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Antiarrhythmic effects of cariporide, a novel Na^+–H^+ exchange inhibitor, on reperfusion ventricular arrhythmias in rat hearts

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Abstract

Cariporide (4-isopropyl-3-methylsulphonylbenzoyl-guanidine methanesulphonate: HOE642) is a novel Na^+–H^+ exchange subtype 1 inhibitor and has antiarrhythmic effects on ischemia/reperfusion arrhythmias without apparent cardiovascular effects in dogs and rats when given before coronary occlusion. The aim of this study was to determine the minimum effective dose and to examine the dose related effects of cariporide when it was administered before and during coronary occlusion as well as simultaneously with reperfusion. In the pre-treatment group, cariporide dose-dependently reduced the ventricular tachycardia duration from 140 to 36 ($P < 0.01$), 59 ($P < 0.05$) and 23 s ($P < 0.01$) with 0.03, 0.1 and 1 mg/kg, respectively, and reduced the incidence of reperfusion-induced ventricular tachycardia from 100 to 50 and 58% ($P < 0.01$), ventricular fibrillation from 83 to 8 and 0% ($P < 0.01$), and mortality from 75 to 8 and 8% ($P < 0.01$) with 0.1 and 1 mg/kg, respectively. In the post-treatment group, cariporide dose-dependently reduced the ventricular tachycardia duration from 92 to 37, 40, 42 ($P < 0.05$) and 24 s ($P < 0.01$) with 0.03, 0.1, 0.3 and 1 mg/kg, respectively, and the incidence of ventricular tachycardia from 100 to 53% ($P < 0.01$) by 1 mg/kg, and ventricular fibrillation from 87 to 33, 7 and 7% ($P < 0.01$), and the mortality from 73 to 27 ($P < 0.05$), 0 and 7% ($P < 0.01$) with 0.1, 0.3 and 1 mg/kg, respectively. In the group with simultaneous injection, both doses of cariporide (1 and 3 mg/kg) reduced the incidence of ventricular fibrillation from 83 to 42% ($P < 0.05$). The heart rate, blood pressure and QT interval did not change after drug treatment. © 1997 Elsevier Science B.V.

Keywords: Cariporide (HOE642); Na^+–H^+ exchange; Arrhythmias; Ischemia; Reperfusion

1. Introduction

Reperfusion injury in the heart is one of the clinical problems causing myocardial damage, arrhythmias and stunning. Compounds that act cytoprotectively against the initial steps of reperfusion may be beneficial under these circumstances. Reperfusion induces ventricular arrhythmias or microvascular injury and accelerates or increases cell necrosis (Opie, 1989). Intracellular accumulation, to an abnormally high level, of Ca^{2+} during ischemia and especially soon after reperfusion is suggested to be the mechanism for reperfusion injury (Nayler et al., 1988; Opie and Coetzee, 1988; Tani and Neely, 1989; Brooks et al., 1995). The increase in intracellular Ca^{2+} is mediated, in part, by a rise in intracellular Na^+ resulting from stimulation of Na^+–H^+ exchange (Piwnica-Worms et al., 1985; Tani and Neely, 1989). Lazdunski et al. (1985) hypothesized that reperfusion activates the Na^+–H^+ exchanger, which would lead to Na^+ overload and consequently Ca^{2+} overload, the putative mediator of arrhythmias and stunning. There are five known isoforms of the mammalian Na^+–H^+ exchanger and these are referred to as Na^+–H^+ exchange subtype 1 to Na^+–H^+ exchange subtype 5 (Fliegel and Dyck, 1995; Klanke et al., 1995). Na^+–H^+ exchange subtype 1 cDNA clones have been isolated from the mammalian myocardium, including the human myocardium (Fliegel et al., 1993), and Dyck et al. (1995) suggested that ischemia and acidosis can increase the amiloride-sensitive Na^+–H^+ exchanger (Na^+–H^+ exchange subtype 1) in the mammalian myocardium. Loh et al. (1996) reported that the native isoform contributing to proton extrusion and hence to pHi regulation in the heart must be Na^+–H^+ exchange subtype 1. Scholz et al. (1993, 1995) discovered a new class of Na^+–H^+ exchange inhibitors, benzoyl guanidine derivatives, HOE694 and cariporide (4-isopropyl-3-methylsulphonylbenzyol-guanidine

