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Cardioprotective effect of TY-12533, a novel Na^+/H^+ exchange inhibitor, on ischemia/reperfusion injury

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Abstract

The effects of 6,7,8,9-tetrahydro-2-methyl-5*H*-cyclohepta[*b*]pyridine-3-carbonylguanidine maleate (TY-12533) on myocardial ischemia/reperfusion injury were evaluated in rats. Inhibitory effects of TY-12533, TY-50893 (the 9-chloro derivative of TY-12533) and cariporide on the platelet Na^+/H^+ exchanger in vitro were almost equal at pH 6.2 and decreased at pH 6.7; but TY-12533 was four times more potent than TY-50893 and cariporide at pH 6.7. TY-12533, TY-50893 and cariporide administered before ischemia (0.01–1 mg/kg, i.v.) suppressed the ischemia/reperfusion-induced arrhythmias to the same extent in vivo; but TY-12533 was more effective than cariporide and TY-50893 when they were administered during ischemia (0.1–1 mg/kg). Similar results were obtained for the inhibitory effects of these drugs administered before ischemia (0.03–0.1 mg/kg, i.v.) and during ischemia (0.1–1 mg/kg) on the ischemia/reperfusion-induced myocardial infarction. These differences between TY-12533 and the other drugs in vitro and in vivo may be ascribed to the pK_a values of the guanidinium moiety of TY-12533 (6.93), TY-50893 (6.35) and cariporide (6.28).

Keywords: TY-12533; Na^+/H^+ exchanger; Ischemia; Reperfusion; Arrhythmia; Myocardial infarction

1. Introduction

In order to restore coronary flow, percutaneous transluminal coronary angioplasty, percutaneous transluminal coronary recanalization or stenting has been applied to patients with acute myocardial infarction (Michels and Yusuf, 1995; Stone et al., 1998). However, the prognosis is not always excellent even if coronary reperfusion is achieved completely. In some cases, the reperfusion itself causes myocardial dysfunction, which has been recognized as ischemia/reperfusion injury based on the findings of animal experiments (Braunwald and Kloner, 1985; Kloner, 1993).

Although there were no clinically successful drugs against ischemia/reperfusion injury Karmazyn (1988) reported that amiloride, by acting as a partial inhibitor of the Na^+/H^+ exchanger, enhanced post-ischemic ventricular recovery in the isolated rat heart. Furthermore, Scholz et al. (1995) discovered the specific Na^+/H^+ exchanger inhibitor cariporide that potently inhibited ischemia/reperfusion-induced arrhythmias in rats and dogs in vivo (Xue et al., 1996). These findings ensured that a close look was taken at the relation between ischemia/reperfusion injury and the Na^+/H^+ exchanger and brought about a breakthrough in research into effective Na^+/H^+ exchanger inhibitors.

The Na^+/H^+ exchanger is activated during ischemia and reperfusion, and the myocardial tissue pH decreases by about 1 pH unit during ischemia and rapidly returns to the

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physiological level after reperfusion in Langendorff-perfused rat hearts (Tani and Neely, 1989; Park et al., 1999). Similar changes in Na^+/H^+ exchanger activity and tissue pH may occur during ischemia/reperfusion in vivo. The guanidino group of amiloride and its derivatives is essential for inhibition of the Na^+/H^+ exchanger, because guanidinium, which equilibrates with guanidine, competes with Na^+ (Vigne et al., 1984; Frelin et al., 1986). Therefore, an acid dissociation constant (pK_a value) for the guanidinium moiety may be an important factor in the design of beneficial Na^+/H^+ exchange inhibitors that are effective during ischemia (at the low pH) and after reperfusion (during the rapid recovery of pH). However, no study has addressed the protective effects of Na^+/H^+ exchange inhibitors against ischemia/reperfusion injury in relation to the pK_a values.

From the above-mentioned viewpoint, this study clarified the pharmacological profile of 6,7,8,9-tetrahydro-2-methyl-5*H*-cyclohepta[*b*]pyridine-3-carbonylguanidine maleate (TY-12533), which we had found to be one of the potent Na^+/H^+ exchange inhibitors with a guanidino group. Using TY-12533, TY-50893 (the 9-chloro derivative of TY-12533) and cariporide, we evaluated the relation between the pK_a values of the guanidinium moiety and the inhibitory effects on the rat platelet Na^+/H^+ exchanger at different pH in vitro. Furthermore, we compared the cardioprotective effects of these drugs administered before and during ischemia in the ischemia/reperfusion-induced arrhythmia rat model and myocardial infarction rat model in vivo.

2. Materials and methods

All animal experiments were reviewed and approved by the Experimental Animal Committee of the Drug Research Department, Toa Eiyo (Fukushima and Oomiya, Japan).

2.1. Drugs

The chemical structure of TY-12533 is shown in Fig. 1. TY-12533, TY-50893 and cariporide were synthesized by the Drug Research Department, Toa Eiyo. The following drugs and reagents were purchased; amiloride hydrochloride (Research Biochemicals, Natick, MA, USA), disopyramide phosphate (Rythmodan® P injection, Chugai Pharmaceutical, Tokyo), aconitine, 2,3,5-triphenyl tetrazolium chloride (TTC) and Evan's blue (Sigma, St. Louis, MO, USA). Other reagents were of the highest quality available (Wako, Osaka, Japan).

