, i.v. (14). In contrast, the current study demonstrates a reduction in ischemia-induced VPC.

In rats, not only pre-ischemic but also post-ischemic administration of NHE-1 inhibitors suppressed the occurrence of ischemia-induced VPC and reperfusion-induced VF (11–13, 15, 18). However, in dogs, there is no report that cariporide suppresses ischemia-induced PVC. Also, there is no report that cariporide suppresses ischemia/reperfusion-induced VF when it was admin-

istered after coronary occlusion. In addition, more importantly, KB-R9032 failed to inhibit ischemia/reperfusion-induced arrhythmias when it was administered 5 min before the onset of reperfusion (25 min after coronary occlusion). It has been reported that BIIB 513 (3 mg/kg, i.v.) suppressed the ischemia-induced VPC and reperfusion-induced VF by post-ischemic administration as well as by the pre-ischemic administration in dogs (10). The affinities of these compounds for NHE-1 are almost equivalent because the  $IC_{50}$  values of KB-R9032, BIIB-513, and cariporide for NHE-1 are 0.12 (16), 0.25 (21), and 0.2  $\mu$ M (8), respectively. Of course, it is known that NHE-1 inhibitors also have affinities for other ion channels, but these effects may not explain the reason why KB-R9032 administered 5 min before reperfusion (25 min after coronary occlusion) failed to

suppress the ischemia/reperfusion-induced arrhythmias. A more possible explanation is that KB-R9032 suppressed an adrenergic pathway which is thought to exacerbate ischemia- (22–24) and reperfusion-induced arrhythmias (9, 25, 26). During myocardial ischemia, energy depletion may lead to an accumulation of acidic metabolites such as lactate and intracellular  $H^+$ , followed by an extrusion of  $H^+$  in exchange for  $Na^+$  via NHE-1. The raised intracellular  $Na^+$  may cause two important events. One is the  $Ca^{2+}$  overload as a consequence of the activation of  $Na^+/Ca^{2+}$  exchange. The other is the  $Na^+$  dependent-nonexocytotic norepinephrine release (27). Under normoxic conditions or during relatively short-term ischemia (i.e., approximately 10 min), norepinephrine is released via a  $Ca^{2+}$ -dependent exocytotic pathway, and it is taken up via a carrier protein. On the other hand, during long-lasting myocardial ischemia (i.e., approximately 20 min), the elevated intracellular  $Na^+$  leads to norepinephrine release from the cytoplasm into the extracellular space via the carrier protein used in the reverse direction (28). The accumulated norepinephrine is suggested to exacerbate ischemia- (22–24) and/or reperfusion-induced arrhythmias (9, 25–26). In addition, it is suggested that norepinephrine stimulates sarcolemmal NHE-1 via  $\alpha_1$  receptors, which led to acceleration of  $Na^+$  overload (29). EIPA blocked norepinephrine extrusion in isolated guinea-pig heart subjected to 20-min global ischemia followed by reperfusion (23, 24). Cariporide also suppressed norepinephrine release in the rat heart (30). On the basis of our results and the accumulated knowledge of the above processes, the mechanism underlying the suppression of ischemia-induced VPC and reperfusion-induced VF by NHE inhibitors may actually derive from two mechanisms: one is an inhibition of  $Ca^{2+}$  overload and the other is an inhibition of excessive norepinephrine release via a suppression of the  $Na^+$ -dependent nonexocytotic process. Therefore, cariporide may also reduce the incidence of ischemia-induced VPC and reperfusion-induced VF when it was administered even after coronary occlusion as well as before, if higher doses had been used.

NHE-1 inhibitors are commonly known to have antiarrhythmic effects, and furthermore, they have cardioprotective effects against ischemia/reperfusion injury. It has been reported that NHE-1 inhibition suppressed ischemia/reperfusion-induced arrhythmias (6, 10, 13, 14, 26, 31) and reduced myocardial infarct size (12, 16, 32, 33). In addition, when rat hearts received NHE-1 inhibitor during VF and were resuscitated, the diastolic dysfunction was prevented and the systolic function recovered earlier (34). These data suggest that the application of NHE-1 inhibitors, such as

KB-R9032, may be an effective clinical approach to prevent sudden death due to cardiac ischemia and/or reperfusion-induced arrhythmias and resuscitation from ventricular fibrillation such as during bypass surgery, cardiac valve surgery, or acute myocardial infarction in patients undergoing thrombolysis or percutaneous coronary intervention.

### Acknowledgment

The authors thank Nippon Organon, Ltd., for supplying us with KB-R9032.

