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Does Cl^-/HCO_3^- exchange play an important role in reperfusion arrhythmias in rats?

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Abstract

The protective effects of Cl^-/HCO_3^- exchange inhibitors, 4,4′-diisothiocyano-stilbene-2,2′-disulfonic acid (DIDS) and 4-acetamido-4′isothiocyanato-stilbene-2,2′-disulfonic acid (SITS), against reperfusion-induced arrhythmias were investigated in anesthetized rats. Rats were subjected to 5-min occlusion of the left coronary artery followed by 10-min reperfusion. All drugs were intravenously administered 5 min before the onset of occlusion. DIDS (75 mg/kg) reduced the incidence of ventricular fibrillation and mortality to 0%, whereas SITS (75 mg/kg) only decreased these parameters to 60%. DIDS simultaneously decreased the mean blood pressure and heart rate, and prolonged PQ and QRS intervals, whereas SITS produced a weaker effect on these parameters and no change in QRS interval. Mexiletine (5 mg/kg), which had been demonstrated to suppress the arrhythmias and reduce the heart rate and mean blood pressure in this model, was shown to prolong PQ and QRS intervals. Verapamil (0.5 mg/kg) or diltiazem (0.4 mg/kg) suppressed the arrhythmias, simultaneously decreasing the heart rate and mean blood pressure and prolonging PQ interval. The results indicate that the protective effect of DIDS on reperfusion arrhythmias in the anesthetized rats is unlikely to be attributed to the inhibitory action on Cl^-/HCO_3^- exchange, but possibly mediated by its blocking effects on cardiac ion channels, such as Na^+ or Ca^{2+} channels.
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Keywords: Cl^-/HCO_3^- exchange; Arrhythmia; Ischemia; Reperfusion; DIDS; SITS

1. Introduction

It is well known that anion exchange mechanisms play a potentially important role in cardiac physiology and pathophysiology (Hume et al., 2000), and the role of anions in initiation of ischemia/reperfusion-induced arrhythmias in the rats has been proposed (Curtis, 1989; Ridley and Curtis, 1992; Curtis et al., 1993). The most extensively characterized anion exchange protein is the Cl^-/HCO_3^- exchanger (Jennings, 1989). The Cl^-/HCO_3^- exchange was shown to be involved in the hypoxia-induced acidification and reoxygenation-induced overload of the intracellular Ca^{2+} ($[Ca^{2+}]_i$) in the rat ventricular myocytes (Kawasaki et al., 2001). Furthermore, the hypoxia/reoxygenation-induced changes in intracellular pH (pH_i) and $[Ca^{2+}]_i$ can be prevented by some anion exchange inhibitors, e.g., 4,4′-diisothiocyano-stilbene-2,2′-disulfonic acid (DIDS) and 4-acetamido-4′isothiocyanato-stilbene-2,2′-disulfonic acid (SITS) (Kawasaki et al., 2001). Similar protective effects of SITS and DIDS were also confirmed in the guinea pig ventricular tissues, using the models of simulated ischemia (Lai et al., 1996; Lai and Nishi, 1998) and ischemia/reperfusion (Tanaka et al., 1996). The evidence obtained in the rat Langendorff hearts indicated that the substitution of NO_3 for Cl^- (Ridley and Curtis, 1992) or inhibition of Cl^-/HCO_3^- exchange by SITS (Curtis, 1989) suppressed ischemia- and reperfusion-induced arrhythmias. Though these in vitro results predict significant roles of Cl^-/HCO_3^- exchange in ischemia/reperfusion arrhythmias, there have been no in vivo data examining the efficacy of these drugs. It has been reported that Ca^{2+} channel antagonists, verapamil and diltiazem (Swies et al., 1990; Lu et al., 1999), and a Na^+ channel antagonist, mexiletine (Saitoh et al., 2000),

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protected against the ischemia and reperfusion arrhythmias in rats. Thus, we investigated the effects of SITS and DIDS on the reperfusion-induced arrhythmias in anesthetized rats in comparison with verapamil, diltiazem and mexiletine in order to clarify the in vivo antiarrhythmic mechanisms of the two compounds.

2. Materials and methods

2.1. Induction of coronary ischemia/reperfusion injury in rats

Male Sprague–Dawley rats (body weight 250–400 g) were purchased from the Animal Laboratory for Research of University of Yamanashi, Faculty of Medicine. The animals were housed under conditions of 23 ± 1 °C with a constant humidity of 55 ± 6%, a cycle of 12-h light and 12-h darkness, and were given free access to food and tap water according to the Guide for the Care and Use of Laboratory Animals as promulgated by the National Research Council. The protocol of this experiment was approved by The Committee of Animal Use and Welfare of the University of Yamanashi, Faculty of Medicine. As reported earlier (Komori et al., 1994; Aye et al., 1997), animals were anesthetized intraperitoneally with pentobarbital sodium (60 mg/kg) and the trachea was cannulated for artificial ventilation. Immediately afterwards, the femoral vein and carotid artery were cannulated for drug administration and continuous blood pressure monitoring, respectively. The electrocardiogram (ECG) was continuously recorded with standard limb lead I on a recorder (Nihon Kohden, RM-62001, Tokyo, Japan) to count arrhythmias and measure the parameters of ECG (Fig. 1). After artificial ventilation was started using room air (volume 1.5 ml/100 g, rate 54 strokes/min) to maintain arterial blood gases within a normal range (pCO_2 26–37 mm Hg, pO_2 80–118 mm Hg, pH 7.38–7.45), the chest was opened by left thoracotomy at approximately 2 mm to the left of the sternum, followed by sectioning the fourth and fifth ribs. After incising the pericardium, the heart was exteriorized by applying gentle pressure on the rib cage, and a 6/0 braided silk suture (attached to a 10-mm micropoint reverse cutting needle) was placed around the left coronary artery. The heart was placed back into the chest and the animal was allowed to stabilize.