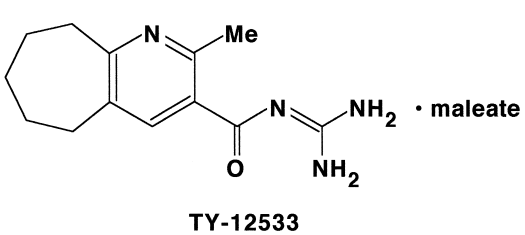

Fig. 1. Chemical structure of TY-12533.

TY-12533, TY-50893, cariporide and amiloride were dissolved in dimethyl sulfoxide (DMSO) and then diluted with reaction buffer for the in vitro studies or with 0.9% saline for the in vivo studies (final concentration of DMSO was 1%). Disopyramide was diluted with 0.9% saline containing 1% DMSO.

2.2. Effects of TY-12533, TY-50893, cariporide and amiloride on Na^+/H^+ exchanger activity in rat platelets (in vitro)

Na^+/H^+ exchanger activity in rat platelets was measured according to the method reported by Scholz et al. (1993). Male Wistar–ST rats weighing 290–330 g (Japan SLC, Hamamatsu, Japan) were anesthetized with diethyl ether. Blood (about 8 ml) was collected from the abdominal aorta and mixed with 1 ml of citric acid-dextrose (composed of 65 mM citric acid, 11 mM glucose, 85 mM trisodium citrate) as anti-coagulant. The mixture was immediately centrifuged at $90 \times g$ for 10 min at room temperature, and the supernatant was collected as platelet-rich plasma and platelets were counted with a microcell counter (F-800, Sysmex, Tokyo, Japan). Next, the platelet-rich plasma was diluted to 20×10^4 platelets/ml with 0.9% saline (platelet-rich plasma/saline). Sodium propionate buffer (250 μl; composed of 140 mM sodium propionate, 20 mM HEPES free acid, 10 mM glucose, 5 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$ and pH 6.2 or 6.7) containing the appropriate concentration of a drug was pre-warmed to 37°C in an aggregometer cuvette, and then platelet-rich plasma/saline (50 μl) was added to the buffer. The decrease in absorbance caused by platelet swelling was periodically measured with an aggregometer (PAT-2M, NBS, Tokyo) at 37°C with stirring in the cuvette at a constant speed of 1100 rpm. Na^+/H^+ exchanger activity was determined by calculating the extinction ratio of absorbance at 20 s after the addition of platelet-rich plasma/saline, and inhibitory ratios of drugs at each concentration were calculated relative to that of amiloride at 3×10^{-4} M, which was defined as 100% inhibition.

2.3. Determination of pK_a values of TY-12533, TY-50893, cariporide and amiloride

The acid dissociation constant (pK_a value) of each drug was determined by a spectrophotometric method (DeVries and Gantz, 1954). The drug was first dissolved in a few drops of DMSO and then diluted to about 100 μg/ml with Britton–Robinson's buffer. Next, the drug solution was exactly diluted with 9 volumes of Britton–Robinson's buffer of various pH values to achieve the desired pH within the range of 1.8–12.0 in 10 steps. The UV–VIS absorption spectra and actual pH of the test solutions were measured with a spectrophotometer (U-3300, Hitachi,

Tokyo) and a pH meter (HM-50G, Toa, Tokyo), respectively, and three wavelengths were chosen visually at which there was a characteristic change in absorbance with the change in pH. The actual pH–absorbance relationships in these three wavelengths were fitted to a pH–absorbance equation, and pK_a values were calculated by non-linear least-squares regression.

2.4. Effects of TY-12533, TY-50893 and cariporide on ischemia / reperfusion-induced arrhythmias in rats (in vivo)

The ischemia/reperfusion-induced arrhythmia model was prepared according to the method reported by Xue et al. (1996). Male Sprague–Dawley rats weighing 320 to 430 g (Japan SLC) were anesthetized with sodium pentobarbital (60 mg/kg, i.p.). The trachea was intubated, and the animal was artificially ventilated with room air by a small animal respirator (SN-480-7, Shinano, Tokyo) with a frequency of 54 stroke/min and a tidal volume of 1.5 ml/100 g. Body temperature was maintained at about 37°C with a thermostated warming pad (HP-4530, Iuchi, Tokyo). The femoral vein was cannulated for intravenous drug administration, and the arterial blood pressure was measured via a polyethylene catheter inserted into the right carotid artery with a pressure transducer (AP-601G, Nihon Kohden, Tokyo, Japan). Heart rate was measured with a cardiotachometer (AT-601G, Nihon Kohden). Left thoracotomy at the fifth intercostal space and pericardiotomy were performed, and an ELP 5-0 nylon ligature (L14-50N, Akiyama Seisakusho, Tokyo) was placed around the left coronary artery about 2–3 mm from its origin. Thereafter, both ends of the nylon ligature were passed through a small polyethylene tube to make a coronary snare. A standard limb lead II electrocardiogram (ECG) was monitored with a cardiograph (ECG-6303, Nihon Kohden), and data of ECG, blood pressure and heart rate were collected by an ECG processor (Softron, Tokyo) and stored on a magnetic optical disk for data analyses. Myocardial ischemia was achieved by tightening the coronary snare, and successful ischemia was confirmed by the typical elevation of the ST segment. At 5 min after ischemia, the heart was reperfused by releasing the snare. Ventricular tachycardia and ventricular fibrillation that occurred within 10 min after reperfusion were evaluated by using the Lambeth Conventions guidelines described by Walker et al. (1988). DMSO (1%, vehicle control), TY-12533, TY-50893 or cariporide was injected (1 ml/kg over 1 min, i.v.) 5 min before ischemia (pre-occlusion treatment) or 1 min before reperfusion (post-occlusion treatment).

