ELSEVIER

European Journal of Pharmacology 404 (2000) 201–212

ejp

www.elsevier.nl/locate/ejphar

Reduction of myocardial infarct size by SM-20550, a novel Na^+/H^+ exchange inhibitor, in rabbits

Kazuto Yamada, Kazuki Matsui *, Kimihiko Satoh, Masahumi Kitano, Setsuko Yamamoto, Naohito Ohashi

Sumitomo Pharmaceuticals Co., Ltd., Research Center 1-98 Kasugadenaka 3-chome, Konohana-ku, Osaka 554-0022, Japan

Received 24 May 2000; received in revised form 2 August 2000; accepted 8 August 2000

Abstract

The effects of *N*-(aminoiminomethyl)-1,4-dimethyl-1*H*-indole-2-carboxamide methanesulfonic acid (SM-20550), a novel potent Na^+/H^+ exchanger, and nicorandil, a K^+ channel opener with nitrate-like activity, were studied in a myocardial ischemia and reperfusion injury model. Anesthetized rabbits underwent occlusion of the coronary artery (30 min) followed by reperfusion (5 h). Intravenous administration of SM-20550 before ischemia reduced the infarct size by approximately 30–70% in a dose-dependent manner, with a significant reduction in serum creatine phosphokinase activity. Similarly, intravenous administration of nicorandil before ischemia reduced the infarct size by 33% with a significant reduction in serum creatine phosphokinase activity. Moreover, intravenous administration of SM-20550 after ischemia resulted in a significant, approximately 20–40% reduction in the infarct size, but the administration of nicorandil after ischemia did not reduce the infarct size. These results indicate that SM-20550 reduced myocardial necrosis when administered either before or after ischemia. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Na^+/H^+ exchange; Myocardial ischemia; Reperfusion injury

1. Introduction

One of the major causes of myocardial ischemia reperfusion injury is thought to be an increase in intercellular Ca^{2+} concentration (i.e., 'Ca^{2+} overload'), which involves the activation of the Na^+/H^+ exchanger (Scholz and Albus, 1993; Avkiran, 1996; Piper et al., 1996). In the course of ischemia, a decrease in intracellular pH activates the Na^+/H^+ exchanger and intracellular H^+ is extruded in exchange for Na^+ to increase intracellular Na^+ concentration. Upon reperfusion, the Na^+/H^+ exchanger is activated again by the washing out of the acidic extracellular fluid, leading to an increased Ca^{2+} concentration, which is caused by the Na^+/Ca^{2+} exchanger. Since a high Ca^{2+} concentration induces cardiac arrhythmia and necrosis (Scholz and Albus, 1993; Avkiran, 1996; Piper et al., 1996), the use of inhibitors of the Na^+/H^+ exchanger has been proposed to reduce arrhythmia and necrosis by preventing Ca^{2+} overload. In fact, inhibitors of the Na^+/H^+ exchanger, ethylisopropyl-amiloride (Bugge et al., 1996), (3-methylsulfonyl-4-piperidinobenzoyl) guanidine methanesulfonate (HOE694) (Klein et al.,1995; Rohmann et al., 1995; Yasutake et al., 1994; Toit and Opie, 1993), 4-isopropyl-3-methylsulphonyl-benzoyl-guanidine methanesulfonate (HOE642) (Garcia-Dorado et al., 1997; Klein et al., 1997, 1998; Miura et al., 1997; Xue et al., 1996; Aye et al., 1997), and 2-methyl-5-methylsulphonyl-1-(1-pyrrollyl)-benzoyl-guanidine (EMD85131) (Gumina et al., 1998) have been reported to inhibit stunning, reperfusion arrhythmia, and myocardial infarction in several animal models.

We recently identified a novel potent and highly selective inhibitor of the Na^+/H^+ exchanger, *N*-(aminoiminomethyl)-1,4-dimethyl-1*H*-indole-2-carboxamide methanesulfonic acid (SM-20550) (Fig. 1), which has IC_{50} of 10 nM for Na^+/H^+ exchange activity in rat cardiomyocytes and little effect on several channels or receptors (Yamamoto et al., 2000). In addition, SM-20550 has been demon-

* Corresponding author. Tel.: +81-6-6466-5205; fax: +81-6-6466-5287.

E-mail address: kmatsui@sumitomopharm.co.jp (K. Matsui).

0014-2999/00/$ - see front matter © 2000 Elsevier Science B.V. All rights reserved.
PII: S0014-2999(00)00610-5

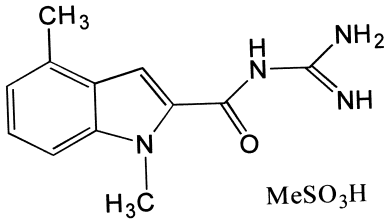

Fig. 1. The structure of SM-20550.

strated to have a cardioprotective effect in isolated perfused rat heart (Yamamoto et al., 2000). The present study focuses on the effect of SM-20550 on myocardial ischemia reperfusion injury in a rabbit model. SM-20550 was administered before or after ischemia. Pre-ischemic treatment with the Na^+/H^+ exchange inhibitors, ethylisopropyl-amiloride (Bugge et al., 1996), HOE694 (Klein et al., 1995; Rohmann et al., 1995), HOE642 (Garcia-Dorado et al., 1997; Klein et al., 1997; Miura et al., 1997), and EMD85131 (Gumina et al., 1998) has been shown to have cardioprotective effects. However, the effects of post-ischemic treatment with these inhibitors have been less clear, with several groups reporting contradictory results. Ethylisopropyl-amiloride, administered after ischemia, failed to reduce myocardial necrosis in a rabbit myocardial ischemia and reperfusion injury model (Bugge et al., 1996). Similarly, post-ischemic treatment with HOE642 could not reduce necrosis in rabbit and porcine model (Garcia-Dorado et al., 1997; Miura et al., 1997). On the other hand, Rohmann reported that HOE694, administered after ischemia, limited the infarct size (Rohmann et al., 1995), although Klein failed to observe reduced necrosis with HOE694 in a porcine models (Klein et al., 1995). A more recent report on EMD85131, however, revealed that EMD85131 could limit the infarct size when administered after ischemia in a canine model (Gumina et al., 1998).

In the present study, we sought to clarify the efficacy of Na^+/H^+ exchanger inhibitors on myocardial infarction, not only when administered before ischemia but also after ischemia, using the novel selective Na^+/H^+ exchanger inhibitor, SM-20550. We also compared the anti-necrotic effects of SM-20550 to those of nicorandil, a K^+ channel opener with nitrate-like activity, which has recently been shown to have cardioprotective effects in patients with anterior wall myocardial infarction (Ito et al., 1999a).