Transient 5-min regional myocardial ischemia was induced by passing the threads through a small plastic tube and pressing the tube against the epicardium, and reperfusion was initiated by releasing the ligature and removing the plastic tube. Consistent with previous studies on the time course of the appearance of ischemia-induced arrhythmias (Curtis et al., 1987; Aye et al., 1997), there were no severe arrhythmias during the 5 min of coronary occlusion. Thus, the ischemia-induced arrhythmias were not evaluated in this study. After reperfusion, the responses were observed for 10 min. Ischemia and reperfusion were confirmed as described previously (Lawson et al., 1993). Successful occlusion was confirmed by the increase of the height of the R wave during the first few seconds of each occlusion (Carbonin et al., 1980) and by a 20–30% reduction in the arterial blood pressure compared to the pre-ischemic values.

2.2. Definition of arrhythmias and analysis

Definitions of arrhythmias were based on the description of the Lambeth conventions (Walker et al., 1988). Ectopic ventricular activity was categorized as a single premature ventricular contraction, ventricular tachycardia (four or more consecutive premature ventricular contractions) or ventricular fibrillation (inability to distinguish individual QRS complexes and to measure the rate). Complex forms (e.g., bigeminy) were included in the count of premature ventricular contractions and were not analyzed separately. Besides the morphology of ECG, the blood pressure tracing was used to confirm which type of ectopic activity was occurring, particularly to distinguish between polymorphic ventricular tachycardia and ventricular fibrillation. When the ventricular tachycardia occurred, the blood pressure was usually pulsatile, whereas with ventricular fibrillation the blood pressure fell rapidly towards zero and was not pulsatile. Ventricular fibrillation may be sustained or may revert spontaneously to normal sinus rhythm in the rat (Curtis et al., 1987). In all experiments, the incidence of ventricular tachycardia, ventricular fibrillation and mortality (due to terminal ventricular fibrillation sustained for 3 min or more) was noted.

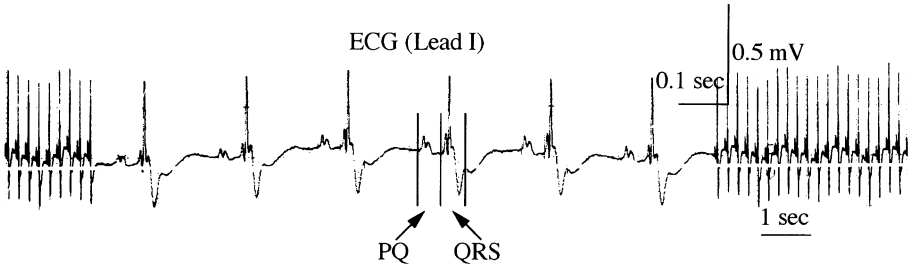

Fig. 1. Actual tracings of ECG showing interval measurement.

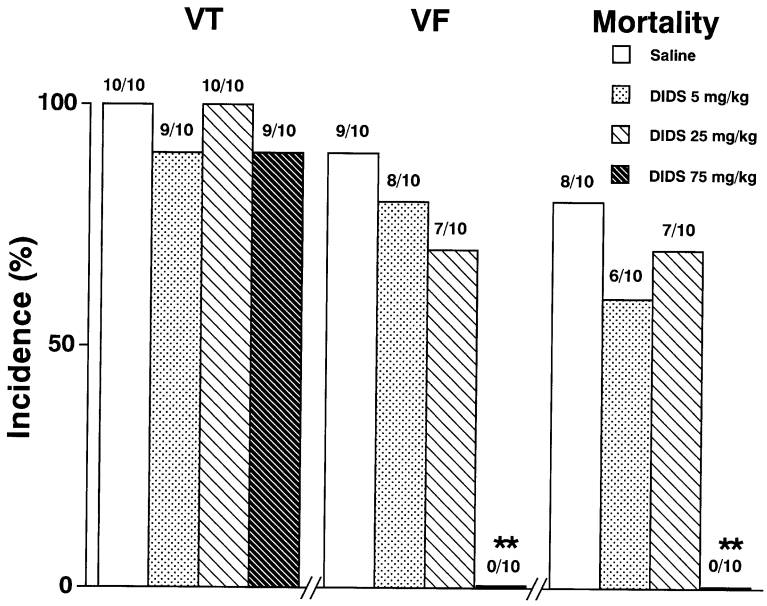

Fig. 2. Effects of DIDS on reperfusion-induced arrhythmias in the anesthetized rats. The incidence of ventricular tachycardia (VT), ventricular fibrillation (VF) and mortality are shown. Rats were subjected to 5-min coronary occlusion and 10-min reperfusion. DIDS (5, 25 or 75 mg/kg) or saline was administered intravenously 5 min before occlusion. Each value represents the mean ± S.E.M. ($n = 10$). $^{**}P < 0.01$ vs. control group.

2.3. Exclusion criteria

Experiments were terminated or excluded from the final data analysis, if any of the following occurred (Komori et al., 1994; Shaw and Coker, 1996): arrhythmias prior to coronary artery occlusion, mean arterial pressure less than 60 mm Hg prior to drug or vehicle administration and atrioventricular block during the first 5-min ischemia (probably caused by ligature occluding the septal branch of the left coronary artery). Eighty-four rats were used in this study. Two of them were excluded for the absence of signs of ischemia and two for too low blood pressure before occlusion.

