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The protective effects of CP-060*S* on ischaemia- and reperfusion-induced arrhythmias in anaesthetized rats

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1 CP-060*S* is a novel sodium and calcium overload inhibitor, and is also characterized as a calcium channel blocker. As these activities have each been shown independently to ameliorate ischaemia damage in the myocardium, the combination may synergistically exert cardioprotection. In this study, therefore, the protective effect of CP-060*S* against ischaemia- and reperfusion-induced arrhythmia was evaluated in anesthetized rats.

2 Rats were anaesthetized with pentobarbitone, and the left anterior descending coronary artery was occluded for either 5 min with subsequent reperfusion (a reperfusion-induced arrhythmia model) or 30 min without (an ischaemia-induced arrhythmia model). All drugs were intravenously administered 1 min before the onset of occlusion.

3 In the reperfusion-induced arrhythmia model, the animals in the vehicle-treated group exhibited ventricular tachycardia (VT) in 100%, ventricular fibrillation (VF) in 89%, and death caused by sustained VF in 56%. CP-060*S* (30–300 μg kg^{-1}) dose-dependently suppressed the incidences of arrhythmias. Significant decreases occurred at 100 μg kg^{-1} in VF (incidence: 42%) and mortality (8%), and at 300 μg kg^{-1} in VT (50%), VF (33%) and mortality (8%). This protective effect of CP-060*S* was 10 times more potent than that of a pure calcium channel blocker, diltiazem (30–1000 μg kg^{-1}) we tested, in terms of effective dose ranges. As both drugs decreased myocardial oxygen consumption estimated by rate-pressure product to a similar extent, the calcium channel blocking activity of CP-060*S* would not seem to be sufficient to explain its potency.

4 In the same model, co-administration of ineffective doses of diltiazem (300 μg kg^{-1}) and a sodium and calcium overload inhibitor, R56865 (100 μg kg^{-1}), produced significant suppression of VT (incidence: 62%), VF (46%) and mortality (8%). By contrast, co-administration of R56865 at the same dose with CP-060*S* (300 μg kg^{-1}) did not add to the effect of a single treatment of CP-060*S*.

5 In the ischaemia-induced arrhythmia model, CP-060*S* (300 μg kg^{-1}) significantly decreased the incidence of VF from 75% to 29%, whereas diltiazem (1 mg kg^{-1}) was ineffective.

6 These results suggest that CP-060*S* inhibits both ischaemia- and reperfusion-induced arrhythmia. The combination of the calcium channel blocking effect and the calcium overload inhibition was hypothesized to contribute to these potently protective effects.

Keywords: CP-060*S*; diltiazem; R56865; ischaemia; reperfusion; arrhythmia; calcium overload; calcium channel blocker

Introduction

CP-060*S*, (−)-(***S***)-2-[3,5-bis(1,1-dimethylethyl)-4-hydroxyphenyl]-3-[3-[*N*-methyl-*N*-[2-(3,4-methylenedioxyphenoxy)ethyl]amino]propyl]-1,3-thiazolidin-4-one hydrogen fumarate (Figure 1), is a novel cardioprotective drug synthesized in our laboratory, which was intentionally designed to induce vasorelaxation and prevent calcium overload in the myocardium. It causes vasorelaxation by acting as an L-type calcium channel blocker (Tamura *et al*., 1996). Since calcium channel blockers such as diltiazem can reduce myocardial oxygen consumption, leading to energy sparing (energy sparing effects) by decreasing heart rate, cardiac contractility and afterload (Nayler *et al*., 1987; Buser *et al*., 1991), they are protective against ischaemia and reperfusion-induced myocardial insults (Thandroyen, 1982; Van Gilst *et al*., 1986; Tosaki *et al*., 1987; Vatner *et al*., 1988; Swies *et al*., 1990). Another activity of CP-060*S* is a calcium overload inhibition, which was confirmed by inhibition of the increase in cytoplasmic free calcium concentration and subsequent shape changes in cardiomyocytes induced by veratridine (Tamura *et al*., 1996). In the same study, diltiazem was ineffective within the tested concentration range in terms of calcium channel blocking activity, but a sodium and calcium inhibitor, R56865 (Donck *et al*., 1993) (Figure 1), exhibited an inhibition similar to CP-060*S*. These observations suggest that CP-060*S* and R56865 share the common property of calcium overload inhibition. Recently, calcium overload inhibition in the myocardium has attracted a great deal of attention because several studies have suggested that it can ameliorate ischaemia and reperfusion-induced myocardial damage in rats, rabbits and pigs (Garner *et al*., 1990; Verscheure *et al*., 1995; Klein *et al*., 1995). In view of its properties of calcium channel blocking effect and calcium overload inhibition, it is our hypothesis that CP-060*S* may ameliorate ischaemia- and reperfusion-induced myocardial damage.

Reperfusion following a relatively short period of myocardial ischaemia elicits arrhythmia, often causing irreversible ventricular fibrillation. A major part of this reperfusion-induced arrhythmia is supposed to be the result of the heterogeneity of damage and recovery in cardiomyocytes during ischaemia and reperfusion, which might lead to re-entry processes (Manning & Hearse, 1984). Behind the

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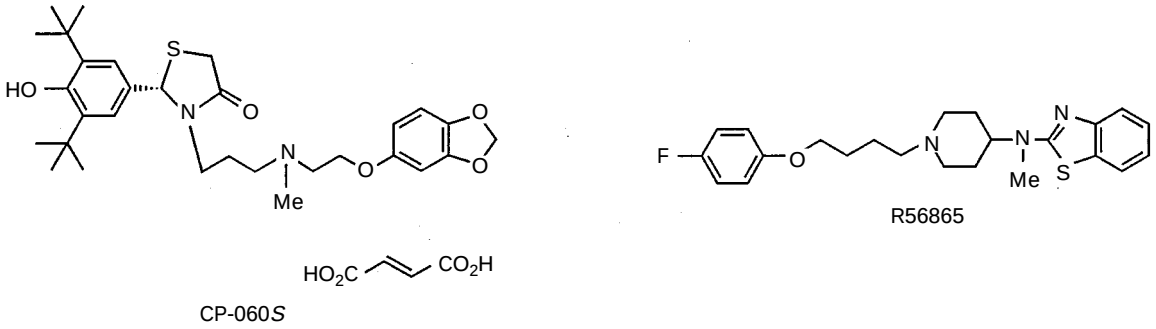