### References

- 1 Pierce GN, Czubyrt MP. The contribution of ionic imbalance to ischemia/reperfusion-induced injury. *J Mol Cell Cardiol.* 1995; 27:53–63.
- 2 Tani M, Neely JR. Role of intracellular  $Na^+$  in  $Ca^{2+}$  overload and depressed recovery of ventricular function of reperfused ischemic rat hearts: possible involvement of  $H^+$ - $Na^+$  and  $Na^+$ - $Ca^{2+}$  exchange. *Circ Res.* 1989;65:1045–1056.
- 3 Lazdunski M, Frelin C, Vigne P. The sodium/hydrogen exchange system in cardiac cells: its biochemical and pharmacological properties and its role in regulating internal concentrations of sodium and internal pH. *J Mol Cell Cardiol.* 1985; 17:1029–1042.
- 4 Clusin WT, Buchbinder M, Harrison DC. Calcium overload, “injury” current, and early ischaemic cardiac arrhythmias – a direct connection. *Lancet.* 1983;1:272–274.
- 5 Dennis SC, Coetzee WA, Cragoe EJ Jr, Opie LH. Effects of proton buffering and of amiloride derivatives on reperfusion arrhythmias in isolated rat hearts: possible evidence for an arrhythmogenic role of  $Na^+$ - $H^+$  exchange. *Circ Res.* 1990;66: 1156–1159.
- 6 Scholz W, Albus U, Linz W, Martorana P, Lang HJ, Scholkens BA. Effects of  $Na^+/H^+$  exchange inhibitors in cardiac coronary occlusion. *J Mol Cell Cardiol.* 1992;24:731–740.
- 7 Scholz W, Albus U, Lang HJ, Linz W, Martorana PA, Englert HC, et al. Hoe 694, a new  $Na^+/H^+$  exchange inhibitor and its effects in cardiac ischaemia. *Br J Pharmacol.* 1993;109:562–568.
- 8 Scholz W, Albus U, Counillon L, Gogelein H, Lang HJ, Linz W, et al. Protective effects of HOE642, a selective sodium-hydrogen exchange subtype 1 inhibitor, on cardiac ischaemia and reperfusion. *Cardiovasc Res.* 1995;29:260–268.
- 9 Yasutake M, Ibuki C, Hearse DJ, Avkiran M.  $Na^+/H^+$  exchange and reperfusion arrhythmias: protection by intracoronary infusion of a novel inhibitor. *Am J Physiol.* 1994;267:H2430–H2440.
- 10 Gumina RJ, Daemmgen J, Gross GJ. Inhibition of the  $Na^+/H^+$  exchanger attenuates phase 1b ischemic arrhythmias and reperfusion-induced ventricular fibrillation. *Eur J Pharmacol.* 2000;396:119–124.
- 11 Wu D, Stassen JM, Seidler R, Doods H. Effects of BIIB513 on ischemia-induced arrhythmias and myocardial infarction in anesthetized rats. *Basic Res Cardiol.* 2000;95:449–456.
- 12 Aihara K, Hisa H, Sato T, Yoneyama F, Sasamori J, Yamaguchi F, et al. Cardioprotective effect of TY-12533, a novel  $Na^+/H^+$