2. Methods

2.1. Surgical preparation

All procedures involving the use of animals were reviewed and approved by the Institutional Animal Care and Use Committee at Sumitomo Pharmaceuticals Research Center (Osaka, Japan).

Animal preparation was based on a minor modification of a previously reported procedure (Chiariello et al., 1988; Yamada et al., 1998). Briefly, male New Zealand White rabbits (2.5–3.5 kg, Kitayama Labes, Ina, Japan) were anesthetized with sodium pentobarbital (40 mg/kg, i.v.). The rabbits were intubated through a tracheotomy and ventilated with a mechanical respirator (SN-480-6 or SN-480-5, Shinano, Tokyo, Japan). A catheter was inserted into the right carotid artery to allow measurement of the mean arterial blood pressure by a pressure transducer (TP-300T or DX-100, Nihon Koden, Tokyo, Japan). Another catheter was inserted into the right jugular vein for the administration of vehicle, SM-20550 or nicorandil. Additional anesthesia was also administered through the right femoral vein as needed. A thoracotomy was performed through the fourth intercostal space and the heart was suspended in a pericardial cradle. A catheter was placed in the left atrium through the left atrial appendage. A 4-0 silk suture was tied around the large marginal branch of the left circumflex coronary artery. The Electrocardiogram lead II was used to monitor the heart rate and ST-segment elevation. The mean arterial blood pressure was continuously monitored by blood pressure meter (AP-621G and AP-611G or AP-641G, Nihon Koden) and the electrocardiogram was monitored by electrocardiogram amplifier (AC-601G, Nihon Koden, Tokyo, Japan) during the experiment. The heart rate was also measured using heart rate counter (AT-600G or AT-601G, Nihon Koden).

After surgery, the rabbits were allowed to stabilize for at least 30 min. The arterial blood pH, pO_2, and pCO_2 were measured by a blood gas system 248 (Ciba Corning, Tokyo, Japan) and maintained within the physiological range by adjusting the respiration rate and oxygen flow. After a baseline recording of the mean arterial blood pressure, heart rate and electrocardiogram was taken, myocardial ischemia was induced by tightening the silk suture to occlude the vessel completely. Coronary artery occlusion was maintained for 30 min and myocardial ischemia was confirmed by the regional cyanosis and ST-segment elevation. The occlusion was then released and reperfusion was allowed for 5 h. For the sham operated group, the coronary artery was not occluded, but all other experimental procedures were performed in the same way as for the group that underwent occlusion.

2.2. Experimental protocol

Vehicle or SM-20550 (Sumitomo Pharmaceutical) was intravenously administered by bolus injection at 10 min prior to ischemia in the first set of experiments, and at 10 min prior to reperfusion in the second set of experiments. After the bolus injection, the infusion of vehicle or SM-20550 was sustained until the termination of the experiment. The rabbits were divided into four groups in the first and second set of experiments. These groups received (1)

10% polyethylene glycol 400 (Nacalai Tesque, Kyoto, Japan) as vehicle, in the same volume as the solutions containing inhibitor, (2) 0.0017 mg/kg by bolus injection and 0.0028 mg/kg/h by continuous infusion as the "Low" dose group of SM-20550, (3) 0.017 mg/kg by bolus injection and 0.028 mg/kg/h by continuous infusion as the "Mid" dose group of SM-20550, and (4) 0.17 mg/kg by bolus injection and 0.28 mg/kg/h by continuous infusion as the "High" dose group of SM-20550.

Similarly, vehicle or nicorandil (SIGMART®Injection, Chugai Pharmaceutical, Tokyo, Japan) was intravenously administered by bolus injection at 10 min prior to ischemia or at 10 min prior to reperfusion. After the bolus injection, vehicle or nicorandil infusion was sustained until the termination of the experiment. The rabbits were divided into two groups in the first and second set of experiments. These groups received (1) 10% polyethylene glycol 400 (Nacalai Tesque) as vehicle, in the same volume as the nicorandil solution, and (2) 0.1 mg/kg nicornadil by bolus injection and 1.5 mg/kg/h by continuous infusion. This dosage of nicorandil was reported to reduce the infarct size in a canine myocardial ischemia and reperfusion model (Gross et al., 1992)

2.3. Quantification of myocardial area-at-risk and necrotic area

At the end of 5 h of reperfusion, the silk suture was tightened again and 1% Evans blue solution was infused