Figure 1 Chemical structures of CP-060*S*, (−)-(***S***)-2-[3,5-bis(1,1-dimethylethyl)-4-hydroxyphenyl]-3-[3-[*N*-methyl-*N*-[2-(3,4-methylenedioxyphenoxy)ethyl]amino]propyl]-1,3-thiazolidin-4-one hydrogen fumarate, and R56865, *N*-[1-[4-(4-fluorophenoxy)butyl]-4-piperidinyl]-*N*-methyl-2-benzothiazolamine.

myocardial damage elicited by ischaemia and reperfusion, calcium overload in cardiomyocytes has been suggested to play a crucial role (Tani & Neely, 1989; Steenbergen *et al*., 1990). Excessive intracellular calcium could be detrimental because it can lead to excessive myofilament activation on reperfusion, activation of proteases which could act to destroy critical intracellular structure, and/or impairment of mitochondrial function (Silverman & Stern, 1994). Therefore, the prevention of intracellular calcium overload with resultant reduction in myocardial damage during ischaemia is a potential way to ameliorate the arrhythmia which occurs at subsequent reperfusion.

In this study, therefore, we evaluated the action of CP-060*S* on ischaemia- and reperfusion-induced arrhythmias in anaesthetized rats in comparison with diltiazem and R56865. To clarify the inhibition mechanism of CP-060*S*, combined administration with R56865 was also attempted.

Methods

Animals

Male Sprague-Dawley rats (280–400 g body weight, Charles River Japan Inc.) were used for all studies.

General procedures

Animals were anaesthetized intraperitoneally with sodium pentobarbitone (50 mg kg^{-1}) and their tracheae intubated for ventilation with room air (10 ml kg^{-1}, 60 strokes min^{-1}). Immediately afterwards, the right femoral vein and artery were cannulated for drug administration and continuous blood pressure monitoring, respectively. Rate-pressure product (RPP), a relative index of myocardial oxygen consumption (Gobel *et al*., 1978), was calculated as the product of systolic blood pressure and heart rate. Anaesthesia was maintained with an intravenous infusion of sodium pentobarbitone (30 mg kg^{-1} h^{-1}). The electrocardiogram (ECG) was continuously recorded with standard limb leads on a Nihon-Kohden continuous ECG recorder (RAG-1100); temporal highspeed recordings for detailed analysis were made with a Nihon-Kohden ECG recorder (Cardiofax V). Rectal body temperature was continuously monitored with a thermometer (TX-100, Shinsei-Rika, Japan) and maintained at 37–38°C with a heating lamp. After left lateral thoracotomy and pericardiectomy, a ligature was placed around the left anterior descending coronary artery (LAD) at a position proximal to a point at one-third the longitudinal axis from the base of the heart, then both ends of the thread passed through a small plastic tube to form a snare around the vessel. After at least 20 min, to allow for a haemodynamic stability, the LAD was occluded by tightening the snare and the thread clamped firmly. Ischaemia was confirmed by change in cardiac colour and elevation in the ST segment of ECG. Following 5 min of occlusion, the clamp was released to allow reperfusion of the ischaemic area for 10 min. Reperfusion was only carried out if the heart was in normal sinus rhythm during the last 5 s of the ischaemic period; otherwise the experiment was terminated. When experiments for sustained ischaemia were performed, the reperfusion produced was omitted. Administration of vehicle or drugs was started 2 min before occlusion as an intravenous bolus for 1 min. When all the experimental procedures were completed, i.e.10 min after reperfusion or 30 min after ischaemia, the LAD was again occluded and blue dye injected intravenously, then the occlusion released and subsequent blue dye injection given in order to confirm visually both ischaemia and reperfusion. Preliminary studies showed that when the ischaemia area covered the apex, it resulted in approximately 40% of the total weight of the left ventricle. When the ischaemia area did not cover the apex, or reperfusion was incomplete, the animals were excluded from the following data analysis. As a result, 21 out of 221 animals were excluded for this reason.

Ventricular arrhythmias were defined and quantified in accordance with the Lambeth Conventions (Walker *et al*., 1988) in which ventricular premature beats (VPBs) are discrete and identifiable premature QRS complexes: a salvo is two or three consecutive VPBs; ventricular tachycardia (VT) is a run of four or more consecutive VPBs; and, ventricular fibrillation (VF) is a signal for which individual QRS deflection can no longer be distinguished from one another, and for which a rate can no longer be measured. VF was considered to occur when gross morphological irregularity of repetitive ectopic complexes was observed for a duration greater than six normal cardiac cycles.

Statistical analysis

Data are expressed as percentages of the incidence of arrhythmia, or mean ± s.e.mean. With regard to haemodynamic parameters and the time to arrhythmic onset, each treated group was compared with a vehicle control by use of Dunnett's test. The Tukey test was used for comparison of CP-060*S* vs diltiazem at the same dose, or single drug treatment vs that of combined treatment. For binomially distributed variables (incidences of arrhythmia), an overall χ^2 test for a $2 \times n$ table was constructed, followed by 2×2 χ^2 tests with

Yates correction to compare individual groups. A difference was considered significant at a level of $P<0.05$.