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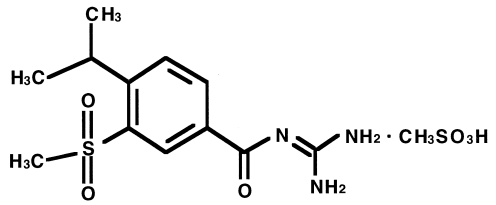

Fig. 1. Chemical structure of cariporide (4-isopropyl-3-methylsulphonyl-benzoyl–guanidine methanesulphonate).

methanesulphonate: HOE642) (Fig. 1) and showed that cariporide was more potent and specific for the Na^+–H^+ exchange subtype 1 than HOE694. Cariporide inhibited Na^+–H^+ exchange in different tissues from different species including humans and it had almost no effects on other transport systems. They also reported that pre-ischemic application of cariporide dose-dependently suppressed ischemia-induced arrhythmias in anesthetized rats. Recently we showed that pre-ischemic application of cariporide protected hearts from ischemia and/or reperfusion-induced arrhythmias in dogs and rats (Xue et al., 1996). The present study was designed to examine dose-related effects of cariporide when it was administered before as well as after ischemic insult and even during reperfusion. Rat ischemia/reperfusion arrhythmia models were used.

2. Methods

2.1. Production of coronary occlusion / reperfusion injury in rats

As reported earlier (Komori et al., 1994; Xue et al., 1996), male Sprague–Dawley rats (body weight 250–400 g) were anesthetized with sodium pentobarbital (60 mg/kg, intraperitoneally). The femoral vein was cannulated to allow drug administration, and the trachea was cannulated for artificial ventilation. The systemic blood pressure was monitored via a catheter inserted into the carotid artery and a standard limb lead II electrocardiogram (ECG) was continuously monitored on a recorder (Nihon Kohden, RM-62001, Tokyo). The chest was opened by a left thoracotomy at a point approximately 2 mm to the left of the sternum, followed by sectioning of the 4th and 5th ribs. Artificial ventilation was immediately started with room air (volume 1.5 ml/100 g, rate 54 strokes/min) to maintain PCO_2, PO_2, and pH within the normal limits. After incision of the pericardium, the heart was exteriorized by applying gentle pressure to 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 for 15 min.

Transient regional myocardial ischemia was induced by passing the threads through a small plastic tube and pulling the suture while pressing the tube against the surface of the myocardium. We chose 5 min of occlusion (i.e. the duration of ischemia) as it has been demonstrated that the incidence of fibrillation occurring upon reperfusion in vivo anesthetized rat reaches a peak 5 min after occlusion and subsides thereafter (Manning and Hearse, 1984) and our preliminary study showed the same result. Ischemia and reperfusion were confirmed as described previously (Lawson et al., 1993). Reperfusion was initiated by releasing the ligature and removing the plastic tube. Responses were observed for 10 min thereafter. Successful occlusion was confirmed by ST segment elevation during the first few seconds of each occlusion and by a 20–30% reduction in the arterial blood pressure compared to the pre-ischemic value. Successful reperfusion was confirmed by the return of arterial blood pressure to the pre-ischemic value.

Animals were obtained through the Animal Laboratory for Research of Yamanashi Medical University. All experiments were carried out according to the Guideline for Animal Experiments of Yamanashi Medical University.

2.2. Experimental protocol

Fig. 2 summarizes the protocols used in this study. Three protocols with separate control groups were carried out. After 15 min of stabilization, cariporide was administered 5 min before coronary artery occlusion (pre-treatment group), 3 min after occlusion but 2 min before reperfusion (post-treatment group) and simultaneously with reperfusion (with-reperfusion group). Cariporide, 0.03–1 mg/kg body weight, was given intravenously to the pre-treatment and post-treatment groups and at 1–3 mg/kg body weight intravenously to the with-reperfusion group. The control groups were given 5% dimethyl sulfoxide (DMSO) as vehicle. The volume of injection was 0.8–0.9 ml; injections were given over 30 s for the pre-treatment and post-treatment groups, and 10 s for the with-reperfu-