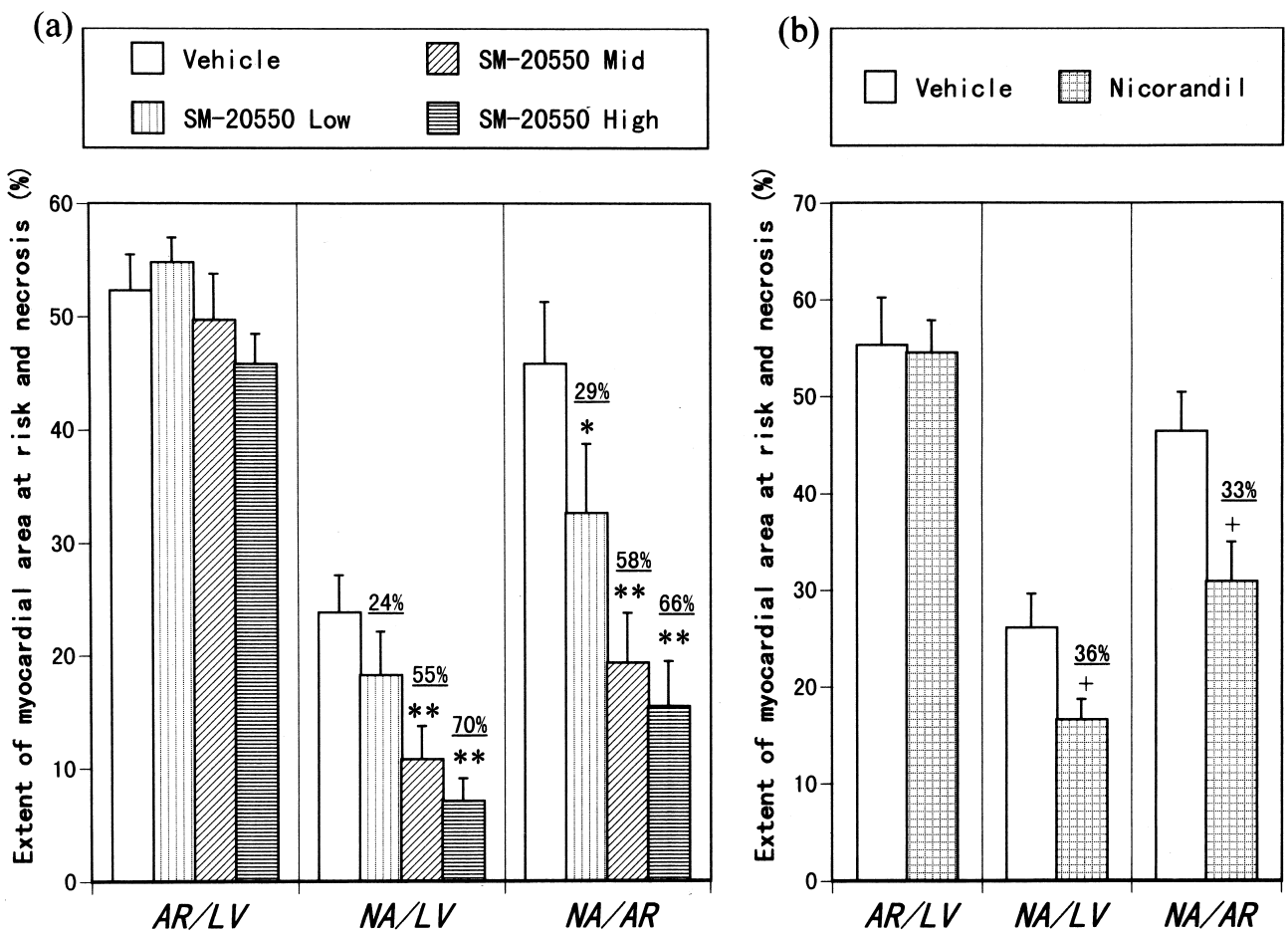

Fig. 2. Effect of pre-ischemic treatment with (a) SM-20550 or (b) nicorandil on myocardial infarct size. Rabbits were subjected to 30 min of coronary artery occlusion (CAO) followed by 5 h of reperfusion. AR, LV, and NA indicate the area at risk, left ventricle area, and necrotic area, respectively. Each bar represents the mean ± S.E. The underlined values indicate percentage of inhibition.

Administration protocol — (a) Vehicle: Bolus intravenous administration of vehicle (10% PEG) at 10 min prior to CAO followed by infusion until termination of the experiment ($n = 8$). SM-20550 Low: Bolus intravenous administration of SM-20550 (0.0017 mg/kg) at 10 min prior to CAO followed by infusion (0.0028 mg/kg/h) until termination of the experiment ($n = 8$). SM-20550 Mid: Bolus intravenous administration of SM-20550 (0.017 mg/kg) at 10 min prior to CAO followed by infusion (0.028 mg/kg/h) until termination of the experiment ($n = 8$). SM-20550 High: Bolus intravenous administration of SM-20550 (0.17 mg/kg) at 10 min prior to CAO followed by infusion (0.28 mg/kg/h) until termination of the experiments ($n = 7$). Statistical significance: *, $P < 0.05$; **, $P < 0.01$ vs. vehicle by Williams' test. (b) Vehicle: Bolus intravenous administration of vehicle (10% PEG) at 10 min prior to CAO followed by infusion until termination of the experiment ($n = 6$). Nicorandil: Bolus intravenous administration of nicorandil (0.1 mg/kg) at 10 min prior to CAO followed by infusion (1.5 mg/kg/h) until termination of the experiment ($n = 7$). Statistical significance: +, $P < 0.05$ vs. vehicle by Student's *t*-test.

quickly via catheter into the left atrium. The heart was then isolated and soaked in ice-cold saline. The left ventricle was dissected free from all other structures and cut into 13 slices that were parallel to the atrioventricular groove. The slices were observed to be either positive or negative staining areas and traced onto transparent plastic sheets. Evans blue-negative staining defines the area-at-risk. The slices were then incubated in a 1% solution of 2,3,5-triphenyltetrazolium chloride (Wako, Osaka, Japan) for 10 min at 37°C and soaked in a 10% formaldehyde neutral buffer solution overnight to enhance the contrast of the staining. The triphenyltetrazolium chloride-positive or negative staining areas were traced onto transparent plastic sheets. Triphenyltetrazolium chloride-negative staining defines the necrotic area.

The total left ventricle area, the area-at-risk, and the necrotic area were measured using image analyzing software (NIH Image, Wayne Rasband National Institutes of Health, USA).

2.4. Measurement of serum creatine phosphokinase

Blood samples (2.5 ml) were withdrawn by catheter before the administration of vehicle, SM-20550 or nicorandil, immediately before ischemia, immediately before reperfusion, and at 1, 3, and 5 h after reperfusion. Whole-