Drugs

CP-060*S*, (−)-(***S***)-2-[3,5-bis(1,1-dimethylethyl)-4-hydroxyphenyl]-3-[3-[*N*-methyl-*N*-[2-(3,4-methylenedioxyphenoxy) ethyl] amino]propyl]-1,3-thiazolidin-4-one hydrogen fumarate, and R56865 were synthesized in our laboratory. Diltiazem was purchased from Sigma Chemical Co. (U.S.A.). CP-060*S* and R56865 were dissolved with heating and sonication in 0.01 N HCl at 2 and 3.3 mg ml^{-1}, respectively, then individually diluted with 0.9% saline. Diltiazem was dissolved at 6.7 mg ml^{-1} in the same manner but without heating or sonication. For combined administration, CP-060*S* or diltiazem was dissolved in a R56865 solution, then further diluted; the resultant solution always exceeded pH 5.

Results

Haemodynamic changes during 5 min ischaemia

Before administration, no significant difference was detected in heart rate, systolic or mean blood pressure, or RPP between any groups (Table 1). In the vehicle-treated group, a transient decrease in blood pressure was observed immediately after the coronary occlusion, resulting in RPP decrease, but recovering within 5 min ($n=18$, Table 1). Maximal changes in heart rate and mean blood pressure after CP-060*S* or diltiazem administration are shown in Figure 2. Compared to the vehicle control, CP-060*S* treatment (30–300 $\mu g\ kg^{-1}$) did not affect heart rate but decreased mean blood pressure and RPP significantly in a dose-dependent manner ($n=10-12$, Figure 2, Table 1); these decreases were transient, reaching a maximum within 1 min of administration and virtually recovering to the vehicle control levels within 1 min after occlusion. Although in two points in the CP-060*S* treated group, i.e., 30 $\mu g\ kg^{-1}$ at 1 min and 100 $\mu g\ kg^{-1}$ at 3 min, significant increases in RPP were detected, these increases were inconsistent and not dose-dependent (Table 1). The ED_{30} value of CP-060*S* for mean blood pressure (a dose which can decrease mean blood pressure by 30%) determined from the dose-response curve shown in Figure 2 was 74 $\mu g\ kg^{-1}$.

Diltiazem up to 300 $\mu g\ kg^{-1}$ did not affect heart rate, whereas, 1 mg kg^{-1} transiently decreased it, but recovery was seen within 5 min after administration ($n=10-12$, Figure 2). Compared with CP-060*S*, diltiazem showed a similar dose-dependent reduction of mean blood pressure and RPP but was slightly less potent at up to 300 $\mu g\ kg^{-1}$, but recovery for both compounds was similar and there was no difference from control at 1 min after occlusion (Figure 2 and Table 1). In

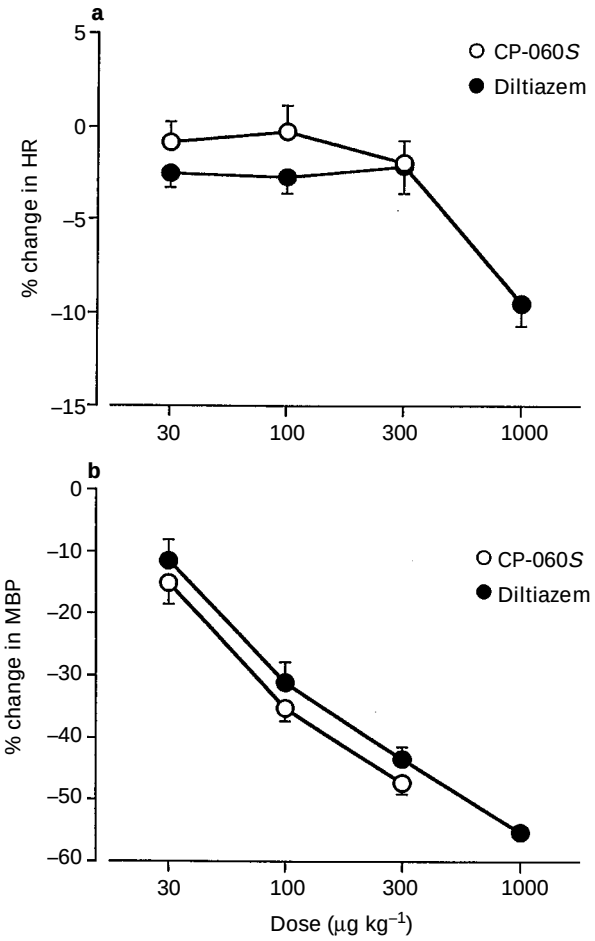

Figure 2 Dose-dependent maximal changes in heart rate (HR) (a) and mean blood pressure (MBP) (b) after the administration of CP-060*S* or diltiazem. Points show mean and vertical lines s.e.mean. The number in each group, and the absolute values of HR and MBP before the administrations, are shown in Table 1.

Table 1 Changes in rate-pressure products during 5 min coronary artery occlusion in rats administered CP-060*S* or diltiazem, intravenously

Drug	*Dose* ($\mu g\ kg^{-1}$)	n	*Before administration (HR, SBP, MBP)*	*Immediately before occlusion*	*Time after occlusion* 1 min	3 min	5 min
Vehicle		18	545±21 (406±8, 134±4, 103±3)	585±23	467±23	504±23	528±23
CP-060*S*	30	10	594±32 (422±9, 140±6, 106±5)	599±18	559±24*	572±27	567±33
	100	12	563±25 (417±7, 135±5, 108±4)	479±17**	530±19	585±11*	571±21
	300	12	557±33 (419±11, 132±5, 104±4)	379±21**	461±20	567±21	562±19
Diltiazem	30	10	564±33 (426±8, 132±7, 105±6)	537±31	516±29	554±20	576±28
	100	10	588±32 (410±11, 143±5, 109±4)	518±23	540±33	599±30*	594±28
	300	11	564±24 (415±6, 136±5, 104±4)	445±25**	501±22	563±32	573±27
	1000	12	578±23 (416±12, 139±3, 107±4)	290±7**	350±12**	443±12	482±12