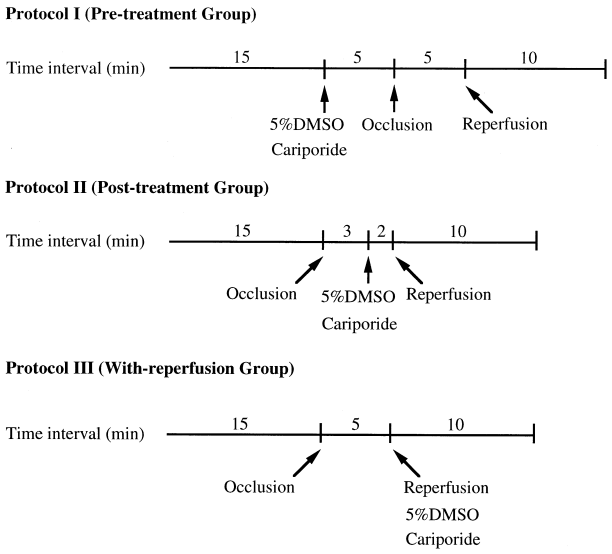

Fig. 2. Diagrammatic representation of the experimental protocols used.

sion group. The ECG and blood pressure were continuously recorded throughout the experiment. The heart rate and QT interval were measured from the lead II ECG. The QT interval was measured at 90% repolarization and is referred to as QT90 (Rees and Curtis, 1993).

2.3. Exclusion criteria

Experiments were terminated and data were excluded from the final data analysis if any of the following occurred (Shaw and Coker, 1996): arrhythmias prior to coronary artery occlusion; mean arterial pressure less than 60 mm Hg prior to drug or vehicle administration; atrioventricular block during the first 5 min of ischemia (probably caused by a ligature occluding the septal branch of the left coronary artery); severe arrhythmias at 5 min post-occlusion that prevented reperfusion, and reperfusion not evident (i.e. maintenance and/or progression of ECG changes (ST, QRS) typical of those occurring during sustained ischemia).

2.4. Definition of arrhythmias and analysis

Definitions of arrhythmias were based on those described in the Lambeth Conventions (Walker et al., 1988). Ectopic activity was categorized as a single ventricular premature beat, ventricular tachycardia (4 or more consecutive ventricular premature beats) or ventricular fibrillation (inability to distinguish individual QRS complexes or measure a rate). Complex forms (e.g. bigeminy) were included in the count of ventricular premature beats and were not analyzed separately. Reference was also made to the blood pressure signal to confirm which type of ectopic activity was occurring, particularly to distinguish between polymorphic ventricular tachycardia and ventricular fibrillation. When the former occurred, the pressure was usually still pulsatile whereas with ventricular fibrillation the blood pressure fell rapidly towards zero and was no longer pulsatile. Ventricular fibrillation may be sustained or may revert spontaneously to a normal sinus rhythm in the rat. 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.

2.5. Drugs

Drugs used in the present study were cariporide (kindly supplied by Hoechst Marion Roussel, Tokyo), dimethyl sulfoxide (DMSO, Wako, Osaka), and pentobarbital sodium (Tokyo Kasei Kogyo).

2.6. Statistics

All data are expressed as means ± S.E.M. Student's *t*-test was used to test drug effects on hemodynamic parameters prior to ischemia. Differences in the durations of