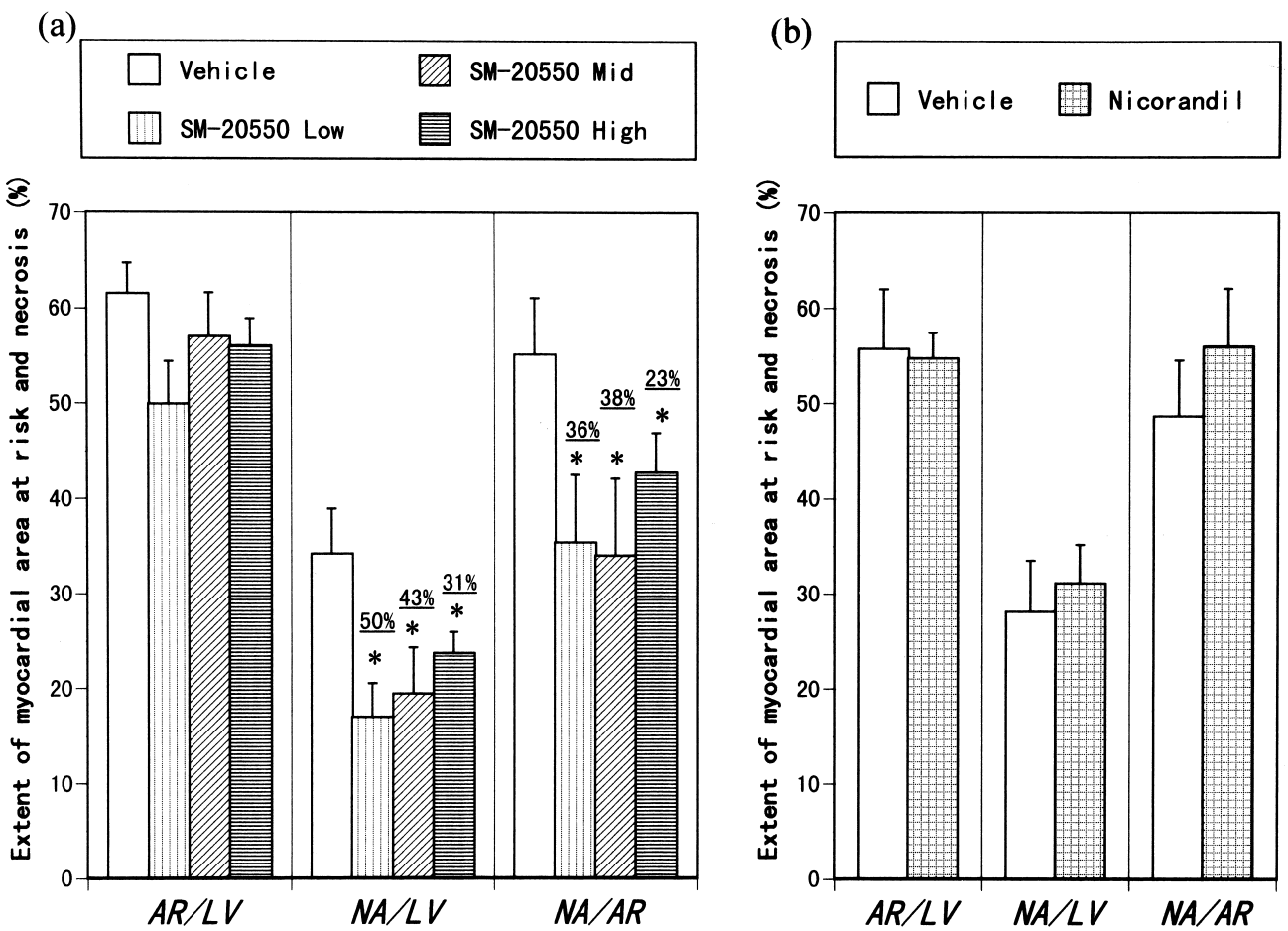

Fig. 3. Effect of post-ischemic treatment with (a) SM-20550 or (b) nicorandil on myocardial infarct size. Rabbits were subjected 30 min of coronary artery occlusion followed by 5 h of reperfusion. AR, LV, and NA indicated the area at risk, left ventricle area, and necrotic area, respectively. Each bar represents the mean ± S.E. The underlined values indicate percentage of inhibition.

Administration protocol — (a) Vehicle: Bolus intravenous administration of vehicle (10% PEG) at 10 min prior to reperfusion followed by infusion until termination of the experiment ($n = 7$). SM-20550 Low: Bolus intravenous administration of SM-20550 (0.0017 mg/kg) at 10 min prior to reperfusion followed by infusion (0.0028 mg/kg/h) until termination of the experiment ($n = 6$). SM-20550 Mid: Bolus intravenous administration of SM-20550 (0.017 mg/kg) at 10 min prior to reperfusion followed by infusion (0.028 mg/kg/h) until termination of the experiment ($n = 6$). SM-20550 High: Bolus intravenous administration of SM-20550 (0.17 mg/kg) at 10 min prior to reperfusion followed by infusion (0.28 mg/kg/h) until termination of the experiment ($n = 6$). Statistical significance: *, $P < 0.05$ vs. vehicle by Williams' test. (b) Vehicle: Bolus intravenous administration of vehicle (10% PEG) at 10 min prior to reperfusion followed by infusion until termination of the experiments ($n = 7$). Nicorandil: Bolus intravenous administration of nicorandil (0.1 mg/kg) at 10 min prior to reperfusion followed by infusion (1.5 mg/kg/h) until termination of the experiment ($n = 6$). Statistical significance: +, $P < 0.05$ vs. vehicle by Student's t-test.

blood samples were transferred to collection tubes and spun at $1000 \times g$ for 20 min at 4°C. The serum samples were collected and stored at −20°C until they were used for the creatine phosphokinase measurement, which was performed using the colorimetric method of Allain et al. (1973) using a CPK II test kit (Wako).

2.5. Serum concentration of SM-20550

The serum sample (1 ml) was added to 50 μl of internal standard solution, 2 ml of 0.2 M sodium carbonate/sodium hydrogen carbonate solution (pH 10), 100 mg of sodium chloride and 6 ml of diethylether. The mixture was shook for 15 min and spun at $6000 \times g$ for 15 min. The organic layer was mixed with 2 ml of 1 N hydrogen chloride and was shook for 10 min and spun at $6000 \times g$ for 5 min. The aqueous layer was added 2 ml of 0.2 M sodium carbonate/sodium hydrogen carbonate solution (pH 10) and 6 ml of diethylether and the mixture was shook for 10 min and spun at $6000 \times g$ for 5 min. The organic layer was evaporated to dryness and the residue was resolved in 200 μl of high-performance liquid chromatography (HPLC) carrier for HPLC analysis or 100 μl of 0.3% acetic acid for liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis.

HPLC analysis: HPLC separation was carried out on a Puresil C18 (4.6 mm i.d. × 15 cm, 5 μm: Waters, MA, USA) with a mobile phase of Pic B7 (Waters)/methanol = 4/6(v/v) at a flow rate of 1 ml/min and the eluent was monitored at 310 nm.

LC-MS/MS analysis: HPLC separation was carried out on a Puresil C18 (2.1 mm i.d. × 15 cm, 5 μm: Waters) with a mobile phase of 0.3% acetic acid/tetrahydrofuran/methanol = 50/10/40(v/v/v) at a flow rate of 0.2 ml/