Values are mean±s.e.mean. HR: heart rate (beats min^{-1}), SBP: systolic blood pressure (mmHg), MBP: mean blood pressure (mmHg) Rate-pressure product (mmHg min^{-1} 100^{-1}) = systolic blood pressure × heart rate/100. *$P<0.05$, **$P<0.01$ vs vehicle control. Before administration, no significant difference was found in the parameters between the groups. No significant difference in RPP was detected between CP-060*S* and diltiazem at the same dose at any time point.

addition, no significant difference in RPP was detected between CP-060*S* and diltiazem at the same dose at any time point (Table 1). At 1 mg kg^{-1}, diltiazem further reduced mean blood pressure and RPP, and this was slow to recover (Figure 2 and Table 1). The calculated ED_{30} value of diltiazem for mean blood pressure was 95 μg kg^{-1}, which was comparable with CP-060*S*, but still 1.3 times less.

Reperfusion-induced arrhythmia

Following 5 min ischaemia and subsequent reperfusion in the vehicle control group, the percentage of animals presenting with the following symptoms was: VPBs and salvos, 100%; VT, 100%; VF, 89%; deaths caused by irreversible VF, 56% ($n=18$, Figure 3). Arrhythmia was observed between 5 and 10 s after the reperfusion and disappeared within 2 min, unless VF was sustained.

CP-060*S* dose-dependently (30–300 μg kg^{-1}) decreased arrhythmia in terms of the percentage of animals afflicted as follows: at 100 μg kg^{-1}, VF and mortality were 42% ($P<0.05$) and 8% ($P<0.05$), respectively, compared to control; at 300 μg kg^{-1}, VPBs and salvos, VT, VF and mortality were 67% ($P<0.05$), 50% ($P<0.01$), 33% ($P<0.01$) and 8% ($P<0.05$), respectively ($n=10-12$, Figure 3). Higher doses could not be assessed because of the poor solubility of CP-060*S*.

In contrast, despite comparable effects with CP-060*S* on haemodynamic parameters, diltiazem failed to inhibit arrhythmia at doses up to 300 μg kg^{-1} ($P>0.05$, $n=10-11$, Figure 3), indicating that diltiazem is much less potent at inhibiting the reperfusion-induced arrhythmias then CP-060*S*. At 1 mg kg^{-1}, diltiazem showed marked suppression of VPBs and salvos (50% incidence), VT (33%), VF (0%) and mortality (0%) (all $P<0.01$, $n=10-12$, Figure 3).