2.5. Effects of TY-12533, cariporide and disopyramide on aconitine-induced arrhythmias in rats (in vivo)

The aconitine-induced arrhythmia model was prepared according to the method reported by Müller and Wilsmann (1982). Male Sprague–Dawley rats weighing 295–420 g (Japan SLC) were anesthetized with urethane (1.25 g/kg, i.p.), and body temperature was maintained at about 37°C with a thermostated warming pad. The trachea was intubated to maintain spontaneous breathing. The jugular and femoral veins were cannulated for administration of aconitine and the test drug, respectively. Arterial blood pressure, heart rate and ECG were measured by the same method as described previously. After the animal was allowed to stabilize for more than 10 min, DMSO (1%, vehicle control), TY-12533 (10 mg/kg), cariporide (10 mg/kg) or disopyramide (5 mg/kg) was injected (2 ml/kg over 2 min, i.v.). Disopyramide was used as a positive control drug as reported by Takahara et al. (1999). One minute after the end of drug administration, aconitine was intravenously infused at 2.5 μg/0.1 ml/min by means of a syringe infusion pump (Model-22, Harvard Apparatus, South Natick, MA, USA). The infusion was continued until ventricular fibrillation appeared. Ventricular tachycardia and ventricular fibrillation were evaluated in a similar manner as described previously.

2.6. Effect of TY-12533, TY-50893 and cariporide on ischemia / reperfusion-induced myocardial infarction in rats (in vivo)

Male Sprague–Dawley rats weighing 320–420 g (Japan SLC) were anesthetized with sodium pentobarbital (60 mg/kg, i.p.) and the femoral vein was cannulated for drug administration. To induce myocardial infarction, myocardial ischemia/reperfusion was performed in a similar manner as described above except that the ischemia was induced for 30 min followed by 24-h reperfusion. DMSO (1%, as vehicle control), TY-12533, TY-50893 or cariporide was injected (1 ml/kg over 1 min, i.v.) 5 min before ischemia (pre-occlusion treatment) or infused by means of a syringe infusion pump (0.05 ml/min over 20 min, i.v.) 10 min before reperfusion (post-occlusion treatment). About 30 min after the start of reperfusion, the incision was closed and the rat was returned to the cage.

Table 1
IC_{50} values of TY-12533, TY-50893, cariporide and amiloride on N^+/H^+ exchanger inhibition, measured as rat platelet swelling induced by sodium propionate (pH 6.2 and 6.7), and the pK_a values of the guanidinium moiety

	IC_{50} (M)		pK_a
	pH 6.2	pH 6.7	
TY-12533	$(1.7 \pm 0.4) \times 10^{-8}$	$(3.2 \pm 0.4) \times 10^{-8}$[a]	6.93
TY-50893	$(1.2 \pm 0.3) \times 10^{-8}$	$(1.3 \pm 0.4) \times 10^{-7}$[a]	6.35
Cariporide	$(2.2 \pm 0.6) \times 10^{-8}$	$(1.2 \pm 0.3) \times 10^{-7}$[a]	6.28
Amiloride	$(1.8 \pm 0.2) \times 10^{-5}$	$(2.0 \pm 0.4) \times 10^{-5}$	8.78

IC_{50} values were calculated by probit method and the values are the means ± S.E.M. of five to six different experiments. Acid dissociation constants (pK_a values) were determined by a spectrophotometric method.
[a] $P < 0.05$ vs. the IC_{50} value at pH 6.2.

Table 2
Effects of TY-12533 and cariporide on hemodynamics in rats

	DMSO		TY-12533		Cariporide	
	Baseline	5 min	Baseline	5 min	Baseline	5 min
HR (beats/min)	374±12	362±11	393±18	387±18	387±14	377±15
SBP (mm Hg)	129±7	135±9	142±11	149±11	138±10	134±10
DBP (mm Hg)	99±6	104±7	107±8	116±7	102±7	98±8

Values (means±S.E.M. for 10–14 rats) were obtained before (baseline) and 5 min after intravenous injection of DMSO (1%), TY-12533 (1 mg/kg) or cariporide (1 mg/kg). HR, heart rate; SBP and DBP, systolic and diastolic blood pressure, respectively. There were no statistically significant differences between the values before and after drug administration.

The effects of the drugs on the ischemia/reperfusion-induced myocardial infarction were evaluated as changes in the area at risk and the infarct size, measured by the TTC-Evan's blue technique (Watanabe et al., 1995). Twenty-four hours after reperfusion, the rat was anesthetized again, and the excised heart was quickly hung on the Langendorff apparatus. After the heart was perfused with 0.9% saline to wash the blood out of the coronary vessels, the coronary artery was religated at the same position, and 1.5 ml Evan's blue dye (1% w/v) was slowly injected to identify the area at risk. The left ventricle was cut into 1.5-mm transverse slices with a slicer (brain slicer, Neuroscience, Tokyo), and the slices were then incubated with 1% TTC in 0.9% saline at 37°C for 20 min to identify the infarct area. After the slices were fixed in 10% phosphate-buffered formalin overnight, the cumulative size of left ventricle, size of area at risk (negative staining with Evan's blue) and infarct size (negative staining with TTC) of each slice were determined by computed planimetry (QUANTIMET 500+, Leica Cambridge, Cambridge, England).