2.4. Experimental protocols

Rats were divided into 8 groups with 10 rats in each group. After 15-min stabilization, saline (control), DIDS (5, 25 or 75 mg/kg), SITS (75 and 110 mg/kg), verapamil (0.5 mg/kg) or diltiazem (0.4 mg/kg) was administered 5 min before the

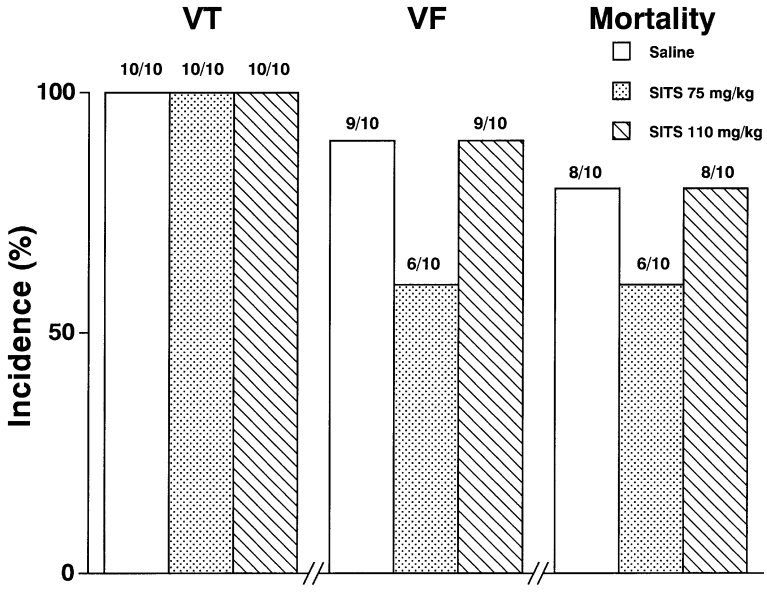

Fig. 3. Effects of SITS on reperfusion-induced arrhythmias in the anesthetized rats. The incidence of VT, VF and mortality are shown. Rats were subjected to 5-min coronary occlusion and 10-min reperfusion. SITS (75–110 mg/kg) or saline was administered intravenously 5 min before occlusion. Each value represents the mean ± S.E.M. ($n = 10$).

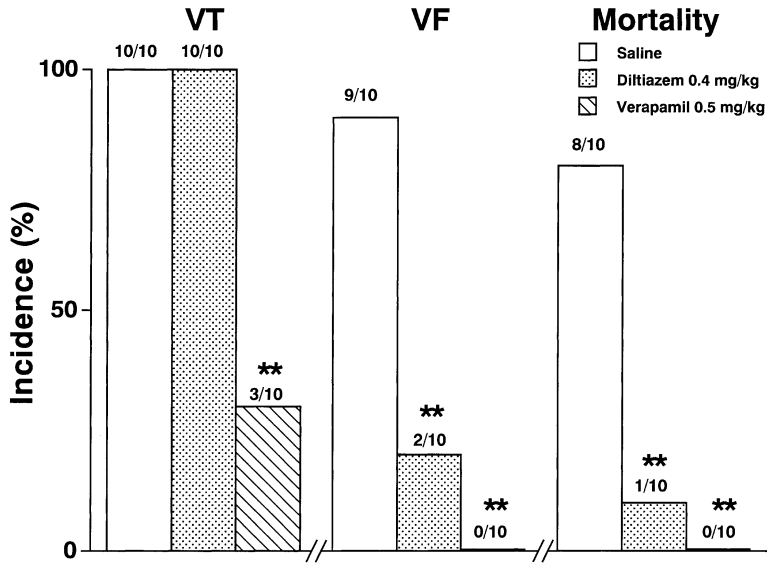

Fig. 4. Effects of diltiazem and verapamil on reperfusion-induced arrhythmias in the anesthetized rats. The incidence of VT, VF and mortality are shown. Rats were subjected to 5-min coronary occlusion and 10-min reperfusion. Diltiazem (0.4 mg/kg), verapamil (0.5 mg/kg) or saline was administered intravenously 5 min before occlusion. Each value represents the mean ± S.E.M. ($n = 10$). **$P < 0.01$ vs. control group.

coronary artery occlusion. Then the coronary artery was occluded for 5 min and reperfused for 10 min. Injections were given within 30–40 s. The ECG and blood pressure were continuously recorded throughout the experiment.

2.5. Drugs

DIDS and SITS were obtained from Sigma (St. Loius, MO, USA) and dissolved in saline on the day of the experiments. Verapamil hydrochloride was purchased from Eisai (Tokyo, Japan) and diltiazem hydrochloride from Tanabe Pharmaceutical (Osaka, Japan). Pentobarbital sodium was purchased from Tokyo Kasei Kogyo (Tokyo, Japan).

2.6. Statistics

Statistical analysis was based upon the guidelines for statistics (Wallenstein et al., 1980) with some modification for the study of arrhythmias using rat hearts (Curtis et al., 1987; Curtis and Hearse, 1989). All data are expressed as mean ± S.E.M. A repeated measured analysis of variance (ANOVA) followed by Dunnett's multiple comparison test was used in the analysis of hemodynamic and ECG parameters of pre-drug (0 min) values and the other values. Differences in the incidence of arrhythmias among groups were analyzed by Fisher's exact probability test.