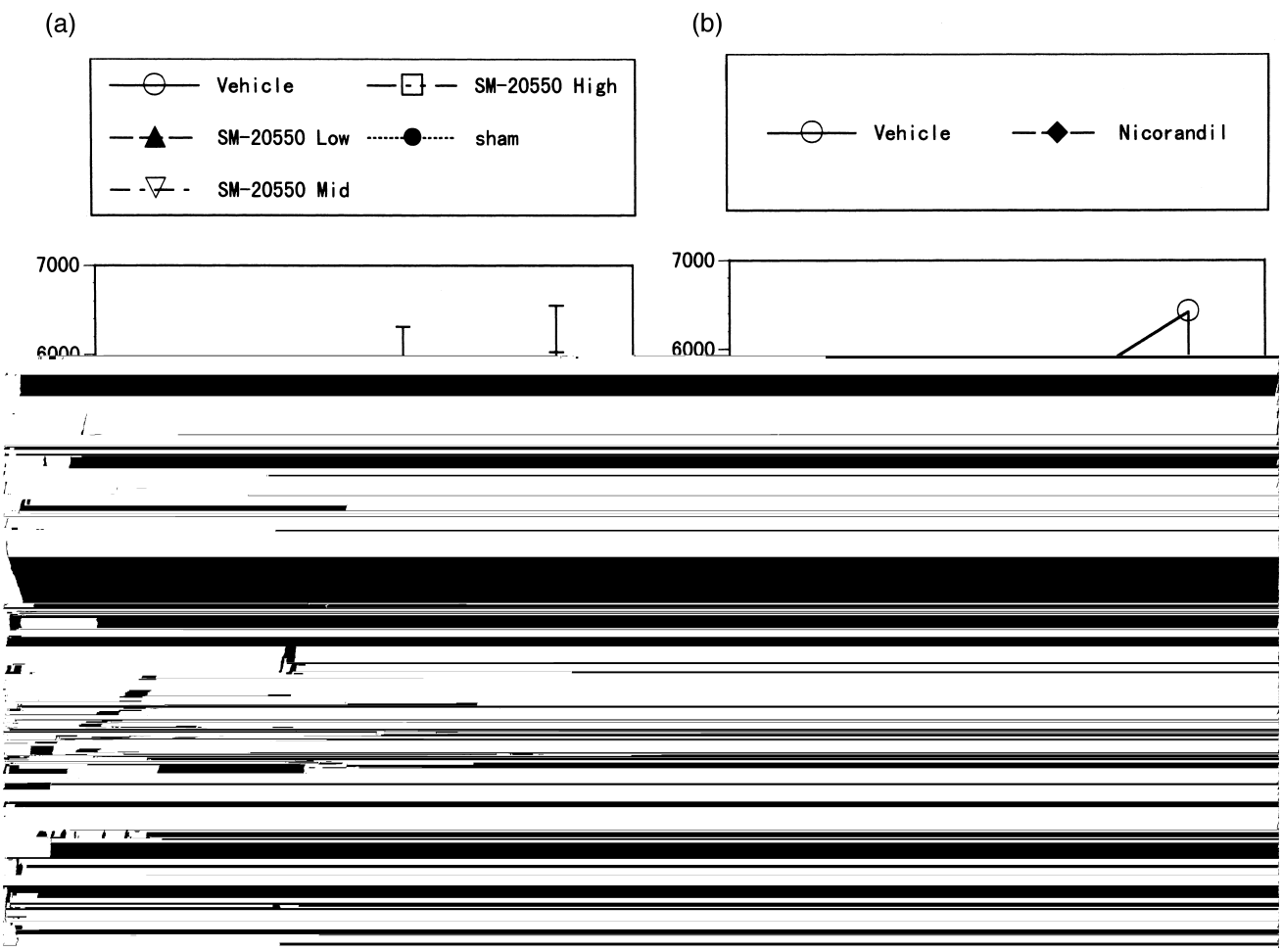

Fig. 4. Effect of pre-ischemic treatment with (a) SM-20550 or (b) nicorandil on serum creatine phosphokinase activity. Rabbits were subjected to 30 min of coronary artery occlusion (CAO) followed by 5 h reperfusion. Blood samples were collected at the indicated time points. The creatine phosphokinase (CPK) activity in the serum was measured. Rabbits in the sham group ($n = 4$) were not subjected to coronary artery occlusion, but were otherwise treated the same as the other groups. Each point represents the mean ± S.E. See the legend for Fig. 2 for details. (a) Vehicle ($n = 8$), SM-20550 Low ($n = 7$), SM-20550 Mid ($n = 8$), SM-20550 High ($n = 7$). Statistical significance: *, $P < 0.05$; **, $P < 0.01$ vs. vehicle by Williams' test, \$, $P < 0.05$; \$\$, $P < 0.01$ vs. sham by Student's *t*-test, #, $P < 0.05$ vs. sham by Welch's test. (b)Vehicle ($n = 6$), nicorandil ($n = 7$). Statistical significance: +, $P < 0.05$ vs. vehicle by Student's *t*-test.

Table 1
Summary of hemodynamic parameters with pre-ischemic treatment

(a)

Hemodynamic parameters	Treatment group	Before treatment	Before ischemia	Post-ischemia		Post-reperfusion						
				10 min	30 min	10 min	30 min	60 min	120 min	180 min	240 min	300 min
Mean arterial blood pressure (mm Hg)	Vehicle ($n=8$)	79.1±2.2	78.3±1.9	65.8±4.3	63.6±2.7	57.5±2.5	59.0±1.9	60.5±1.8	62.3±2.4	61.9±3.3	60.5±3.3	56.8±2.8
	SM-20550 Low ($n=8$)	79.0±2.4	78.0±2.6	69.1±4.0	63.5±5.0	57.3±4.4	59.3±4.0	56.5±3.9	53.9±3.2	55.5±3.4	50.4±3.5	52.1±3.8
	SM-20550 Mid ($n=8$)	76.4±2.4	75.0±1.6	66.3±1.8	58.8±4.1	55.0±3.3	53.0±3.0	55.3±2.8	59.6±2.5	57.9±3.0	54.9±2.6	57.8±2.1
	SM-20550 High ($n=7$)	77.0±3.4	74.1±3.9	68.6±4.7	69.3±4.0	60.4±3.7	59.0±4.3	55.7±4.1	60.7±3.2	59.7±5.1	61.9±3.5	62.9±3.7
Heart rate (beats/min)	Vehicle ($n=8$)	261.6±8.6	259.8±8.8	252.3±9.8	252.9±9.2	248.0±10.2	243.0±8.9	237.5±10.0	232.4±10.5	222.4±10.2	215.4±11.1	203.8±12.8
	SM-20550 Low ($n=8$)	263.0±8.5	267.5±8.8	265.6±10.3	260.0±10.3	246.1±6.6	242.0±9.1	235.4±8.0	227.4±9.2	218.1±9.0	208.6±10.5	204.6±11.8
	SM-20550 Mid ($n=8$)	254.6±5.9	255.6±7.8	247.9±8.5	242.4±10.1	230.0±9.0	229.3±9.7	228.5±7.2	210.6±8.9	201.5±9.6	190.6±10.5	186.3±12.2
	SM-20550 High ($n=7$)	265.0±7.2	271.0±7.5	255.6±9.6	255.4±7.4	250.7±6.7	245.7±7.7	238.9±9.4	226.1±9.6	217.7±13.5	208.9±15.4	210.1±17.0

(b)

Mean arterial blood pressure (mm Hg)	Vehicle ($n=6$)	76.2±2.7	75.0±2.8	66.5±3.9	57.8±4.0	52.3±4.2	53.0±3.4	54.0±1.9	56.8±1.6	52.5±1.2	53.0±3.7	51.3±4.2
	Nicorandil ($n=7$)	76.7±2.1	68.1±3.8	60.4±3.1	51.3±3.5	47.9±2.9	44.9±3.5	46.6±2.2[b]	45.0±2.8[c]	44.9±2.8[b]	42.9±3.0	42.3±3.6
Heart rate (beats/min)	Vehicle ($n=6$)	273.5±13.1	274.2±13.4	273.4±19.5	265.7±12.2	265.3±9.5	257.7±10.5	247.3±12.5	233.7±10.8	220.7±7.9	216.7±10.0	213.2±13.2
	Nicorandil ($n=7$)	279.3±10.8	281.1±9.1	276.6±9.9	271.9±10.8	268.4±11.5	258.6±10.7	250.7±13.1	239.7±11.6	230.6±11.2	219.6±13.7	212.0±20.9

(a) SM-20550 or (b) nicorandil was administered at 10 min prior to coronary artery occlusion (see the legend in Fig. 2 for details).
Each point represents the mean±S.E.
(a) [a] $P<0.05$ vs. vehicle by Williams' test.
(b) [b] $P<0.05$ vs. vehicle by Student's t-test, [c] $P<0.01$ vs. vehicle by Student's t-test.