2.7. Statistics

The results are expressed as means ± S.E.M. except the pK_a value, the incidence of arrhythmia and the mortality. SPSS statistical package (SPSS Japan, Tokyo) was used for statistical analyses. The median (50%) inhibitory concentrations (IC_{50} values) of drugs were calculated by the probit method, and the IC_{50} values at pH 6.2 and 6.7 were compared by Student's unpaired *t*-test. Hemodynamic values before and after drug treatment were compared by Student's paired *t*-test. The incidence of arrhythmia and the mortality in the drug treatment groups and the DMSO group were compared by Fisher's exact probability test. Other comparisons between groups were made with one-way analysis of variance followed by Dunnett's test. Differences reaching $P < 0.05$ were considered to be statistically significant.

3. Results

3.1. Na^+/H^+ exchanger inhibition and pK_a values

The IC_{50} values of the drugs for inhibition of the Na^+/H^+ exchanger and the pK_a values of the guanidinium moiety are shown in Table 1.

The activation of the Na^+/H^+ exchanger by sodium propionate buffer (pH 6.2 or 6.7) caused platelet swelling with a decrease in the absorbance of the platelet solution. TY-12533, TY-50893 and cariporide inhibited Na^+/H^+ exchanger activity. At pH 6.2, IC_{50} values of these drugs were almost equal and in the order of 10^{-8} M. By

Table 3
Effects of pre- or post-occlusion treatment with TY-12533 and cariporide on ischemia/reperfusion-induced arrhythmias (incidence) and mortality in rats

	Dose (mg/kg)	*n*	Incidence of arrhythmias (%)		Mortality (%)
			VT	VF	
Pre-occlusion treatment					
DMSO		14	100	86	64
TY-12533	0.01	11	91	18[a]	18
	0.1	10	40[a]	10[a]	0[a]
	1	10	20[a]	0[a]	0[a]
Cariporide	0.01	10	100	40	10
	0.1	10	30[a]	10[a]	0[a]
	1	10	40[a]	0[a]	0[a]
Post-occlusion treatment					
DMSO		10	100	90	70
TY-12533	0.1	10	100	40	10
	1	10	70	0[a]	0[a]
Cariporide	0.1	10	100	60	20
	1	10	90	40	20

DMSO (1%), TY-12533 or cariporide at each dose was intravenously injected 5 min before ischemia (pre-occlusion treatment) or 1 min before reperfusion (post-occlusion treatment) over 1 min. VT, ventricular tachycardia; VF, ventricular fibrillation.

[a] $P < 0.05$ vs. the DMSO group.

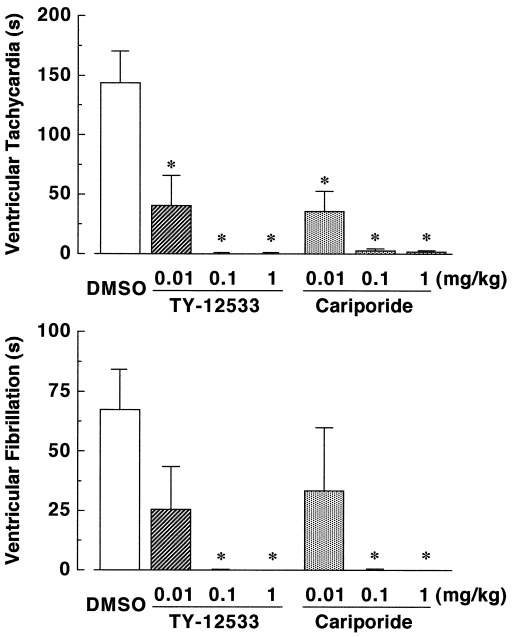

Fig. 2. Effects of pre-occlusion treatment with TY-12533 and cariporide on ischemia/reperfusion-induced arrhythmias in rats. The drug at each dose or DMSO (1%) was intravenously injected (1 ml/kg, over 1 min) 5 min before ischemia. Values are total duration of ventricular tachycardia and fibrillation observed within 10 min after reperfusion. Each column represents the mean ± S.E.M. for 10–14 rats. * $P < 0.05$ vs. the DMSO group.

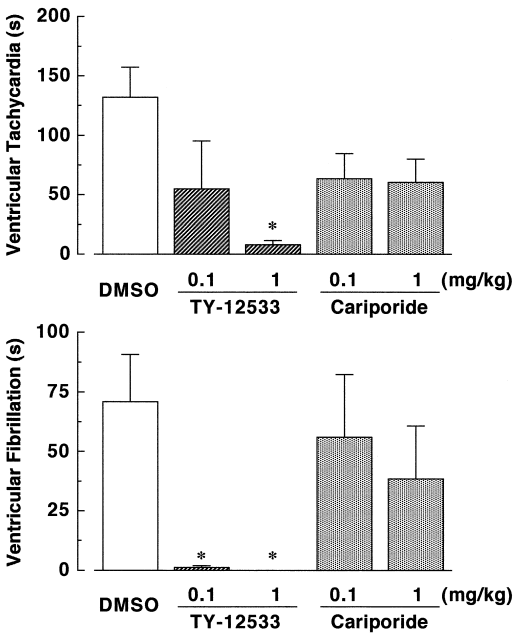

Fig. 3. Effects of post-occlusion treatment with TY-12533 and cariporide on ischemia/reperfusion-induced arrhythmias in rats. The drug at each dose or DMSO (1%) was intravenously injected (1 ml/kg, over 1 min) 1 min before reperfusion. Values are total duration of ventricular tachycardia and fibrillation observed within 10 min after reperfusion. Each column represents the mean ± S.E.M. for 10 rats. * $P < 0.05$ vs. the DMSO (1%) group.