3. Results

3.1. Effects of DIDS on reperfusion-induced arrhythmias

In the saline control group, the incidence of reperfusion-induced ventricular tachycardia, ventricular fibrillation and mortality was 100%, 90% and 80%, respectively (Fig. 2). Administration of 75 mg/kg DIDS completely attenuated both ventricular fibrillation incidence and mortality to 0% ($P < 0.01$), whereas there was no significant reduction in the

Table 1
Effects of DIDS, SITS, verapamil and diltiazem on the heart rate and mean blood pressure of the anesthetized rats

Groups (mg/kg, i.v.)	Predrug	1 min	2 min	3 min	4 min	5 min	Predrug	1 min	2 min	3 min	4 min	5 min
	Heart rate (beats/min)						*Mean blood pressure (mm Hg)*					
Saline control	382 ± 16	382 ± 17	384 ± 17	380 ± 17	377 ± 14	376 ± 15	73 ± 6	81 ± 6[a]	78 ± 6[a]	75 ± 6	74 ± 5	73 ± 5
DIDS (5)	425 ± 13	407 ± 15	407 ± 21	411 ± 15	409 ± 14	405 ± 12	81 ± 3	92 ± 5[a]	91 ± 5[a]	87 ± 6[b]	80 ± 5	80 ± 5
DIDS (25)	400 ± 18	385 ± 19	396 ± 17	394 ± 15	395 ± 13	392 ± 17	76 ± 4	61 ± 3[a]	67 ± 4[b]	69 ± 4	73 ± 4	70 ± 4
DIDS (75)	368 ± 23	347 ± 16[b]	340 ± 17[a]	334 ± 20[a]	339 ± 20[a]	334 ± 18[a]	76 ± 6	49 ± 2[a]	49 ± 2[a]	46 ± 3[a]	49 ± 6[a]	53 ± 6[a]
SITS (75)	402 ± 15	370 ± 8[b]	359 ± 21[a]	375 ± 14	383 ± 13	399 ± 13	72 ± 3	38 ± 6[a]	43 ± 7[b]	65 ± 2	94 ± 14[b]	87 ± 8
SITS (110)	418 ± 20	343 ± 28[a]	340 ± 31[a]	363 ± 23[a]	378 ± 18[b]	385 ± 16[b]	79 ± 5	39 ± 4[a]	48 ± 6[a]	54 ± 9[a]	67 ± 9	74 ± 10
Verapamil (0.5)	395 ± 15	377 ± 13[b]	367 ± 8[a]	367 ± 10[a]	362 ± 8[a]	362 ± 8[a]	83 ± 4	36 ± 2[a]	42 ± 3[a]	54 ± 3[a]	64 ± 3[a]	67 ± 5[a]
Diltiazem (0.4)	402 ± 12	362 ± 14[a]	369 ± 18[a]	368 ± 16[a]	376 ± 16[a]	378 ± 16[a]	80 ± 6	52 ± 3[a]	62 ± 4[a]	69 ± 5[a]	73 ± 5[b]	71 ± 5[a]

Each value represents the mean ± S.E.M. ($n = 10$).
[a] $P < 0.01$ vs. predrug value of each group.
[b] $P < 0.05$ vs. predrug value of each group.

Table 2
Effects of DIDS, SITS, verapamil and diltiazem on the PQ interval and QRS duration of the anesthetized rats

Groups (mg/kg, i.v.)	Predrug	1 min	2 min	3 min	4 min	5 min	Predrug	1 min	2 min	3 min	4 min	5 min
	PQ interval (ms)						*QRS width (ms)*					
Saline control	50 ± 3	51 ± 2	49 ± 3	51 ± 2	50 ± 2	50 ± 2	51 ± 2	54 ± 3	53 ± 3	52 ± 2	53 ± 3	53 ± 3
DIDS (5)	57 ± 5	57 ± 4	54 ± 1	56 ± 3	55 ± 1	54 ± 2	57 ± 4	59 ± 5	60 ± 6[b]	58 ± 5	60 ± 6[b]	60 ± 5
DIDS (25)	53 ± 3	54 ± 4	56 ± 5	58 ± 5[a]	56 ± 4	57 ± 4	58 ± 3	61 ± 3	61 ± 3	62 ± 3	60 ± 4	61 ± 3
DIDS (75)	48 ± 1	66 ± 2[a]	76 ± 2[a]	87 ± 3[a]	92 ± 3[a]	95 ± 3[a]	53 ± 4	60 ± 5[b]	56 ± 5	61 ± 5[b]	62 ± 5[a]	65 ± 5[a]
SITS (75)	48 ± 1	49 ± 1	50 ± 3	55 ± 7	53 ± 5	52 ± 3	50 ± 2	54 ± 2	55 ± 2	47 ± 3	48 ± 5	49 ± 5
SITS (110)	47 ± 1	49 ± 1	49 ± 2	51 ± 1[b]	52 ± 1[a]	52 ± 2[a]	52 ± 2	55 ± 3	51 ± 3	47 ± 4	49 ± 3	49 ± 3
Verapamil (0.5)	46 ± 1	51 ± 2[a]	53 ± 1[a]	52 ± 1[a]	51 ± 1[a]	50 ± 1[a]	56 ± 2	57 ± 2	56 ± 2	54 ± 2	56 ± 2	57 ± 2
Diltiazem (0.4)	46 ± 1	52 ± 1[a]	51 ± 1[a]	51 ± 1[a]	51 ± 1[a]	52 ± 2[a]	52 ± 2	50 ± 3	50 ± 3	50 ± 3	50 ± 3	50 ± 3

Each value represents the mean ± S.E.M. ($n = 10$).
[a] $P < 0.01$ vs. predrug value of each group.
[b] $P < 0.05$ vs. predrug value of each group.

ventricular tachycardia incidence. Administration of a lower dose of 5 or 25 mg/kg of DIDS did not show any significant effect on the incidence of ventricular tachycardia, ventricular fibrillation and mortality.