Table 2
Summary of hemodynamic parameters with post-ischemic treatment

(a)

Hemodynamic parameters	Treatment group	Before ischemia	Post-ischemia			Post-reperfusion						
			10 min	20 min	30 min	10 min	30 min	60 min	120 min	180 min	240 min	300 min
Mean arterial blood pressure (mm Hg)	Vehicle ($n=7$)	84.3±2.1	70.9±3.5	69.4±3.1	69.7±3.0	60.6±2.6	58.9±2.6	57.7±1.8	63.6±2.0	61.3±2.8	59.3±2.5	58.4±2.9
	SM-20550 Low ($n=6$)	77.5±2.9	67.8±4.4	62.3±3.4	6.23±4.0	59.3±3.3	59.7±2.9	61.7±2.6	59.8±3.9	60.2±3.7	60.2±3.1	62.5±3.9
	SM-20550 Mid ($n=6$)	80.2±3.0	71.0±5.9	69.0±5.9	66.3±4.6	60.7±4.7	61.8±4.3	61.7±5.2	64.5±3.8	62.5±3.8	57.0±2.5	58.8±2.1
	SM-20550 High ($n=6$)	79.7±3.0	72.5±6.5	65.3±8.0	66.5±7.9	63.5±7.5	64.7±7.0	64.0±6.6	65.3±6.9	59.3±5.5	60.2±4.7	62.7±4.1
Heart rate (beats/min)	Vehicle ($n=7$)	275.0±10.4	281.3±15.5	259.1±11.9	260.1±11.2	258.0±10.8	252.0±7.9	242.1±7.6	236.1±6.7	227.4±7.2	216.0±7.3	208.1±9.1
	SM-20550 Low ($n=6$)	268.0±12.7	240.5±3.8	242.8±9.1	239.2±8.6	250.3±9.6	245.8±8.8	236.7±6.0	230.7±7.8	223.0±6.8	215.7±8.7	214.8±8.5
	SM-20550 Mid ($n=6$)	271.7±12.4	255.8±5.1	255.2±5.7	254.5±6.2	245.2±6.8	250.0±5.7	241.0±5.2	234.8±3.4	220.7±2.6	216.3±6.2	211.2±3.1
	SM-20550 High ($n=6$)	260.7±5.7	253.8±9.5	249.5±11.0	246.0±9.8	250.5±7.1	250.5±5.6	249.8±5.6	238.3±4.5	224.0±4.7	230.7±9.0	229.5±9.4[a]

(b)

Mean arterial blood pressure (mm Hg)	Vehicle ($n=7$)	74.1±1.5	66.0±4.6	58.0±2.9	61.3±3.5	56.6±2.5	56.7±2.5	54.7±3.9	55.1±4.8	45.4±5.9	47.0±6.2	42.0±5.5
	Nicorandil ($n=6$)	76.2±1.7	71.7±2.6	61.5±6.9	64.3±5.7	48.3±5.5	45.5±4.7	47.2±3.8	44.5±3.3	42.5±3.5	44.5±3.9	45.8±4.4
Heart rate (beats/min)	Vehicle ($n=7$)	280.4±9.2	278.7±8.6	270.0±7.3	266.1±8.5	268.3±6.2	264.7±5.6	258.1±11.5	248.9±10.5	225.4±11.1	219.9±13.5	211.4±13.0
	Nicorandil ($n=6$)	267.0±8.0	280.3±17.6	244.3±11.8	252.2±9.6	260.5±7.7	260.3±7.4	259.2±6.0	238.8±8.3	238.7±5.6	229.0±8.3	222.2±12.0

(a) SM-20550 or (b) nicorandil was administered at 10 min prior to reperfusion (see the legend in Fig. 3 for details).
Each point represents the mean±S.E.
(a) [a] $P < 0.05$ vs. vehicle by Williams' test.
(b) [b] $P < 0.05$ vs. vehicle by Student's *t*-test.

significant effect on heart rate was observed at any recorded time points for the nicorandil-treated group compared with the vehicle-treated group (Tables 1b and 2b).

4. Discussion

In myocardial ischemia and reperfusion injury, inhibition of the Na^+/H^+ exchanger prevents stunning, reperfusion arrhythmia, and myocardial infarction in several animal models. Recently, a novel potent and highly selective inhibitor of the Na^+/H^+ exchanger, SM-20550 (Fig. 1), has been demonstrated to inhibit Na^+/H^+ exchange activity in rat cardiomyocytes with its IC_{50} of 10 nM, which is approximately 10 times lower than that of ethylisopropyl-amiloride, and have little effect on several channels or receptors other than Na^+/H^+ exchanger (Yamamoto et al., 2000). In the present study, pre-ischemic treatment with SM-20550 reduced the infarct size (Fig. 2a) and serum creatine phosphokinase activity (Fig. 4a) in a dose-dependent manner, without affecting hemodynamic parameters (Table 1a). In the course of ischemia, the Na^+/H^+ exchanger is activated as a result of cellular acidosis (Tani and Neely, 1989; Imahashi et al., 1998), and upon reperfusion, the Na^+/H^+ exchanger is activated again by the washing out of acidic extracellular fluid, which induces an intracellular and extracellular pH imbalance (Tani and Neely, 1989). The activation of the Na^+/H^+ exchanger during myocardial ischemia and reperfusion induces an increase in the intracellular Na^+ concentration, which ultimately leads to Ca^{2+} overload due to activation of the Na^+/Ca^{2+} exchanger (Scholz and Albus, 1993; Avkiran, 1996; Piper et al., 1996). The inhibitory effect of SM-20550 on the activation of the Na^+/H^+ exchanger during ischemia and reperfusion could lead to reduced Ca^{2+} overload and inhibition of myocardial necrosis. In fact, SM-20550 reduced the increases in tissue Na^+ and Ca^{2+} contents and improved cardiac function during global ischemia and reperfusion in isolated perfused rat heart (Yamamoto et al., 2000). The efficacy of SM-20550 in preventing myocardial necrosis when administered before ischemia observed here is consistent with the results of previous studies using other Na^+/H^+ exchanger inhibitors such as ethylisopropyl-amiloride (Bugge et al., 1996), HOE694 (Klein et al., 1995; Rohmann et al., 1995), HOE642 (Garcia-Dorado et al., 1997; Klein et al., 1997; Miura et al., 1997), and EMD85131 (Gumina et al., 1998).