changing pH of reaction buffer from 6.2 to 6.7, the IC_{50} value of TY-12533 was significantly increased but remained in the order of 10^{-8} M. In contrast, IC_{50} values of TY-50893 and cariporide were increased to about 10^{-7} M by the change in pH to 6.7, and the values were about four times higher than the IC_{50} value of TY12533 at pH 6.7. Amiloride also inhibited Na^+/H^+ exchanger activity; but its IC_{50} values were about 600–1000 times higher than those of TY-12533. There was no difference between the IC_{50} values of amiloride at pH 6.2 and 6.7.

The pK_a values of TY-12533, TY-50893 and cariporide were below physiological pH; but the value of TY-12533 was higher than those of TY-50893 and cariporide. The pK_a value of amiloride was far higher than physiological pH.

3.2. Effects on ischemia / reperfusion-induced arrhythmias

3.2.1. Effects of pre-occlusion treatment

TY-12533 and cariporide (1 mg/kg, i.v.) administered before ischemia had no significant effect on heart rate or blood pressure (Table 2).

In the vehicle control (1% DMSO) group, nine out of 14 animals died of ventricular fibrillation within 10 min after reperfusion (mortality 64%, Table 3). The total duration and the incidence of ischemia/reperfusion-induced ventricular tachycardia within 10 min after reperfusion

Table 4
Effects of pre- or post-occlusion treatment with TY-50893 on ischemia/reperfusion-induced arrhythmias and mortality in rats

	Dose (mg/kg)	n	Incidence (%)		Duration (s)		Mortality (%)
			VT	VF	VT	VF	
Pre-occlusion treatment							
TY-50893	0.01	6	83	17[a]	26.3 ± 14.8	0.4 ± 0.4[a]	0
	0.1	6	50	0[a]	5.1 ± 3.2[a]	0.0 ± 0.0[a]	0
Post-occlusion treatment							
TY-50893	0.1	6	100	67	97.7 ± 57.6	44.1 ± 24.1	33
	1	6	67	33	42.9 ± 19.4	42.8 ± 36.3	17

TY-50893 at each dose was intravenously injected 5 min before ischemia (pre-occlusion treatment) or 1 min before reperfusion (post-occlusion treatment) over 1 min. Values of the incidence and the duration of arrhythmias observed within 10 min after reperfusion are presented as percentages and the mean ± S.E.M., respectively. VT, ventricular tachycardia; VF, ventricular fibrillation.

[a] $P < 0.05$ vs. the DMSO group shown in Fig. 2 (pre-occlusion treatment) and Fig. 3 (post-occlusion treatment).

were 143.8 ± 26.6 s (Fig. 2) and 100% (Table 3), respectively, and those of ventricular fibrillation were 67.3 ± 16.9 s (Fig. 2) and 86% (Table 3), respectively. TY-12533 and cariporide at 0.01 mg/kg significantly reduced the total duration of ventricular tachycardia to 40.4 ± 25.4 s and 35.6 ± 17.0 s (Fig. 2), respectively. Both drugs significantly reduced all the arrhythmic parameters at doses higher than 0.1 mg/kg and completely abolished the ventricular fibrillation at 1 mg/kg (Table 3, Fig. 2). TY-50893 (0.01 and 0.1 mg/kg) also suppressed the ventricular tachycardia and fibrillation (Table 4).

3.2.2. Effects of post-occlusion treatment

In the DMSO (1%) group, seven out 10 animals died of ventricular fibrillation (mortality 70%, Table 3). The total duration and the incidence of ischemia/reperfusion-induced ventricular tachycardia within 10 min after reperfusion were 131.8 ± 25.5 s (Fig. 3) and 100% (Table 3), respectively, and those of ventricular fibrillation were 70.8 ± 20.0 s (Fig. 3) and 90% (Table 3), respectively. TY-12533 at 0.1 mg/kg significantly reduced the total duration of ventricular fibrillation to 1.3 ± 0.6 s (Fig. 3). TY-12533 at 1 mg/kg abolished the ventricular fibrillation and significantly reduced the total duration of ventricular tachycardia to 7.9 ± 3.5 s (Fig. 3) and mortality to 0% (Table 3). In contrast, cariporide at 1 mg/kg did not exert statistically significant effects on the arrhythmic parameters (Fig. 3, Table 3); it only tended to reduce mortality (20%, Table 3) and ventricular fibrillation (incidence, 40%; total duration, 38.5 ± 22.2 s; Fig. 3). TY-50893 tended to reduce mortality and ventricular tachycardia and fibrillation to the same extent as cariporide, and the effects were not statistically significant (Table 4).