3.2. Effects of SITS on reperfusion-induced arrhythmias

SITS at doses of 25–50 mg/kg did not affect the incidence of arrhythmias ($n = 6$, data not shown). SITS at a dose of 75 mg/kg reduced the incidence of ventricular fibrillation and mortality to 60% (Fig. 3), although it was not statistically significant. A higher dose of SITS (110 mg/kg) failed to produce any decrease of the arrhythmia incidence (Fig. 3). Conversely, SITS at a high dose (150 mg/kg) induced a third degree A-V block in three of five rats, but did not affect the incidence of arrhythmias (data not shown).

3.3. Effects of diltiazem and verapamil on reperfusion-induced arrhythmias

Diltiazem (0.4 mg/kg) significantly decreased the incidence of reperfusion-induced ventricular fibrillation and mortality to 20% and 10% ($P < 0.01$), respectively, but did not alter ventricular tachycardia incidence. Verapamil (0.5 mg/kg) significantly decreased the incidence of ventricular tachycardia, ventricular fibrillation and mortality to 30% ($P < 0.01$), 0% ($P < 0.01$) and 0% ($P < 0.01$), respectively (Fig. 4).

3.4. Effects of DIDS, SITS, diltiazem and verapamil on hemodynamics and ECG parameters (Tables 1 and 2)

DIDS (75 mg/kg), diltiazem (0.4 mg/kg) or verapamil (0.5 mg/kg) markedly decreased the mean blood pressure and heart rate within 5 min after administration, just before occlusion of the coronary artery. DIDS at 5 mg/kg increased mean blood pressure, but at 25 mg/kg transiently decreased mean blood pressure, without altering the heart rate. Administration of 75 mg/kg SITS decreased and then increased the mean blood pressure to more than the pre-drug value, whereas the administration of 110 mg/kg decreased it. These two high doses of SITS decreased the heart rate dose-dependently (Table 1).

In the ECG (Table 2), DIDS (75 mg/kg), diltiazem (0.4 mg/kg) and verapamil (0.5 mg/kg) prolonged the PQ intervals, and DIDS prolonged the QRS duration, but the latter two have no effect on QRS duration. SITS at 75 and 110 mg/kg did not change the QRS duration, but a higher dose, 110 mg/kg, slightly prolonged the PQ interval.

3.5. Effects of mexiletine on ECG and hemodynamic parameters

We recently reported that mexiletine reduced the incidences of reperfusion-induced ventricular tachycardia, ventricular fibrillation and mortality in anesthetized rats (Saitoh et al., 2000). In that study, mexiletine (5 mg/kg) signifi-

Table 3
Effects of mexiletine (5 mg/kg, i.v.) on the parameters of hemodynamics and ECG of the anesthetized rats

	Predrug	1 min	2 min	3 min	4 min	5 min	Predrug	1 min	2 min	3 min	4 min	5 min
	Heart rate (beats/min)						*PQ interval (ms)*					
Saline control	413 ± 14	411 ± 15	411 ± 16	410 ± 16	403 ± 14	401 ± 14	41 ± 2	42 ± 1	42 ± 2	42 ± 1	42 ± 1	41 ± 2
Mexiletine	443 ± 18	362 ± 18[a]	388 ± 18[a]	389 ± 18[a]	385 ± 17[a]	381 ± 17[a]	41 ± 1	47 ± 1[a]	46 ± 1[a]	44 ± 1[a]	44 ± 1[a]	43 ± 1
	Mean blood pressure (mm Hg)						*QRS width (ms)*					
Saline control	84 ± 6	90 ± 8[b]	88 ± 7	85 ± 5	81 ± 5	80 ± 4	44 ± 3	45 ± 1	45 ± 2	44 ± 1	44 ± 3	43 ± 2
Mexiletine	81 ± 6	83 ± 6	75 ± 5[b]	70 ± 5[a]	68 ± 5[a]	64 ± 5[a]	42 ± 1	47 ± 2[a]	47 ± 2[a]	44 ± 1	44 ± 1	44 ± 1

Each value represents the mean ± S.E.M. ($n = 10$).
[a] $P < 0.01$ vs. predrug value of each group.
[b] $P < 0.05$ vs. predrug value of each group.

cantly decreased the heart rate and mean blood pressure (Table 3). The PQ interval and QRS duration were significantly prolonged as shown in Table 3, which were not analyzed in the previous paper (Saitoh et al., 2000).

4. Discussion

DIDS and SITS were previously demonstrated to be anion exchange inhibitors in vitro and to have antiarrhythmic effects on in vitro ischemia models. The present study was performed to investigate whether they also suppress reperfusion arrhythmias in in vivo rats and to speculate the mechanisms of action by comparing them with other drugs including Na^+ and Ca^{2+} channel blockers. The results were contradictory to in vitro studies and only DIDS, not SITS, showed a powerful antiarrhythmic effect.