In contrast to the pre-ischemic treatment, the effects of post-ischemic treatment with Na^+/H^+ exchanger inhibitors have been controversial. Here, SM-20550 significantly decreased myocardial necrosis by approximately 20–40% when administered 10 min prior to reperfusion (Fig. 3a) and serum creatine phosphokinase activity (Fig. 5a). On the other hand, ethylisopropyl-amiloride (Bugge et al., 1996) and HOE642 (Miura et al., 1997) do not inhibit the infarct size when administered 10 or 5 min prior to reperfusion in a rabbit model with 30 min of myocardial ischemia and 3 h of reperfusion. Furthermore, HOE642 failed to reduce necrosis when administered 10 min prior to reperfusion in a model with 55 min of ischemia and 5 h of reperfusion (Garcia-Dorado et al., 1997), and when administered 15 min prior to reperfusion in a pig model with 60 min of low-flow ischemia and 24 h of reperfusion (Klein et al., 1998), although HOE642 reduced the infarct size when administered 45 min prior to reperfusion in the same low-flow ischemia model (Klein et al., 1998). Additional conflicting results have been reported for the effect of post-ischemic treatment with HOE694. Klein demonstrated that 3 mg/kg of HOE694, administered 10 min prior to reperfusion, failed to reduce the infarct size in a porcine model with 45 min of ischemia and 24 h of reperfusion (Klein et al., 1995), while Rohmann demonstrated that 7 mg/kg of HOE694, administered 15 min prior to reperfusion, reduced the infarct size in a porcine model with 60 min of ischemia and 2 h of reperfusion (Rohmann et al., 1995). In addition, Rohmman proposed that 10 times higher dosages of a Na^+/H^+ exchanger inhibitor are necessary to protect during reperfusion than during ischemia (Rohmann et al., 1995). Among the conflicting results for post-ischemic effect of Na^+/H^+ exchange inhibitors, the effect of SM-20550 on myocardial necrosis appeared to be similar to that of EMD85131 (Gumina et al., 1998). We demonstrated that post-ischemic treatment with SM-20550 reduced the infarct size, and this inhibition did not require higher dosages than those used for the pre-ischemic treatment. Similarly, the same dosage of EMD85131 administered post- and pre-ischemically reduces the infarct size in a canine myocardial ischemia and reperfusion model (Gumina et al., 1998). The reason for these conflicting results for post-ischemic administration of Na^+/H^+ exchange inhibitors may not be clear at present, but may include the differences in their potency to inhibit Na^+/H^+ exchanger, in their distribution rate to heart, or in experimental protocols.

As discussed above, upon reperfusion, the Na^+/H^+ exchanger is reactivated by the washing out of the acidic extracellular fluid. Tani and Neely showed that there is an overshoot of intracellular Na^+ concentration upon reperfusion in the isolated perfused rat heart (Tani and Neely, 1989), which suggests that Na^+/H^+ exchange inhibitors could reduce the Na^+ influx to some extent, even if administered just before reperfusion. Naturally, treatment with Na^+/H^+ exchanger inhibitors before ischemia reduces the increase in intracellular Na^+ concentration during ischemia (Tani and Neely, 1989; Imahashi et al., 1998) and would also reduce the increased Na^+ upon reperfusion, while treatment with Na^+/H^+ exchanger inhibitors just before reperfusion would only reduce the increase in intracellular Na^+ concentration that is associated with reperfusion. As shown in Fig. 6, the serum concentration of SM-20550 reached a steady state by 10 min after

administration and remained almost constant during the experiment. This could explain why the pre-ischemic treatment with SM-20550 showed a greater inhibitory effect on myocardial necrosis (approximately 30–70%) than the post-ischemic treatment (approximately 20–40%). Recently, SM-20550 has been reported to reduce the infarct size in canine myocardial injury after reperfusion when administered either pre- or post-ischemically (Ito et al., 1999b). The anti-necrotic effect of SM-20550 in rabbits observed in this study supports the results obtained in dogs (Ito et al., 1999b).

The pre-ischemic treatment with nicorandil reduced the infarct size (Fig. 2b) and serum creatine phosphokinase activity (Fig. 4b). In contrast, post-ischemic nicorandil treatment did not reduce the infarct size (Fig. 3b) and serum creatine phosphokinase activity (Fig. 5b). These results support previous reports that used a rabbit infarct model (Imagawa et al., 1998) and a canine model of myocardial ischemia and reperfusion (Gross et al., 1992; Mizumura et al., 1996). Treatment with nicorandil tended to reduce the mean arterial blood pressure, with statistically significant reductions in some time points (Tables 1b and 2b). It is possible that nicorandil, which is a K^+ channel opener that also has nitrate-like activity, reduced the mean arterial blood pressure by causing vasodilatation. Recently, the effect of intravenous nicorandil has been examined in patients with anterior wall myocardial infarction (Ito et al., 1999a). Regional left ventricular function, wall motion score, and regional wall motion were significantly improved by the treatment with nicorandil (Ito et al., 1999a). Considering that SM-20550 reduced the infarct size when administered both pre- and post-ischemically, while post-ischemic nicorandil treatment did not reduce the infarct size in the present study (Figs. 2, 3 and 4), clinical efficacy of SM-20550 in patients with myocardial infarction would be expected.

In summary, we demonstrated that SM-20550, a novel Na^+/H^+ exchanger inhibitor, reduced myocardial infarction when administered either pre- or post-ischemically, without affecting hemodynamic parameters. In contrast, nicorandil reduced myocardial infarction only when administered pre-ischemically. Thus, treatment with SM-20550 would be expected to be beneficial for myocardial ischemia and reperfusion injury.

Acknowledgements

We thank Ms. Masako Toda and Mr. Kenji Miyazaki for excellent technical assistance during the course of this study. We are grateful to Mr. Takao Watanabe in the Pharmacokinetics Group and other members of the Cardiovascular Group for their contribution to this work and for valuable discussion.

References

Allain, C.C., Henson, C.P., Nadel, M.K., Knoblesdorff, A.J, 1973. Rapid single-step kinetic colorimetric assay for lactate dehydrogenase in serum. Clin. Chem. 19, 223–227.

Avkiran, M., 1996. Sodium–hydrogen exchange in myocardial ischemia and reperfusion: a critical determinant of injury? In: Karmazyn, M. (Ed.), Myocardial Ischemia: Mechanism, Reperfusion, Protection. Birkhäuser Verlag, Basel, Switzerland, pp. 299–311.