3.3. Effects on aconitine-induced arrhythmias

In the DMSO (1%) group, the cumulative doses of aconitine required to induce ventricular tachycardia and ventricular fibrillation were 55.0 ± 5.6 and 59.4 ± 4.5 μg/kg, respectively (Fig. 4). Disopyramide (5 mg/kg), but not TY-12533 or cariporide (10 mg/kg), significantly increased the arrhythmogenic doses of aconitine (Fig. 4).

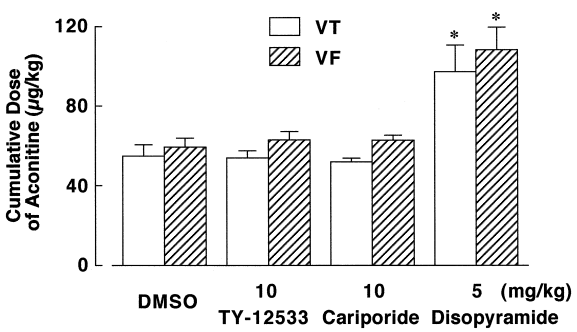

Fig. 4. Effects of TY-12533, cariporide and disopyramide on aconitine-induced ventricular tachycardia (VT) and ventricular fibrillation (VF) in rats. The drug or DMSO (1%) was intravenously injected (2 ml/kg, over 2 min) 3 min before intravenous infusion of aconitine (2.5 μg/0.1 ml/min). Each column represents the mean ± S.E.M. for eight rats. * $P < 0.05$ vs. the DMSO (1%) group.

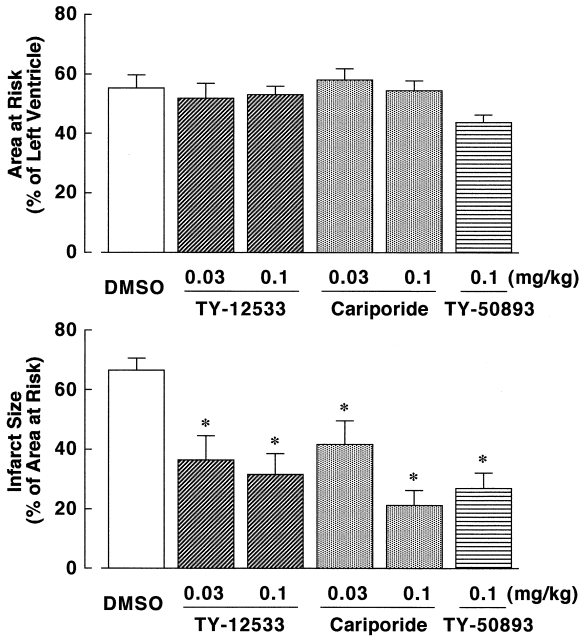

Fig. 5. Effects of pre-occlusion treatment with TY-12533, TY-50893 and cariporide on ischemia/reperfusion-induced myocardial infarction in rats. The drug at each dose or DMSO (1%) was intravenously injected (1 ml/kg, over 1 min) 5 min before ischemia. Each column represents the mean ± S.E.M. for six rats (TY-50893 group) or 10 rats (other groups). * $P < 0.05$ vs. the DMSO group.

3.4. Effects on ischemia / reperfusion-induced myocardial infarction

3.4.1. Effects of pre-occlusion treatment

Fig. 5 shows the myocardial area at risk as a percentage of left ventricle and the infarct size as a percentage of the area at risk. The area at risk was about 55% and was not significantly different among the groups. The infarct size in the DMSO (1%) group was 66.5 ± 4.0%. TY-12533 (0.03 and 0.1 mg/kg), cariporide (0.03 and 0.1 mg/kg) and TY-50893 (0.1 mg/kg) significantly reduced the infarct size; the values at 0.1 mg/kg were 31.6 ± 7.0% (about 50% inhibition), 21.3 ± 5.0% (about 70% inhibition) and 27.0 ± 5.2% (about 60% inhibition), respectively.

3.4.2. Effects of post-occlusion treatment

The myocardial area at risk and the infarct size (Fig. 6) were calculated as described above. The area at risk in each group was almost the same (about 50%), and the infarct size in the DMSO (1%) group was 62.8 ± 9.5%. TY-12533 at 0.1–1 mg/kg significantly reduced the infarct size in a dose-dependent manner. The value at 1

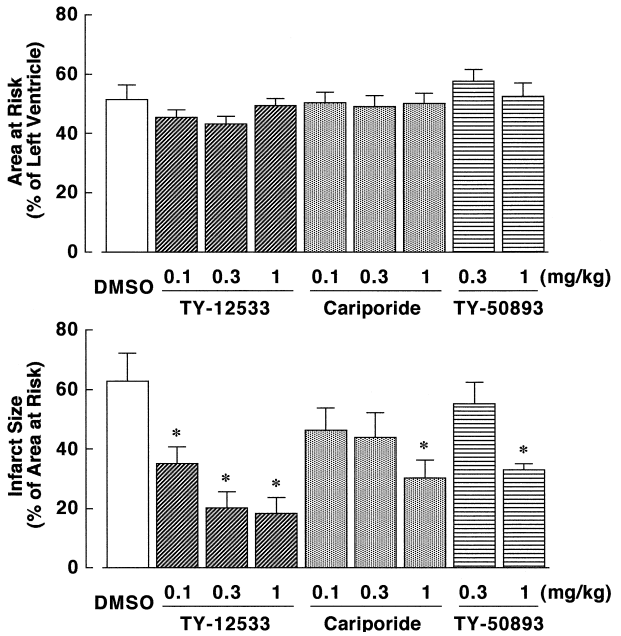

Fig. 6. Effects of post-occlusion treatment with TY-12533, TY-50893 and cariporide on ischemia/reperfusion-induced myocardial infarction in rats. The drug at each dose or DMSO (1%) was intravenously infused (0.05 ml/min, over 20 min), beginning 10 min before reperfusion. Each column represents the mean ± S.E.M. for six rats (TY-50893 group) or 10 rats (other groups). * $P < 0.05$ vs. the DMSO group.