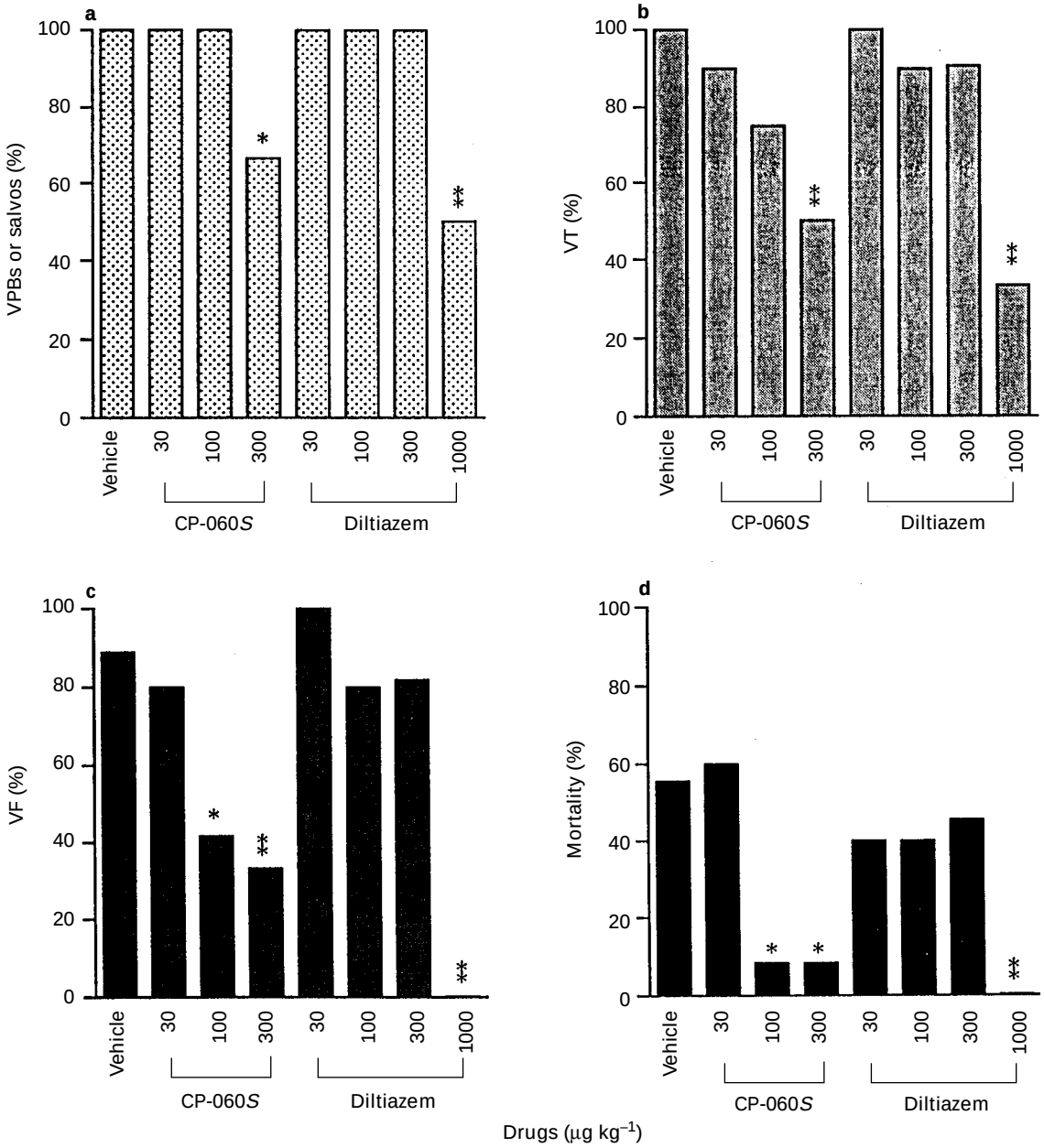

Figure 3 Effects of CP-060*S* or diltiazem on reperfusion-induced arrhythmias. (a) Incidence of ventricular premature beats (VPBs) or salvos. (b) Incidence of ventricular tachycardia (VT). (c) Incidence of ventricular fibrillation (VF). (d) Mortality. Rats were subjected to 5 min coronary artery occlusion and 10 min reperfusion. Administration of vehicle or drugs was started 2 min before occlusion as intravenous bolus for 1 min. $*P<0.05$; $**P<0.01$ compared with vehicle control. Numbers of each group are shown in Table 1.

R56865 at 500 μg kg^{-1} significantly decreased the incidences of VT, VF and mortality to 58% ($P<0.05$), 42% ($P<0.05$) and 0% ($P<0.01$), respectively ($n=12$, Figure 4). At 100 μg kg^{-1} it was ineffective ($P>0.05$, $n=12$, Figure 4). As shown by Garner *et al*. (1990), R56865 at 500 μg kg^{-1} slightly reduced the heart rate, resulting in decrease in RPP, but this was not significantly different from vehicle control (Table 2).

Combination of CP-060S or diltiazem with R56865

In order to clarify whether the potent inhibition of reperfusion-induced arrhythmia by CP-060*S* involved a common mechanism with R56865, combined administration of CP-060*S* or diltiazem with R56865 was attempted. Before administration, the heart rate, systolic and mean blood pressure, and RPP were not significantly different between the treatment groups (Table 2). A combination of 300 μg kg^{-1} CP-060*S*, with 100 μg kg^{-1} R56865 had no further additive effects to the single treatment ($n=12$, Figure 4 and Table 2). In contrast and compared to the vehicle control, 300 μg kg^{-1} diltiazem with 100 μg kg^{-1} R56865 significantly reduced the incidences of VT, VF and mortality to 62%, 46% and 8%, respectively (all $P<0.05$, $n=13$), whereas a single same dose of either compound had little or no effect ($P>0.05$, $n=11-12$, Figure 4). R56865, in combination with the maximal dose of diltiazem at 1 mg kg^{-1} also tended additionally to suppress arrhythmias compared to a single administration of diltiazem (both $n=12$,