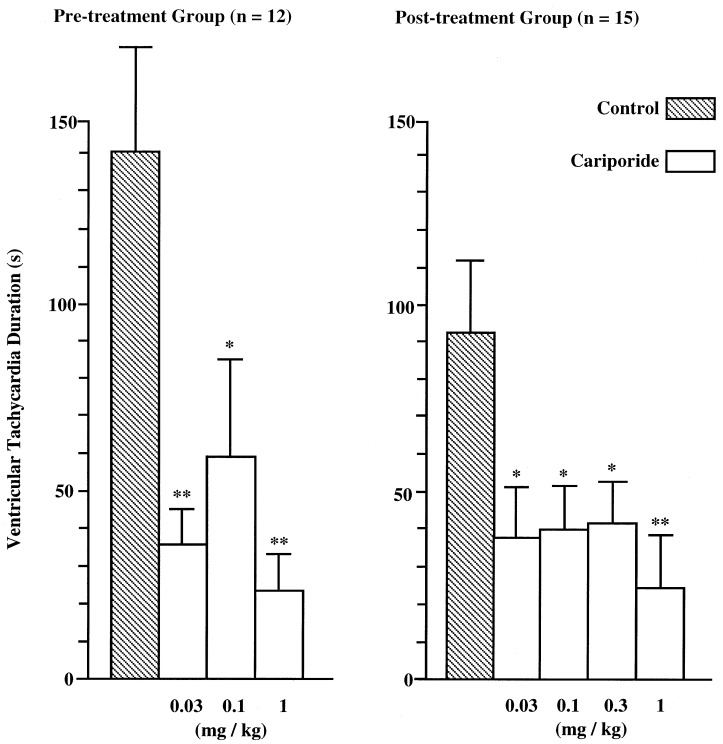

Fig. 3. Dose-dependent effects of intravenous injection of cariporide (0.03, 0.1, 0.3 and 1 mg/kg), on the total ventricular tachycardia duration after reperfusion in rat hearts. Results are means ± S.E.M. ($n = 12$ in the pre-treatment group, $n = 15$ in the post-treatment group). Error bars = S.E.M. $^{*}P < 0.05$, $^{**}P < 0.01$, statistically significant difference from the 5% DMSO treated control group.

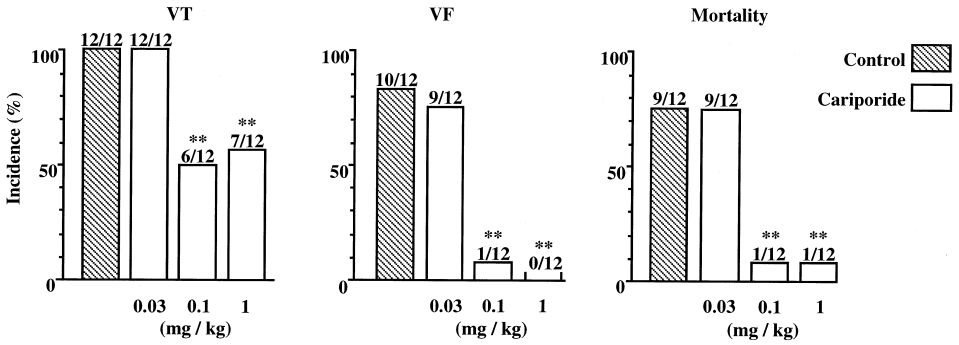

Fig. 4. Prevention of reperfusion-induced ventricular tachycardia (VT), ventricular fibrillation (VF) and mortality in rat hearts by intravenous pre-treatment with cariporide 0.03, 0.1 and 1 mg/kg ($n = 12$ for control as well as drug treatments). Numbers above bars give incidence of ventricular tachycardia and ventricular fibrillation. $^{**}P < 0.01$, statistically significant difference from the 5% DMSO treated control group.

ventricular tachycardia between control and drug groups were tested by analysis of variance combined with Dunnett's multiple comparison test (Wallenstein et al., 1980). Differences in the incidence of arrhythmias between cariporide groups and 5% DMSO groups were analyzed with Fisher's exact probability test. A P value of less than 0.05 was considered statistically significant.

3. Results

3.1. Effects of cariporide on reperfusion-induced arrhythmias in the pre-treatment group

In the 5% DMSO control group, the total duration of reperfusion-induced ventricular tachycardia was 140 s (Fig. 3, $n = 12$), and cariporide 0.03, 0.1 and 1 mg/kg significantly reduced the ventricular tachycardia duration to 36 s ($P < 0.01$), 59 s ($P < 0.05$) and 23 s ($P < 0.01$), respectively. Cariporide at 0.03 mg/kg reduced the total duration of reperfusion induced ventricular tachycardia more than it did at 0.1 mg/kg, because with the former, severe ventricular fibrillation occurred within seconds. The incidence of reperfusion-induced ventricular tachycardia, ventricular fibrillation and mortality in the control group was high (ventricular tachycardia 100%, ventricular fibrillation 83% and mortality 75%, Fig. 4), and cariporide 0.1 and 1 mg/kg significantly decreased the incidence of ventricular tachycardia to 50% and 58%, ventricular fibrillation to 8% and 0% and mortality to 8% and 8%, respectively ($P < 0.01$). Cariporide 0.03 mg/kg did not reduce these parameters.