mg/kg of TY-12533 was 18.4 ± 5.4% (about 70% inhibition). In contrast, cariporide and TY-50893 significantly reduced infarct size, to 30.3 ± 6.0% and 33.0 ± 2.0%, respectively (about 50% inhibition), at the highest dose (1 mg/kg) only.

4. Discussion

This study demonstrated that TY-12533, a newly synthesized inhibitor of the Na^+/H^+ exchanger, markedly suppressed the arrhythmias and the myocardial infarction induced by ischemia/reperfusion in rats, even when the drug was administered during ischemia.

The underlying mechanisms of myocardial ischemia/reperfusion injury are suggested as follows. Protons accumulate intra- and extracellularly during ischemia, and the rapid washout of extracellular protons by reperfusion makes an intracellular to extracellular proton gradient, which activates the Na^+/H^+ exchanger (Park et al., 1999; Xiao and Allen, 1999). Na^+ also accumulates intracellularly during ischemia because Na^+ influx via the Na^+/H^+ exchanger or Na^+ channel is increased (Tani et al., 1996; Hartmann and Decking, 1999; Lu et al., 1999) or Na^+ efflux via Na^+/K^+ ATPase is limited (Karmazyn et al., 1999). However, the Na^+/H^+ exchanger activated by reperfusion further increases Na^+ influx to induce Na^+ overload and, consequently, intracellular Ca^{2+} overload occurs via the reverse-mode Na^+/Ca^{2+} exchanger and gives rise to cellular damage (Scholz and Albus, 1993; Lazdunski et al., 1985; Tani and Neely, 1989; Tani et al., 1996; Karmazyn et al., 1999). Thus, Na^+ influx via the Na^+/H^+ exchanger after reperfusion may be a critical trigger for ischemia/reperfusion injury. The effect of TY-12533 administered during ischemia on ischemia/reperfusion injury may be due to suppression of the intracellular Ca^{2+} overload, which is a conceivable consequence of inhibition of the Na^+/H^+ exchanger after reperfusion.

In the in vitro study, we assessed the relation between pH and inhibitory effects of TY-12533, TY-50893 and cariporide on the Na^+/H^+ exchanger. Na^+/H^+ exchanger activity was evaluated by the rat platelet swelling induced by sodium propionate buffer at pH 6.2 and 6.7. The IC_{50} value of cariporide obtained in this study (1.2×10^{-7} M, at pH 6.7) was slightly lower than the result for human platelets (2×10^{-7} M, at pH 6.7) reported by Scholz et al. (1995). At pH 6.2, the inhibitory effects of TY-12533, TY-50893, and cariporide were almost equal (IC_{50} values were in the order of 10^{-8} M). However, a change in pH from 6.2 to 6.7 largely reduced the inhibitory effects of cariporide and TY-50893 (IC_{50} values were in the order of 10^{-7} M); but the effect of TY-12533 was relatively resistant to the change in pH (IC_{50} value remained in the order of 10^{-8} M). Frelin et al. (1986) has shown that guanidinium, which is equilibrated with guanidine, competes with Na^+ at the external Na^+ transport site of the Na^+/H^+ exchanger. In our results, the order of the pK_a values of the guanidinium moiety were cariporide (6.28) ≤ TY-50893 (6.35) < TY-12533 (6.93) ≪ amiloride (8.78). Although the basic chemical structure of TY-50893 is the same as that of TY-12533, the electron-withdrawing 9-chloro group of TY-50893 may lower the pK_a value. Considering their pK_a values, it is probable that TY-12533, TY-50893 and cariporide are sufficiently protonated in the active form (guanidinium) to inhibit the Na^+/H^+ exchanger at pH 6.2 and less protonated at pH 6.7; but the protonation of TY-12533 is higher than that of TY-50893 or cariporide at pH 6.7. Amiloride, with its higher pK_a value, may be protonated to the same extent at both pH values and, thereby, its inhibitory effect (although much less than those of other drugs) is not affected by the change in pH. Thus, differences in the inhibitory profiles of the drugs at pH 6.2 and 6.7 can be related to the pK_a values of the guanidinium moiety.

In the in vivo study, TY-12533, TY-50893 and cariporide given as pre-occlusion treatment (0.01–1 mg/kg) reduced or abolished the ventricular tachycardia and fibrillation that occurred after reperfusion. Thus, the anti-arrhythmic effects of these drugs are almost equal after pre-occlusion treatment. These effects are not due to improvement of the myocardial oxygen balance secondary to hemodynamic changes, because neither TY12533 nor cariporide affected systemic blood pressure or heart rate. However, the anti-arrhythmic effects of these drugs were

different after post-occlusion treatment. TY-12533 suppressed the ischemia/reperfusion-induced arrhythmias, and especially at 1 mg/kg, it abolished the ventricular fibrillation. In contrast, TY-50893 and cariporide at 1 mg/kg did not significantly reduce the ventricular fibrillation. These data indicate that TY-12533 is more effective than TY-50893 and cariporide when administered during ischemia.