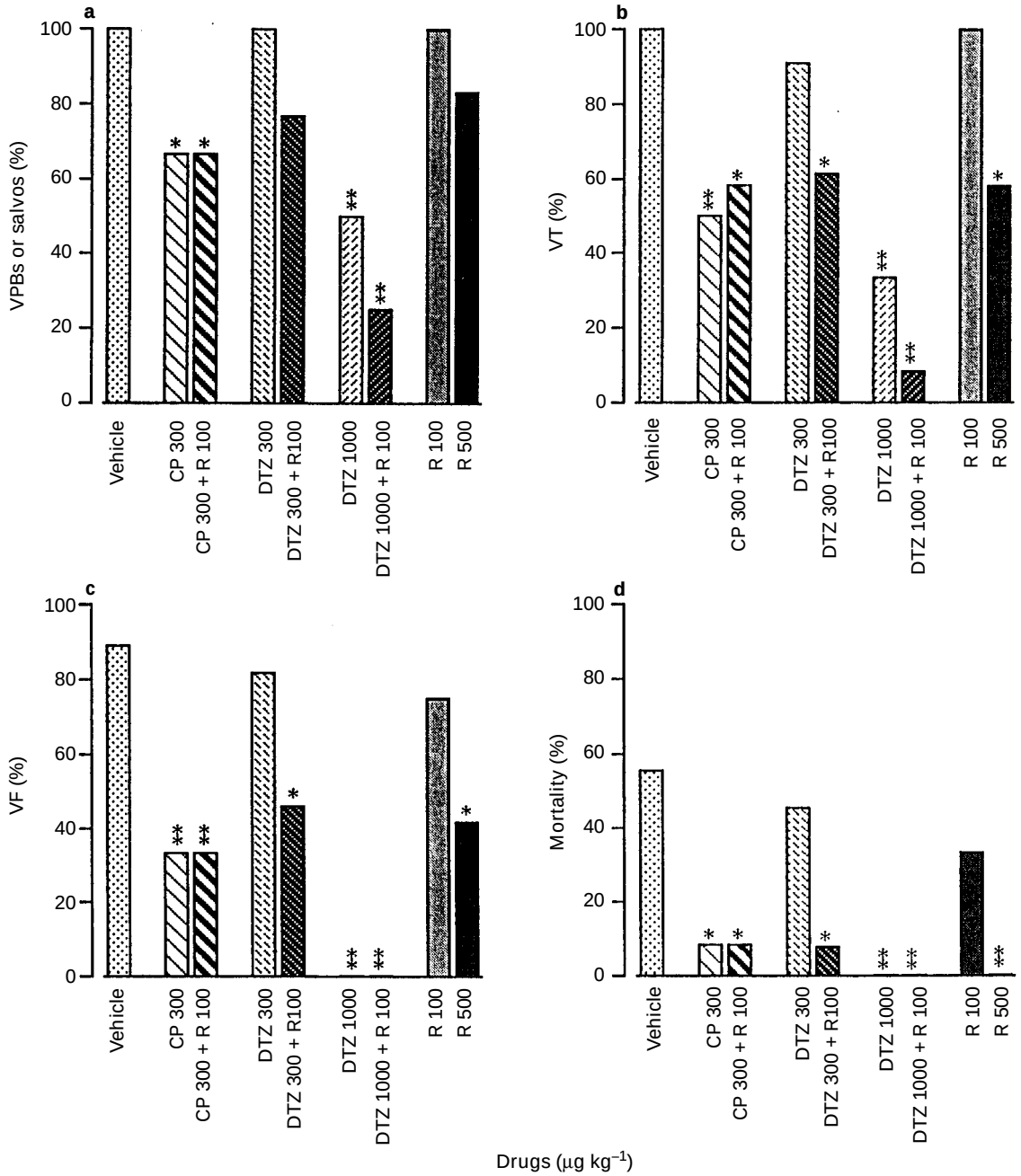

Figure 4 Effects of combination of CP-060*S* (CP) or diltiazem (DTZ) with R56865 (R) on reperfusion-induced arrhythmias. (a) Incidence of ventricular premature beats (VPBs) or salvos. (b) Incidence of ventricular tachycardia (VT). (c) Incidence of ventricular fibrillation (VF). (d) Mortality. Rats were subjected to 5 min coronary artery occlusion and 10 min reperfusion. Administration of vehicle or drugs was started 2 min before occlusion as intravenous bolus for 1 min. *$P<0.05$; **$P<0.01$ compared with vehicle control. Numbers of each group are shown in Table 2.

Figure 4). In any case, combined administration of R56865 did not affect RPP in the single treatment groups (Table 2).

Thirty minutes ischaemia-induced arrhythmia

Before administration, no significant difference was detected in heart rate, systolic or mean blood pressure, or RPP between any groups (Table 3). For the vehicle control group, continuous occlusion for 30 min caused: VPBs or salvos in all animals; 94% had VT; 75%, VF; and death, caused by sustained VF, occurred in 19% ($n=16$, Figure 5). Arrhythmias were observed between 4 and 20 min after the onset of occlusion. CP-060*S* at 300 μg kg^{-1} significantly decreased the incidence of VF to 29% ($P<0.05$, $n=14$) and insignificantly delayed the time to onset of VT and VF ($P>0.05$, $n=10$ and 4, respectively, Figure 5). In contrast, diltiazem at 1 mg kg^{-1} was ineffective on VT and mortality and exhibited only a slight, insignificant decrease in VF. Diltiazem delayed the time to onset of VT significantly ($P<0.05$, $n=12$, Figure 5). Both CP-060*S* and diltiazem significantly decreased RPP to a similar extent, which recovered to the vehicle control level at the end of 30 min of ischaemia (Table 3).

Discussion

The data presented in this study revealed the protective effect of CP-060*S* against both ischaemia- and reperfusion-induced arrhythmias. Since CP-060*S* has a dual activity, i.e., sodium and calcium overload inhibition and calcium channel blocking effect, and that the two forms of cardioprotection occur independently, it is reasonable to hypothesize that the potent protection exhibited by CP-060*S* involves to some degree the two actions simultaneously.

A number of studies have demonstrated the protective effects of calcium channel blockers against ischaemia and reperfusion-induced arrhythmias in various animal species (Thandroyen, 1982; Van Gilst *et al*., 1986; Tosaki *et al*., 1987; Vatner *et al*., 1988; Swies *et al*., 1990). Several underlying mechanisms have been proposed for this protection: inhibition of platelet aggregation and thromboxane A_2 release (Swies *et al*., 1990), reduction in noradrenaline overflow (Van Gilst *et al*., 1986), and increase in blood flow in the ischaemic zone (Vatner *et al*., 1988). However, the main mechanism of action for calcium channel blockers is generally accepted to involve their energy sparing effects, because primarily their effects work through decreases in heart rate, cardiac contractility and afterload (Nayler *et al*., 1987; Buser *et al*., 1991).

In this study, diltiazem at 1 mg kg^{-1} considerably reduced RPP, and this was the only and highest dose shown to suppress reperfusion-induced arrhythmias. This suggests that the energy sparing effect may contribute to the protective effect of diltiazem. By contrast, while the effects of CP-060*S* on haemodynamic parameters were comparable with diltiazem, the antiarrhythmic effect was almost ten times more potent. If it were supposed that CP-060*S* inhibited the arrhythmias

Table 2 Changes in rate-pressure products during coronary artery occlusion in rats administered CP-060*S* or diltiazem, in combination with R56865, intravenously

Drug	*Dose* (μg kg^{-1})	n	*Before administration* (*HR, SBP, MBP*)	*Immediately before occlusion*	*Time after occlusion* 1 min	3 min	5 min
Vehicle		18	545±21 (406±8, 134±4, 103±3)	585±23	467±23	504±23	528±23
CP-060*S*	300	12	557±33 (419±11, 132±5, 104±4)	379±21**	461±20	567±21	562±19
CP-060*S*+R56865	300+100	12	584±26 (417±8, 140±5, 102±5)	430±21**	506±17	546±13	515±19
Diltiazem	300	11	564±24 (415±6, 136±5, 104±4)	445±25**	501±22	563±32	573±27
Diltiazem+R56865	300+100	13	551±26 (402±7, 136±5, 104±4)	413±31**	486±22	547±16	529±19
Diltiazem	1000	12	578±23 (416±12, 139±3, 107±4)	290±7**	350±12**	443±12	482±12
Diltiazem+R56865	1000+100	12	581±40 (402±10, 144±7, 109±6)	257±19**	328±23**	421±26	449±28
R56865	100	12	561±29 (410±10, 137±5, 105±4)	553±29	508±25	555±32	568±33
	500	12	544±25 (404±8, 134±4, 105±4)	488±21	444±24	447±22	439±19

Values are mean±s.e.mean. HR: heart rate (beats min^{-1}), SBP: systolic blood pressure (mmHg), MBP: mean blood pressure (mmHG) Rate-pressure product (mmHg min^{-1} 100^{-1})=systolic blood pressure×heart rate/100. *$P<0.05$, **$P<0.01$ vs vehicle control. Before administration, no significant difference was found in the parameters between the groups. No significant difference in RPP was detected between single drug treatment and that of combined treatment.