- exchange inhibitor, on ischemia/reperfusion injury. *Eur J Pharmacol.* 2000;404:221–229.
- 13 Zhu BM, Miyamoto S, Kamiya K, Komori S, Hashimoto K. Inhibitory effects of pre-ischemic and post-ischemic treatment with FR 168888, a  $\text{Na}^+/\text{H}^+$  exchange inhibitor, on reperfusion-induced ventricular arrhythmias in anesthetized rat. *Jpn J Pharmacol.* 2002;88:93–99.
  - 14 Xue YX, Aye NN, Hashimoto K. Antiarrhythmic effects of HOE642, a novel  $\text{Na}^+/\text{H}^+$  exchange inhibitor, on ventricular arrhythmias in animal hearts. *Eur J Pharmacol.* 1996;317:309–316.
  - 15 Aye NN, Xue YX, Hashimoto K. Antiarrhythmic effects of cariporide, a novel  $\text{Na}^+/\text{H}^+$  exchange inhibitor, on reperfusion ventricular arrhythmias in rat hearts. *Eur J Pharmacol.* 1997;339:121–127.
  - 16 Yamamoto T, Hori M, Watanabe I, Tsutsui H, Harada K, Ikeda S, et al. Synthesis and quantitative structure-activity relationships of N-(3-oxo-3,4-dihydro-2H-benzo[1,4]oxazine-6-carbonyl)guanidines as  $\text{Na}/\text{H}$  exchange inhibitors. *Chem Pharm Bull (Tokyo).* 1998;46:1716–1723.
  - 17 Harada K, Shimamoto A, Wakabayashi S, Shigekawa M, Nishimura N. Comparative study of inhibitory activities of KB-R9032, a novel  $\text{Na}^+/\text{H}^+$  exchange inhibitor, for NHE isoforms expressed in a fibroblast cell line. *Jpn J Pharmacol.* 1998;76 Suppl I:170P.
  - 18 Harada K, Sugimoto H, Shimamoto A, Watano T, Nishimura N. Cardioprotective effects of a novel  $\text{Na}^+/\text{H}^+$  exchange inhibitor, KBR-9032, on cardiac ischemia-reperfusion injury in rats. *Jpn J Pharmacol.* 1997;73 Suppl I:232P.
  - 19 Hashimoto K, Haruno A, Matsuzaki T, Hirasawa T, Awaji T, Uemura Y. Effects of a new class III antiarrhythmic drug (E-4031) on canine ventricular arrhythmia models. *Asia Pacific J Pharmacol.* 1991;6:127–137.
  - 20 Naito M, Michelson EL, Kmetzo JJ, Kaplinsky E, Dreifus LS. Failure of antiarrhythmic drugs to prevent experimental reperfusion ventricular fibrillation. *Circulation.* 1981;63:70–79.
  - 21 An J, Varadarajan SG, Camara A, Chen Q, Novalija E, Gross GJ, et al. Blocking  $\text{Na}^+/\text{H}^+$  exchange reduces  $[\text{Na}^+]_i$  and  $[\text{Ca}^{2+}]_i$  load after ischemia and improves function in intact hearts. *Am J Physiol Heart Circ Physiol.* 2001;281:H2398–H2409.
  - 22 Schömig A. Catecholamines in myocardial ischemia: Systemic and cardiac release. *Circulation.* 1990;82 Suppl II:13–22.
  - 23 Schömig A, Haass M, Richardt G. Catecholamine release and arrhythmias in acute myocardial ischaemia. *Eur Heart J.* 1991;12 Suppl F:38–47.
  - 24 Imamura M, Lander HM, Levi R. Activation of histamine  $\text{H}_3$ -receptors inhibits carrier-mediated norepinephrine release during protracted myocardial ischemia: comparison with adenosine  $\text{A}_1$ -receptors and  $\alpha_2$ -adrenoceptors. *Circ Res.* 1996;78:475–481.
  - 25 Yasutake M, Avkiran M. Exacerbation of reperfusion arrhythmias by  $\alpha_1$  adrenergic stimulation: a potential role for receptor mediated activation of sarcolemmal sodium-hydrogen exchange. *Cardiovasc Res.* 1995;29:222–230.
  - 26 Yasutake M, Ibuki C, Hearse DJ, Avkiran M.  $\text{Na}^+/\text{H}^+$  exchange and reperfusion arrhythmias: protection by intracoronary infusion of a novel inhibitor. *Am J Physiol.* 1994;267:H2430–H2440.
  - 27 Schömig A, Dart AM, Dietz R, Mayer E, Kubler W. Release of endogenous catecholamines in the ischemic myocardium of the rat. Part A: Locally mediated release. *Circ Res.* 1984;55:689–701.
  - 28 Kurz T, Richardt G, Hagl S, Seyfarth M, Schomig A. Two different mechanisms of noradrenaline release during normoxia and simulated ischemia in human cardiac tissue. *J Mol Cell Cardiol.* 1995;27:1161–1172.
  - 29 Yokoyama H, Yasutake M, Avkiran M.  $\alpha_1$ -adrenergic stimulation of sarcolemmal  $\text{Na}^+/\text{H}^+$  exchanger activity in rat ventricular myocytes: evidence for selective mediation by the  $\alpha_{1A}$ -adrenoceptor subtype. *Circ Res.* 1998;82:1078–1085.
  - 30 Hatta E, Yasuda K, Levi R. Activation of histamine  $\text{H}_3$  receptors inhibits carrier-mediated norepinephrine release in a human model of protracted myocardial ischemia. *J Pharmacol Exp Ther.* 1997;283:494–500.
  - 31 Scholz W, Albus U, Counillon L, Gogelein H, Lang HJ, Linz W, et al. Protective effects of HOE642, a selective sodium-hydrogen exchange subtype 1 inhibitor, on cardiac ischaemia and reperfusion. *Cardiovasc Res.* 1995;29:260–268.
  - 32 Aye NN, Komori S, Hashimoto K. Effects and interaction, of cariporide and preconditioning on cardiac arrhythmias and infarction in rat in vivo. *Br J Pharmacol.* 1999;127:1048–1055.
  - 33 Gumina RJ, Mizumura T, Beier N, Schelling P, Schultz JJ, Gross GJ. A new sodium/hydrogen exchange inhibitor, EMD 85131, limits infarct size in dogs when administered before or after coronary artery occlusion. *J Pharmacol Exp Ther.* 1998;286:175–183.
  - 34 Gazmuri RJ, Ayoub IM, Kolarova JD, Karmazyn M. Myocardial protection during ventricular fibrillation by inhibition of the sodium-hydrogen exchanger isoform-1. *Crit Care Med.* 2002;30:S166–S171.