Aye et al. (1997) demonstrated that cariporide suppressed ischemia/reperfusion-induced ventricular tachycardia and ventricular fibrillation in rats when it was given either 5 min before or 3 min after ischemia. Our data obtained with cariporide as pre-occlusion treatment were almost the same as their results. However, after post-occlusion treatment, the anti-arrhythmic effect of cariporide observed in our study was less potent than that demonstrated in their study. The difference may be due to the experimental protocols; in our study, reperfusion was initiated simultaneously at the end of drug administration, whereas in their study, 90 s were allowed to start reperfusion after drug administration.

The myocardial tissue pH decreases by about 1 pH unit within 5 min after the onset of global zero-flow ischemia and rapidly returns to the physiological level after reperfusion in Langendorff-perfused rat hearts (Park et al., 1999). Considering the pK_a values of the guanidinium moiety, the drugs administered before ischemia may be sufficiently protonated in the active form during ischemia in the ischemia/reperfusion-induced arrhythmia model. Therefore, the effects of pre-occlusion treatment with TY-12533, TY-50893 and cariporide were almost equal, as assumed from their potency to inhibit the Na^+/H^+ exchanger at pH 6.2 in vitro. However, the drugs administered during ischemia could not reach the ischemic zone until reperfusion, because there are no functional coronary collaterals in rats (Winkler et al., 1984). In this case, the drug actions appear after reperfusion. It should be noted that there are differences between TY-12533 and TY-50893 in their effects as pre- or post-occlusion treatment even though they have the same basic chemical structure. Judging from the pK_a values and the potency to inhibit the Na^+/H^+ exchanger at pH 6.2 and 6.7 in vitro, TY-12533 administered during ischemia may be protonated and, thus, inhibit the Na^+/H^+ exchanger more sufficiently than TY-50893 and cariporide during the period of rapid recovery of pH to the physiological level soon after reperfusion. The pK_a value of TY-12533 may, thus, contribute to the beneficial effect of this drug on ischemia/reperfusion-induced arrhythmias when given as post-occlusion treatment.

Pre-occlusion treatment with a class I anti-arrhythmic drug also inhibits ischemia/reperfusion-induced arrhythmias (Lu et al., 1999). In this regard, we examined the anti-arrhythmic potency of TY-12533 and cariporide in the aconitine-induced arrhythmia model. Aconitine prolongs the open state of the Na^+ channel, leading to intracellular Na^+ overload and consequently Ca^{2+} overload via the reverse-mode Na^+/Ca^{2+} exchanger (Sawanobori et al., 1987). Disopyramide inhibited the aconitine-induced arrhythmias as previously reported (Takahara et al., 1999). However, neither TY-12533 nor cariporide at an excessive dose (10 mg/kg) affected the arrhythmias, indicating that TY-12533 and cariporide have no class I anti-arrhythmic action.

Ischemia/reperfusion also caused myocardial infarction, the size of which was significantly reduced by the pre-treatment with TY-12533, TY-50893 or cariporide at 0.1 mg/kg. However, it is important to evaluate whether the post-occlusion treatment with a Na^+/H^+ exchanger inhibitor reduces myocardial infarct size, because pre-occlusion treatment is less likely to occur in a clinical setting (Gumina et al., 1998). Therefore, to mimic the adjunctive clinical therapy for acute myocardial infarction, we infused the drugs intravenously, starting after onset of the occlusion in the ischemia/reperfusion-induced myocardial infarction model. As post-occlusion treatment, TY-12533 at 0.1 mg/kg significantly reduced the infarct size, whereas TY-50893 and cariporide were less effective and a higher dose (1 mg/kg) was required to cause a significant reduction of the infarct size. The present results, thus, indicate the usefulness of TY-12533 for the clinical situation, an effect which could also be ascribable to the pK_a value of TY-12533, which is nearer to the physiological pH than those of TY-50893 and cariporide. Numerous mechanisms other than intracellular Ca^{2+} overload have been suggested to participate in the pathogenesis of myocardial infarction: inhibition of the Na^+/H^+ exchanger attenuates neutrophil-mediated reperfusion injury (Faes et al., 1995) and peroxide-induced myocardial derangement (Myers et al., 1998; Hara et al., 1999). These effects might also contribute to the curative effect of TY-12533 on the myocardial infarction. These issues require further elucidation.

In summary, this study demonstrated that the novel Na^+/H^+ exchange inhibitor TY-12533 has curative and preventive effects against myocardial ischemia/reperfusion injury such as fatal arrhythmia and myocardial infarction. The effects of TY-12533 were more potent than those of TY-50893 and cariporide as post-occlusion treatment, which could be ascribed to the pK_a value-related inhibitory effects of these drugs on the Na^+/H^+ exchanger. These pharmacological profiles suggest that TY-12533 could be beneficial as adjunct to coronary interventions such as percutaneous transluminal coronary angioplasty, percutaneous transluminal coronary recanalization and coronary graft surgery.

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