Previous studies showed that verapamil and diltiazem at doses of 0.3–0.5 mg/kg suppressed coronary occlusion/reperfusion arrhythmias in anesthetized or conscious rats (Swies et al., 1990; Kinoshita et al., 1989). A similar dose of verapamil or diltiazem was antiarrhythmic in the present reperfusion-induced model. Mexiletine at 5 mg/kg was also effective (Saitoh et al., 2000; Komori et al., 1994). DIDS and SITS at such small doses did not exert significant antiarrhythmic effects. The results obtained in in vitro show that high concentrations (100 or 500 μM) of DIDS or SITS are necessary for suppressing the Cl^-/HCO_3^- exchanger (Vaughan-Jones, 1979, 1986; Lai et al., 1996; Kawasaki et al., 2001) and arrhythmias (Curtis, 1989, 1990). Also, it is reported that the concentration of SITS (500 μM) to inhibit action potential changes during simulated ischemia was five times higher than that of DIDS (100 μM) in the guinea pig ventricular papillary muscle (Lai et al., 1996), indicating that a high dose of SITS may be necessary for its antiarrhythmic effect. Our results showed that the antiarrhythmic dose for DIDS was 75 mg/kg, but SITS at the dose of 75 mg/kg only tended to suppress the arrhythmias. Further increase of the dose of SITS (150 mg/kg) showed cardiac toxicity including A-V block. Thus, it is possible that addition of some other cardiac action induced by a high dose of SITS or lack of some action might have obscured its antiarrhythmic effect in vivo. It has been speculated that both DIDS and SITS inhibit Cl^-/HCO_3^- exchange during ischemia/reperfusion, which may account for their antiarrhythmic effects in vitro (Lai et al., 1996; Lai and Nishi, 1998; Hume et al., 2000; Kawasaki et al., 2001). Thus, we consider that the additional effects of DIDS other than the inhibition of Cl^-/HCO_3^- exchange may be important in suppressing the reperfusion-induced arrhythmias in the anesthetized rats in vivo.

We observed that DIDS at 75 mg/kg decreased the blood pressure and heart rate and prolonged the PQ interval when it showed antiarrhythmic action on the reperfusion arrhythmias. Similar results were obtained by Ca^{2+} channel blockers such as verapamil and diltiazem, not only in the present study but also in previous studies (Swies et al., 1990; Lu et al., 1999). DIDS has been shown to block L-type Ca^{2+} channels in canine colonic myocytes (Dick et al., 1999). We therefore speculate that the antiarrhythmic effect of DIDS in the anesthetized rats was likely to be attributed in part to its Ca^{2+} channel-blocking action. These calcium antagonistic actions reduce myocardial oxygen consumption by decreasing the blood pressure, heart rate and cardiac contractility (Nayler et al., 1987; Buser et al., 1991), and inhibit Ca^{2+} overload-induced delayed afterdepolarization and triggered activity (El-Sherif et al., 1983; Gough et al., 1984), which can explain their protection against ischemia and reperfusion arrhythmias (Van Gilst et al., 1986; Tosaki et al., 1987; Swies et al., 1990). However, SITS had no effect on the arrhythmias, although it also decreased the mean blood pressure and heart rate, and prolonged the PQ interval slightly and transiently. This weak effect of SITS on Ca^{2+} channel-blocking activity compared with DIDS may be related to the lack of the antiarrhythmic effect of this drug in the present study.

Na^+ channel inhibitors usually prolong PQ (PR) interval and QRS duration (Priori et al., 1987; Yang et al., 1995; Barrett et al., 2000). Mexiletine, a Na^+ channel inhibitor, or DIDS had such effects in the present results, suggesting that DIDS may also have an inhibitory effect on Na^+ channels. In the rat Langendorff hearts, it has been shown that SITS reduced ventricular excitability, indicating SITS also inhibited Na^+ channels (Curtis, 1990). However, we did not observe any effects of SITS on the QRS duration. Thus, these results may reflect the difference between these stilbene derivatives in inhibiting Na^+ channels. Consistent with this idea, DIDS appears to block voltage-activated Na^+ channels in guinea pig cardiac ventricular cells, whereas SITS has only a weak effect (Liu et al., 1998). The different potency of DIDS and SITS in blocking Na^+ channels is likely to be another cause for the absence of the antiarrhythmic effect of SITS.

Quantitatively different effects of DIDS and SITS in inhibiting anion exchanger and Cl^- channels have been reported (Vaughan-Jones, 1979, 1986; Dick et al., 1999). In isolated sheep cardiac Purkinje fibres, SITS at concentrations of 80–100 μM and DIDS at a higher concentration of 150 μM completely inhibited Cl^-/HCO_3^- exchange activity (Vaughan-Jones, 1979, 1986). Dick et al. (1999) analyzed DIDS and SITS, as inhibitors of the volume-sensitive, outwardly rectifying Cl^- current, and reported that, in canine colonic myocytes, DIDS is a strong Cl^- channel inhibitor (IC_{50} of DIDS and SITS was 0.84 and 226 μM, respectively). These results suggest that SITS is more potent and selective on anion exchanger than DIDS, but DIDS exerts a much greater inhibition on Cl^- channels. Under conditions of Ca^{2+} overload, spontaneous intracellular Ca^{2+} release may activate arrhythmogenic transient inward current, partially mediated by $I_{Cl(Ca)}$ (Cl^- current regulated by $[Ca^{2+}]_i$), to cause delayed afterdepolarization (Hiraoka et al., 1998). It has been reported that DIDS inhibits this current in sheep Purkinje and ventricular myocytes (Verkerk

et al., 2000). In the present in vivo study, we could not determine whether or not the antiarrhythmic effects of DIDS were attributed to the inhibition of Cl^- channels; however, the doses of 75 mg/kg must be enough for this inhibition, as judged by Cl^- channel-mediated depolarization in rat resistance arteries (Lamb et al., 2000). Thus, we speculate that, in the present study, the antiarrhythmic mechanisms of DIDS may also be related to inhibition of the Cl^- channels.