Table 3 Changes in rate-pressure products during 30 min coronary artery occlusion in rats administered CP-060*S* or diltiazem, intravenously

Drug	*Dose* (μg kg^{-1})	*Before administration* (*HR, SBP, MBP*)	*Immediately before occlusion*	*30 min after occlusion*
Vehicle		608±32 (442±10, 137±5, 108±4) (16)	649±29 (16)	628±47 (13)
CP-060*S*	300	606±34 (449±10, 134±6, 107±5) (14)	369±19** (14)	608±25 (13)
Diltiazem	1000	592±30 (448±11, 132±4, 106±4) (14)	303±18** (14)	596±39 (9)

Values are mean±s.e.mean. Numbers of each group are in parentheses. HR: heart rate (beats min^{-1}), SBP: systolic blood pressure (mmHg), MBP: mean blood pressure (mmHg). Rate-pressure product (mmHg min^{-1} 100^{-1})=systolic blood pressure×heart rate/100. **$P<0.01$ vs vehicle control. Before administration, no significant difference was found in the parameters between the groups.

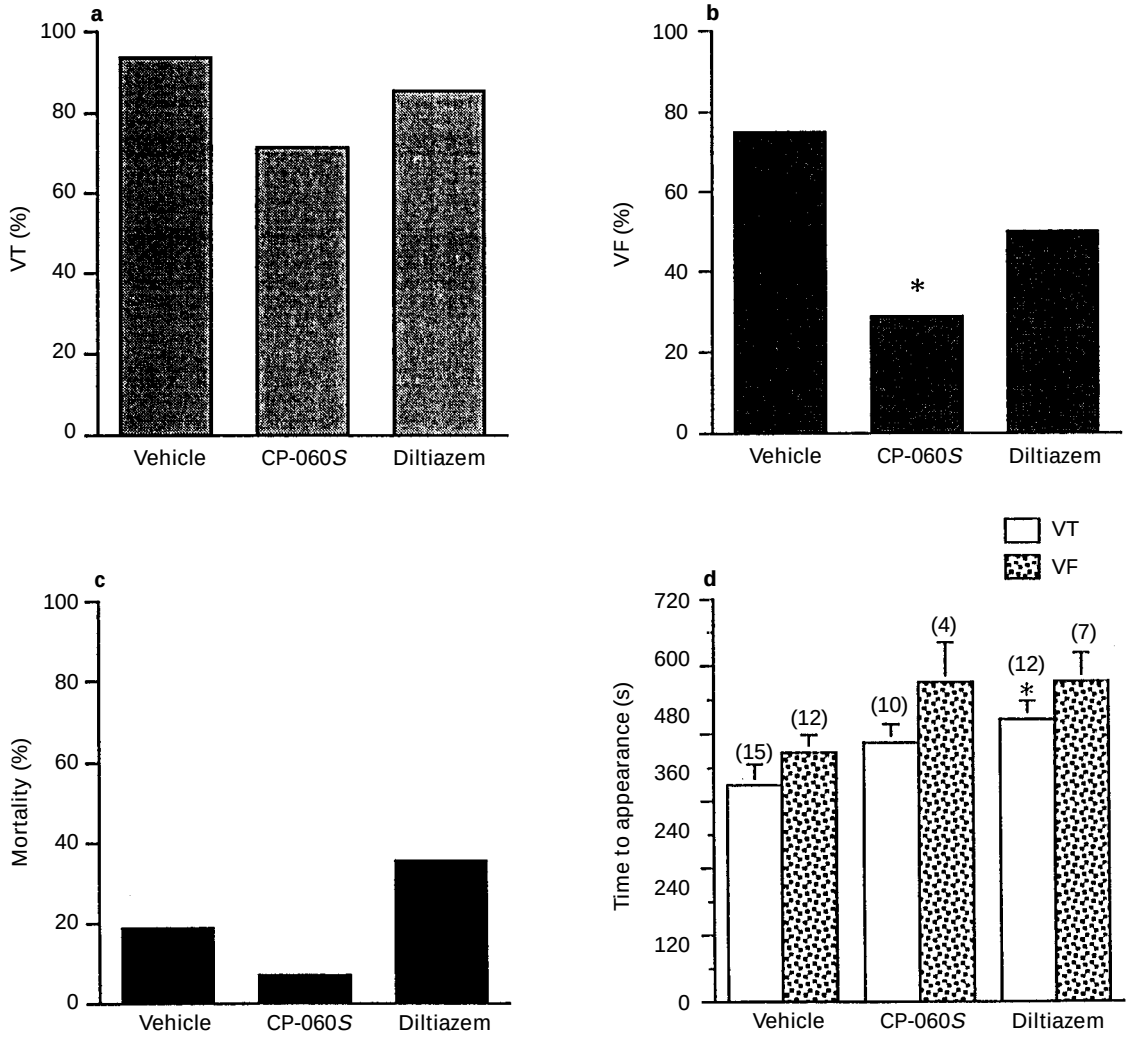

Figure 5 Effects of CP-060*S* (300 μg kg^{-1}) and diltiazem (1000 μg kg^{-1}) on ischaemia-induced arrhythmias. (a) Incidence of ventricular tachycardia (VT). (b) Incidence of ventricular fibrillation (VF). (c) Mortality. (d) Time to appearance of VT or VF from the onset of ischaemia. Rats were subjected to 30 min coronary artey occlusion. Administration of vehicle or drugs was started 2 min before occlusion as intravenous bolus for 1 min. $*P<0.05$ compared with vehicle control. Numbers of each group are shown in Table 3, except in (d) where they are shown in parentheses. Columns in (d) are mean $\pm$ s.e.mean.

through only the energy sparing effect, its effective dose should have been much higher than in the present results. This discrepancy seems irresolvable unless some additional therapeutic effect in CP-060*S* exists which is lacking in diltiazem.