Cariporide at the highest dose of 1 mg/kg had no significant effect on the heart rate, blood pressure and QT90 in the control rats (Table 1).

3.2. Effects of cariporide on reperfusion-induced arrhythmias in the post-treatment group

In the 5% DMSO control group, the total duration of reperfusion-induced ventricular tachycardia was 92 s (Fig. 3, $n = 15$), and cariporide 0.03, 0.1, 0.3 and 1 mg/kg significantly reduced the duration of ventricular tachycardia to 37 s, 40 s, 42 s ($P < 0.05$), and 24 s ($P < 0.01$), respectively. The incidence of reperfusion-induced ventricular tachycardia and ventricular fibrillation and mortality in the control group was also high (ventricular tachycardia 100%, ventricular fibrillation 87% and mortality 73%, Fig.

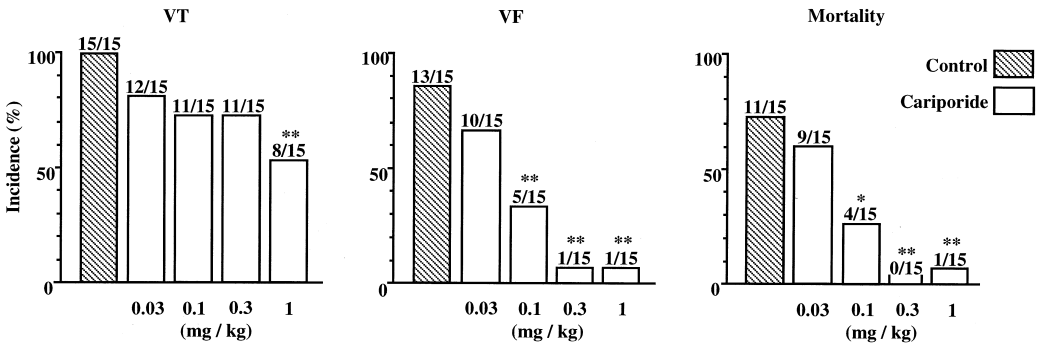

Fig. 5. Dose-dependent prevention of reperfusion-induced ventricular tachycardia (VT), ventricular fibrillation (VF) and mortality in rat hearts by intravenous post-treatment with cariporide 0.03, 0.1, 0.3 and 1 mg/kg ($n = 15$ for control as well as drug treatments). Numbers above bars give incidence of ventricular tachycardia and ventricular fibrillation. $^{*}P < 0.05$, $^{**}P < 0.01$, statistically significant difference from the 5% DMSO treated control group.

Table 1
Effects of cariporide on the hemodynamic indices of the rat heart (pre-treatment group)

	Before treatment	5 min after treatment
5% DMSO ($n = 12$)		
HR (beats/min)	439 ± 13	426 ± 15
QT_{90} (ms)	43 ± 2	44 ± 3
SBP (mm Hg)	108 ± 8	97 ± 7
DBP (mm Hg)	79 ± 7	70 ± 6
Cariporide ($n = 12$)		
HR (beats/min)	458 ± 12	446 ± 14
QT_{90} (ms)	47 ± 2	46 ± 2
SBP (mm Hg)	115 ± 7	102 ± 4
DBP (mm Hg)	87 ± 6	80 ± 4

HR: heart rate. SBP: systolic blood pressure. DBP: diastolic blood pressure.

5), but cariporide 0.1, 0.3 and 1 mg/kg significantly decreased the incidence of ventricular fibrillation to 33%, 7% and 7% ($P < 0.01$), and mortality to 27% ($P < 0.05$), 0% and 7% ($P < 0.01$), respectively. Cariporide 0.03 mg/kg did not significantly reduce these parameters. With regard to the incidence of ventricular tachycardia, only 1 mg/kg significantly decreased it to 53% ($P < 0.01$).