DIDS at the lowest dose of 5 mg/kg increased the mean blood pressure, probably via the influence of the saline injected, since the same phenomenon was observed in the control group. The increase of the mean blood pressure at 4 and 5 min after administration of SITS at 75 mg/kg is difficult to explain.

In conclusion, a dose of 75 mg/kg of DIDS was the threshold concentration for the significant antiarrhythmic effect. This may or may not correlate with a threshold concentration for inhibition of Cl^-/HCO_3^- exchange. This is unknown since no assay of Cl^-/HCO_3^- exchange activity was performed. However, this antiarrhythmic dose was associated with ECG interval changes indicating non-selectivity of action of DIDS. Therefore, the antiarrhythmic action is likely to be attributed to these non-selective actions. On the other hand, the ineffectiveness of SITS to protect against arrhythmias even at high doses suggests that Cl^-/HCO_3^- exchange is not important for reperfusion arrhythmogenesis.

References

Aye, N.N., Xue, Y.X., Hashimoto, 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.

Barrett, T.D., Hayes, E.S., Yong, S.L., Zolotoy, A.B., Abraham, S., Walker, M.J.A., 2000. Ischaemia selectivity confers efficacy for suppression of ischaemia-induced arrhythmias in rats. Eur. J. Pharmacol. 398, 365–374.

Buser, P.T., Wagner, S., Wu, S., Higgins, C.B., Wikman-Coffelt, J., 1991. Protective effects of calcium antagonists on energy and substrate metabolism during ischemia and reperfusion in hypertensive myocardial hypertrophy. J. Cardiovasc. Pharmacol. 18 (Suppl. 10), S87–S92.

Carbonin, P., Di Gennaro, M., Valle, R., Beranbei, R., Habed, A., 1980. Intracellular calcium and electrogram in ischemic isolated rat heart. Am. J. Physiol. 239, H380–H390.

Curtis, M.J., 1989. Inhibition of chloride–bicarbonate exchange: a new approach to the attenuation of ischaemia-induced and reperfusion-induced arrhythmias. J. Mol. Cell. Cardiol. 21 (Suppl. II), S-141.

Curtis, M.J., 1990. SITS (4-acetamido-4′isothiocyanato-stilbene-2, 2′-disulfonic acid) reduces ventricular excitability. J. Mol. Cell. Cardiol. 22 (Suppl. III), S-75.

Curtis, M.J., Hearse, D.J., 1989. Ischaemia-induced and reperfusion-induced arrhythmias differ in their sensitivity to potassium: implications for mechanisms of initiation and maintenance of ventricular fibrillation. J. Mol. Cell. Cardiol. 21, 21–40.

Curtis, M.J., Macleod, B.A., Walker, M.J.A., 1987. Models for the study of arrhythmias in myocardial ischaemia and infarction: the use of the rat. J. Mol. Cell. Cardiol. 19, 399–419.

Curtis, M.J., Garlick, P.B., Ridley, P.D., 1993. Anion manipulation, a novel antiarrhythmic approach: mechanism of action. J. Mol. Cell. Cardiol. 25, 417–436.

Dick, G.M., Kong, I.D., Sanders, K.M., 1999. Effects of anion channel antagonists in canine colonic myocytes: comparative pharmacology of Cl^-, Ca^{2+} and K^+ currents. Br. J. Pharmacol. 127, 1819–1831.

El-Sherif, N., Gough, W.B., Zeiler, R.H., Mehra, R., 1983. Triggered ventricular rhythms in 1-day-old myocardial infarction in the dog. Circ. Res. 52, 566–579.

Gough, W.B., Zeiler, R.H., El-Sherif, N., 1984. Effects of diltiazem on triggered activity in canine 1 day old infarction. Cardiovasc. Res. 18, 339–343.

Hiraoka, M., Kawano, S., Hirano, Y., Furukawa, T., 1998. Role of cardiac chloride currents in changes in action potential characteristics and arrhythmias. Cardiovasc. Res. 40, 23–33.

Hume, J.R., Duan, D., Collier, M.L., Yamazaki, J., Horowitz, B., 2000. Anion transport in heart. Physiol. Rev. 80, 31–81.

Jennings, M.L., 1989. Structure and function of the red blood cell anion transport protein. Annu. Rev. Biophys. Biophys. Chem. 18, 397–430.

Kawasaki, H., Otani, H., Mishima, K., Imamura, H., Inagaki, C., 2001. Involvement of anion exchange in the hypoxia/reoxygenation-induced changes in pH_i and $[Ca^{2+}]_i$ in cardiac myocyte. Eur. J. Pharmacol. 411, 35–43.

Kinoshita, K., Mitani, A., Hearse, D.J., Braimbridge, M.V., Manning, A.S., 1989. Reperfusion-induced arrhythmias in the conscious rat: a comparative study with three calcium antagonists. J. Surg. Res. 47, 166–172.

Komori, S., Sawanbori, T., Tamura, K., Kane, K.A., Parratt, J.R., 1994. Effects of NS-2, a new class I antiarrhythmic agent, and AFD-19, its active metabolite, on ventricular arrhythmias induced by coronary artery occlusion and reperfusion in anesthetized rats: comparison with disopyramide and mexiletine. Jpn. J. Pharmacol. 65, 193–200.

Lai, Z.F., Nishi, K., 1998. Intracellular chloride activity increases in guinea pig ventricular muscle during simulated ischemia. Am. J. Physiol. 275, H1613–H1619.