Aye, N.N., Xue, Y.X., Hasimoto, K., 1997. Antiarrhythmic effects of cariporide, a novel Na^+–H^+ exchange inhibitor, on reperfusion ventricular arrhythmias in rat hearts. Eur. J. Pharmacol. 339, 121–127.

Bugge, E., Munch-Ellingsen, J., Ytrehus, K., 1996. Reduced infarct size in the rabbit heart in vivo by ethylisopropyl-amiloride. A role for Na^+/H^+ exchange. Basic Res. Cardiol. 91, 203–209.

Chiariello, M., Golino, P., Cappelli-Bigazzi, M., Ambrosio, G., Tritto, I., Salvatore, M., 1988. Reduction in infarct size by the prostacyclin analogue iloprost (ZK 36374) after experimental coronary artery occlusion–reperfusion. Am. Heart J. 115, 499–504.

Garcia-Dorado, D., Gonzaláz, M.A., Barrabés, J.A., Ruiz-Meana, J.A., Solares, M., Lidon, J., Blanco, R-M., Puigfel, J., Piper, Y., Soler-Soler, H.M., 1997. Prevention of ischemic rigor contracture during coronary occlusion by inhibition of Na^+–H^+ exchange. Cardiovasc. Res. 35, 80–89.

Gross, G.J., Auchampach, J.A., Maruyama, M., Warltier, D.C., Pieper, G.M., 1992. Cardioprotective effect of Nicorandil. J. Cardiovasc. Pharmacol. 20 (Suppl. 3), S22–S28.

Gumina, R.J., Mizumura, T., Beier, N., Schelling, P., Schultz, J.J., Gross, G.J., 1998. 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. 286, 175–183.

Imagawa, J., Baxter, G.F., Yellon, D.M., 1998. Myocardial protection afforded by Nicorandil and ischaemic preconditioning in a rabbit infarct model in vivo. J. Cardiovasc. Pharmacol. 31, 74–79.

Imahashi, K., Hashimoto, K., Yamaguchi, H., Nishimura, T., Kusuoka, H., 1998. Alteration of intracellular Na^+ during ischemia in diabetic rat hearts: the role of reduced activity in Na^+/H^+ exchange against stunning. J. Moll. Cell Cardiol. 30, 509–517.

Ito, H., Taniyama, Y., Iwakura, K., Nishikawa, N., Masuyama, T., Kuzuya, T., Hori, M., Higashino, Y., Fujii, K., Minamino, T., 1999a. Intravenous Nicorandil can preserve microvascular integrity and myocardial viability in patients with reperfused anterior wall myocardial infarction. J. Am. Coll. Cardiol. 33, 654–660.

Ito, Y., Imai, S., Ui, G., Nakao, M., Imai, K., Kamiyama, H., Naganuma, F., Matsui, K., Ohashi, N., Nagai, R., 1999b. A Na^+–H^+ exchange inhibitor (SM-20550) protects from microvascular deterioration and myocardial injury after reperfusion. Eur. J. Pharmacol. 374, 355–366.

Klein, H.H., Pich, S., Bohle, R.M., Wollenweber, J., Nebendahl, K., 1995. Myocardial protection by Na^+–H^+ exchange inhibition in ischemic, reperfused porcine hearts. Circulation 92, 912–917.

Klein, H.H., Bohle, R.M., Pich, S., Lindert-Heimberg, S., Wollenweber, J., Nebendahl, K., 1997. Time delay of cell death by Na^+/H^+-exchange inhibition in regionally ischemic, reperfused porcine hearts. J. Cardiovasc. Pharmacol. 30, 235–240.

Klein, H.H., Bohle, R.M., Pich, S., Lindert-Heimberg, S., Wollenweber, J., Schade-Brittinger, C., Nebendahl, K., 1998. Time-dependent protection by Na^+/H^+ exchange inhibition in a regionally ischemic, reperfused porcine heart preparation with low residual blood flow. J. Moll. Cell Cardiol. 30, 795–801.

Miura, T., Ogawa, T., Suzuki, K., Goto, M., Simamoto, K., 1997. Infarct size limitation by a new Na^+–H^+ exchange inhibitor, Hoe642: difference from preconditioning in the role of protein kinase C. J. Am. Coll. Cardiol. 29, 693–701.

Mizumura, T., Nithipatikom, K., Gross, G.J., 1996. Infarct size-reducing effect of nicorandil is mediated by the KATP channel but not by its nitrate-like properties in dogs. Cardiovasc. Res. 32, 274–285.

Piper, H.M., Basler, C., Ladilov, Y.V., Schäfer, M., Siegmund, B., Ruiz-Meana, M., Garcia-Dorado, D., 1996. The role of Na^+/H^+ exchange in ischemia–reperfusion. Basic Res. Cardiol. 91, 191–202.

Rohmann, S., Weygandt, H., Minck, K.–O., 1995. Preischaemic as well as postischaemic application of Na^+/H^+ exchange inhibitor reduces infarct size in pigs. Cardiovasc. Res. 30, 945–951.

Scholz, W., Albus, U., 1993. Na^+/H^+ exchange and its inhibition in cardiac ischemia and reperfusion. Basic Res. Cardiol. 88, 443–455.

Tani, M., Neely, J.R., 1989. 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. 65, 1045–1056.

Toit, E.F., Opie, L.H., 1993. Role for the Na^+/H^+ exchanger in reperfusion stunning in isolated perfused rat heart. J. Cardiovasc. Pharmacol. 22, 877–883.

Xue, Y.X., Aye, N.N., Hashimoto, K., 1996. Antiarrhythmic effects of HOE642, a novel Na^+–H^+ exchange inhibitor, on ventricular arrhythmias in animal hearts. Eur. J. Pharmacol. 317, 309–316.

Yamada, K., Tojo, S.J., Hayashi, M., Morooka, S., 1998. The role of P-selectin, sialyl Lewis X and sulfatide in myocardial ischemia and reperfusion injury. Eur. J. Pharmacol. 346, 217–225.

Yamamoto, S., Matsui, K., Kitano, M., Ohashi, N., 2000. SM-20550, a new Na^+/H^+ exchange inhibitor and its cardioprotective effect in ischemic–reperfused isolated rat hearts by preventing Ca^{2+} overload. J. Cardiovasc. Pharmacol. 35, 855–862.

Yasutake, M., Ibuki, C., Hearse, D.J., Avkiran, M., 1994. Na^+/H^+ exchange and reperfusion arrhythmias: protection by intracoronary infusion of a novel inhibitor. Am. J. Physiol. 267, H2430–H2440.