3.3. Effects of cariporide on reperfusion-induced arrhythmias in the with-reperfusion group

The incidence of reperfusion-induced ventricular tachycardia, ventricular fibrillation and mortality in the 5% DMSO control group was 100%, 83% and 67%, respectively (Fig. 6, $n = 12$). Cariporide 1 and 3 mg/kg significantly reduced reperfusion-induced ventricular fibrillation to 42% ($P < 0.05$), but did not significantly reduce mortality or the incidence of ventricular tachycardia.

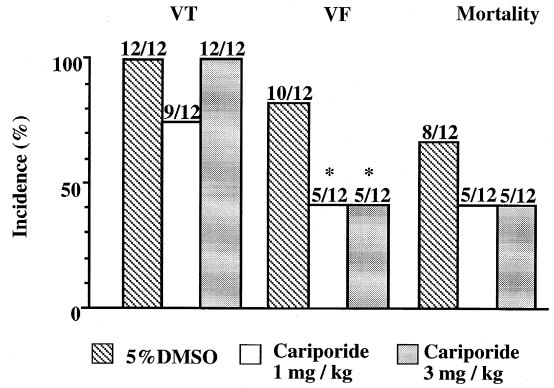

Fig. 6. Effects of cariporide 1 and 3 mg/kg given at the same time as reperfusion on reperfusion-induced ventricular tachycardia (VT), ventricular fibrillation (VF) and mortality in rats ($n = 12$ for control as well as drug treatments). Numbers above bars give incidence of ventricular tachycardia and ventricular fibrillation. $^{*}P < 0.05$, statistically significant difference from the 5% DMSO treated control group.

4. Discussion

Recently we reported that the pre-ischemic application of cariporide 1 mg/kg significantly suppressed reperfusion-induced ventricular tachycardia, ventricular fibrillation and mortality in anesthetized rats (Xue et al., 1996). In the present study, we demonstrated that cariporide suppressed the total duration of reperfusion-induced ventricular tachycardia and the incidence of reperfusion-induced ventricular tachycardia, ventricular fibrillation and mortality when given both before or after ischemia. In the pre-treatment group, cariporide produced a significant suppression of these parameters at 0.03 mg/kg for the duration of ventricular tachycardia and at 0.1 mg/kg for the other parameters in an-all-or-nothing pattern, as shown in Figs. 3 and 4. These effective doses are consistent with those reported by Scholz et al. (1995) for ischemia-induced ventricular arrhythmias. Thus cariporide is effective on both ischemia-induced and reperfusion-induced arrhythmias. Using the same dose range of 0.3–0.6 mg/kg, Miura et al. (1997) reported that cariporide reduced the infarct size in the rabbit hearts when it was given before ischemia. It is difficult to know how much Na^+–H^+ exchange was reduced in these hearts. Scholz et al. (1995) showed in the working rat heart preparation that 0.01–1 μM of cariporide reduced lactate, creatine kinase and LDH during ischemia and reperfusion. They used a similar concentration of cariporide and showed that sodium influx into rabbit erythrocytes was reduced to 90% and 39% with 0.03 and 3 μM, that Na^+–H^+ exchange in human platelets was reduced to 67% and 27% with 0.1 and 10 μM, and that the recovery of pH after a prepulse of NH_4Cl in rat cardiomyocytes was reduced to 31% and 7% with 1 and 10 μM. With regard to post-ischemic application, the effective minimum dose for suppressing ventricular tachycardia was 1 mg/kg and for the other parameters it was 0.1 mg/kg. There were dose-related effects at higher doses, as shown in Fig. 5. Higher doses are needed for an effect after the post-ischemic application, because it is known that there are no functional coronary collaterals in rats (Maxwell et al., 1984), and thus no inhibitor might reach the myocardium during coronary artery occlusion.