Lai, Z.F., Liu, J., Nishi, K., 1996. Effects of stilbene derivatives SITS and DIDS on development of intracellular acidosis during ischemia in isolated guinea pig ventricular papillary muscle in vitro. Jpn. J. Pharmacol. 72, 161–174.

Lamb, F.S., Kooy, N.W., Lewis, S.J., 2000. Role of Cl^- channel in α-adrenoceptor-mediated vasoconstriction in the anesthetized rat. Eur. J. Pharmacol. 401, 403–412.

Lawson, C.S., Coltart, D.J., Hearse, D.J., 1993. 'Dose'-dependency and temporal characteristics of protection by ischaemic preconditioning against ischaemia-induced arrhythmias in rat hearts. J. Mol. Cell. Cardiol. 25, 1391–1402.

Liu, J., Lai, Z.F., Wang, X.D., Tokutomi, N., Nishi, K., 1998. Inhibition of sodium current by chloride channel blocker 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid (DIDS) in guinea pig cardiac ventricular cells. J. Cardiovasc. Pharmacol. 31, 558–567.

Lu, H.R., Yang, P., Remeysen, P., Saels, A., Dai, D.Z., De Clerck, F., 1999. Ischemia/reperfusion-induced arrhythmias in anaesthetized rats: a role of Na^+ and Ca^{2+} influx. Eur. J. Pharmacol. 365, 233–239.

Nayler, W.G., Panagiotopoulos, S., Elz, J.S., Sturrock, W.J., 1987. Fundamental mechanisms of action of calcium antagonists in myocardial ischemia. Am. J. Cardiol. 59, 75B–83B.

Priori, S.G., Bonazzi, O., Facchini, M., Varisco, T., Schwartz, P.J., 1987. Antiarrhythmic efficacy of penticainide and comparison with disopyramide, flecainide, propafenone and mexiletine by acute oral drug testing. Am. J. Cardiol. 60, 1068–1072.

Ridley, P.D., Curtis, M.J., 1992. Anion manipulation: a new antiarrhythmic approach. Action of substitution of chloride with nitrate on ischemia- and reperfusion-induced ventricular fibrillation and contractile function. Circ. Res. 70, 617–632.

Saitoh, M., Aye, N.N., Komori, S., Nakazawa, T., Hashimoto, K., 2000. Effects of HNS-32, a novel antiarrhythmic drug, on ventricular arrhythmias induced by coronary artery occlusion and reperfusion in anesthetized rats. Mol. Cell. Biochem. 205, 133–140.

Shaw, L.A., Coker, S.J., 1996. Suppression of reperfusion-induced arrhythmias with combined administration of $5\text{-}HT_2$ and thromboxane A_2 antagonists. Br. J. Pharmacol. 117, 817–822.

Swies, J., Omogbai, E.K.I., Smith, G.M., 1990. Occlusion and reperfusion-induced arrhythmias in rats: involvement of platelets and effects of calcium antagonists. J. Cardiovasc. Pharmacol. 15, 816–825.

Tanaka, H., Matsui, S., Kawanishi, T., Shigenobu, K., 1996. Use of chloride blockers: a novel approach for cardioprotection against ischemia–reperfusion damage. J. Pharmacol. Exp. Ther. 278, 854–861.

Tosaki, A., Szekeres, L., Hearse, D.J., 1987. Diltiazem and the reduction of reperfusion-induced arrhythmias in the rat: protection is secondary to modification of ischemic injury and heart rate. J. Mol. Cell. Cardiol. 19, 441–451.

Van Gilst, W.H., De Graeff, P.A., Kingma, J.H., De Langen, C.D., Wesseling, H., 1986. Effects of diltiazem on reperfusion-induced arrhythmias in vitro and in vivo. J. Mol. Cell. Cardiol. 18, 1255–1266.

Vaughan-Jones, R.D., 1979. Regulation of chloride in quiescent sheep-heart Purkinje fibres studied using intracellular chloride and pH-sensitive micro-electrodes. J. Physiol. (Lond.) 295, 111–137.

Vaughan-Jones, R.D., 1986. An investigation of chloride–bicarbonate exchange in the sheep cardiac Purkinje fibre. J. Physiol. (Lond.) 379, 377–406.

Verkerk, A.O., Veldkamp, M.W., Bouman, L.N., van Ginneken, A.C., 2000. Calcium-activated Cl^- current contributes to delayed afterdepolarizations in single Purkinje and ventricular myocytes. Circulation 101, 2639–2644.

Walker, M.J., Curtis, M.J., Hearse, D.J., Campbell, R.W., Janse, M.J., Yellon, D.M., Cobbe, S.M., Coker, S.J., Harness, J.B., Harron, D.W., Higgins, A.J., Julian, D.G., Lab, M.J., Manning, A.S., Northover, B.J., Parratt, J.R., Riemersma, R.A., Riva, E., Russell, D.C., Sheridan, D.C., Winslow, E., Wooward, B., 1988. The Lambeth conventions: guidelines for the study of arrhythmias in ischaemia, infarction and reperfusion. Cardiovasc. Res. 22, 447–455.

Wallenstein, S., Zucker, C.L., Fleiss, J.L., 1980. Some statistical methods useful in circulation research. Circ. Res. 47, 1–9.

Yang, Q., Padrini, R., Bova, S., Piovan, D., Magnolfi, G., 1995. Electrocardiographic interactions between pinacidil, a potassium channel opener and class I antiarrhythmic agents in guinea-pig isolated perfused heart. Br. J. Pharmacol. 114, 1745–1749.