Since Hearse and Bolli (1992) suggested that acceptable evidence for drug-induced protection against reperfusion-induced injury requires the drug to be given at the onset of reperfusion, we tested the effects of the drug when given simultaneously with reperfusion. Though the time course of inhibition of Na^+–H^+ exchange by cariporide in the rat heart is not known, we administered the drug at the onset of reperfusion. We assumed that the inhibitor took only tens of seconds to reach the myocardium, so if a sufficiently high dose were given it would prevent reperfusion-induced arrhythmias, because the delay before the onset of ventricular arrhythmias was less than two minutes in all control groups (data not shown). Meng and Pierce (1990) and Yasutake et al. (1994) have already shown that the

inhibitor reached the binding site within one minute of reperfusion and prevented lethal arrhythmias and the development of cardiac dysfunction and damage. In the with-reperfusion group, the highest dose of cariporide used in the pre- as well as post-treatment groups, 1 mg/kg, afforded a significant protection against reperfusion-induced ventricular fibrillation. However a higher dose of 3 mg/kg did not provide further protection against reperfusion-induced ventricular fibrillation, probably indicating that the inhibitor reached the receptor site too late when it was injected at the onset of reperfusion to inhibit Na^+–H^+ exchange enough to suppress the arrhythmias. Although a number of in vitro studies showed protective effects of Na^+–H^+ exchange inhibitors when they were given at the time of reperfusion (Du Toit and Opie, 1993; Meng et al., 1993; Moffat and Karmazyn, 1993; Yasutake et al., 1994; Myers et al., 1995; Yasutake and Avkiran, 1995), there has been no study examining the effects of Na^+–H^+ exchange inhibitors administered at the onset of reperfusion in vivo. Thus for the clinical application of cariporide, it is noteworthy that it showed an antifibrillatory effect even when given simultaneously with reperfusion.

Na^+–H^+ exchange plays an important role in the pathophysiology of cardiac ischemia (Lazdunski et al., 1985). During ischemia, protons generated by a variety of metabolic processes (Dennis et al., 1991) induce rapid acidification of the intracellular space. This acidosis increases intracellular Ca^{2+}, partly by activation of the Na^+–H^+ exchange mechanism in response to the rise in intracellular H^+, which leads to a rise in intracellular Na^+ and hence increases Ca^{2+} influx by a Na^+–Ca^{2+} exchange mechanism (Bountra and Vaughan-Jones, 1989; Harrison et al., 1992).

In regard to reperfusion-induced arrhythmias, restoration of intracellular pH during reperfusion may be partially related to Na^+-dependent proton extrusion, which aggravates Ca^{2+} overload by slowing Ca^{2+} extrusion. Na^+–H^+ exchange blockers may be effective in limiting the increase in intracellular Na^+ during ischemia or in inhibiting the fast rise in intracellular Na^+ on reperfusion when the partial block of Na^+–H^+ exchange by extracellular acidification is suddenly relieved early in reperfusion. It appears that myocardial protection can be achieved best by inhibiting the Na^+–H^+ exchange system during ischemia (Karmazyn, 1988; Anderson et al., 1990; Murphy et al., 1991). However, beneficial effects have also been observed by blocking this system after the start of reperfusion, as Meng et al. (1993), Yasutake et al. (1994), and Yasutake and Avkiran (1995) showed in vitro and as shown by us in vivo.

Cariporide did not affect hemodynamic parameters as shown during the 5 min recording of the stabilization period and 5 min after drug administration, as has been reported previously (Scholz et al., 1995; Xue et al., 1996).

Coronary reperfusion by thrombolytic therapy, percutaneous transluminal angioplasty or bypass surgery has emerged as being effective in the management of acute ischemic syndromes. Spontaneous reperfusion after coronary spasm or thrombosis is a common occurrence and has been suggested as a cause of sudden cardiac death in patients with coronary artery diseases. Moreover, it has been suggested that the incidence and severity of reperfusion arrhythmias may increase if the period of myocardial ischemia is shortened by early reperfusion (for example, with pre-hospital thrombolysis), or following abrupt myocardial reperfusion after spontaneous relief of coronary artery spasm (Hansen, 1995). Our data suggest that cariporide could be used to prevent lethal ventricular arrhythmias under such clinical conditions.

Acknowledgements

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