In the heart subjected to ischaemia and subsequent reperfusion, calcium overload in cardiomyocytes causes arrhythmia, mechanical dysfunction, and eventually cell death (Tani & Neely, 1989; Steenbergen *et al*., 1990). The transsarcolemmal routes of this excess calcium influx have not been fully elucidated, but the L-type calcium channel or impairment of calcium ATPase have been excluded (Nalyer *et al*., 1987; Donck *et al*., 1993). Instead, studies show that the calcium overload is triggered by an early rise in intracellular sodium concentration during ischaemia and a further rise upon reperfusion (Tani & Neely, 1989; Donck *et al*., 1993). The increased intracellular sodium concentration has been proposed to increase calcium influx via stimulation of the sodium-calcium exchanger, leading to calcium overload (Tani & Neely, 1989). One of the possible events leading to this rise in sodium involves tetrodotoxin-sensitive non-inactivating sodium channels (Verdonck *et al*., 1991; Silverman & Stern, 1994). In fact, impaired sodium channel inactivation in depolarized cardiac cells, leading to excessive sodium influx, has been observed in association with substances which may be released during ischaemia and reperfusion (Bhatnagar *et al*., 1990; Burnashev *et al*., 1991; Undrovinas *et al*., 1992). In addition, recently, Ju *et al*. (1996) indicated that hypoxia increased the probability of tetrodotoxin-sensitive, inactivation-resistant sodium channels being open in rat ventricular myocytes. By blocking this channel with a sodium and calcium overload inhibitor, R56865, cardioprotection against reperfusion-induced injury can be achieved (Donck *et al*., 1993). R56865 has been shown to suppress reperfusion-induced arrhythmia in anaesthetized rats (Garner *et al*., 1990) and rabbits (Verscheure *et al*., 1995), and to decrease reperfusion-induced myocardial necrosis in pigs (Klein 1995). These observations suggest that blocking tetrodotoxin-sensitive non-inactivating sodium channels can reduce excess sodium influx and subsequent calcium overload in cardiomyocytes subjected to ischaemia and reperfusion, potentially resulting in cardioprotection.

Apart from its calcium channel blocking effect, CP-060*S* has some similarities with factors which inhibit calcium overload. Firstly, CP-060*S*, as well as R56865, inhibit increases in the free cytoplasmic calcium concentration and subsequent shape

changes in cardiomyocytes induced by veratridine (Tamura *et al*., 1996). As veratridine slows inactivation of the sodium current and increases the probability of the sodium channel being open (Sunami *et al*., 1993), the veratridine-induced sodium overload and subsequent increase in intracellular calcium concentration is a model of ischaemic calcium overload (Donck & Borgers, 1991). Secondly, the inhibition of both drugs on calcium overload in this myocyte model may involve the blockade of veratridine-induced tetrodotoxin-sensitive non-inactivating sodium current (Donck & Borgers, 1991; Fukazawa *et al*., 1997). As diltiazem was ineffective in concentration ranges sufficient to block the calcium channel in this myocyte model, the calcium channel blocking effect of CP-060*S* may not be involved in its inhibition of the veratridine-induced sodium and calcium overload (Tamura *et al*., 1996). Thirdly, although various class I antiarrhythmic agents can attenuate arrhythmia or injury of the heart following ischaemia and reperfusion (Winslow *et al*., 1983; Curtis *et al*., 1987; Takeo *et al*., 1995), neither R56865 nor CP-060*S* is categorized as a class I antiarrhythmic agent, since they do not change dV/dt_{max} of the action potential in cardiac cells or tissues under physiological conditions (Carmeliet & Tytgat, 1991; Fukazawa *et al*., 1997). In view of these similarities, it seems highly likely that CP-060*S* exerts its potently protective effects on reperfusion-induced arrhythmia in a manner similar to R56865, in addition to the calcium channel blocking effect.

In order to investigate this hypothesis, we attempted the combined administration of CP-060*S*, or diltiazem, with R56865. The combination of CP-060*S* with R56865 did not elicit additional protection against arrhythmias. On the other hand, the combination of diltiazem with R56865 elicited additional protection both at lower, ineffective and higher, strongly effective doses. These results suggest that in addition to its calcium channel blocking effect, CP-060*S* shares a common mechanism of protection with R56865 against reperfusion-induced arrhythmia. Therefore, the combination of calcium channel blocking effect and sodium calcium overload inhibition can elicit synergistic amelioration of reperfusion-induced arrhythmias, and this combination can be achieved with a single treatment of CP-060*S*.

Adding to the suppression of reperfusion-induced arrhythmias, CP-060*S* also reduced the arrhythmias which occurred during sustained ischaemia. In contrast, diltiazem failed to affect these arrhythmias despite inducing a hypotension which resulted in a decrease in RPP. The ineffectiveness of diltiazem could be due to the excessive hypotension; hypotension could be detrimental by reducing coronary flow and thus masking the beneficial effect. However, in this study, this may not have been the case because the coronary artery was completely occluded and rats are known to have little coronary collateral circulation (Maxwell *et al*., 1987). Therefore, the effects of diltiazem on coronary flow can be reasonably understood as negligible in this study. As diltiazem delayed the time to onset of arrhythmias, its energy sparing effect may lead to the delayed onset of arrhythmias, but it failed to reduce the incidence. On the other hand, with CP-060*S*, both calcium channel blocking effect and calcium overload inhibition may have contributed to the reduction in arrhythmias, as shown in the case of the reperfusion-induced arrhythmias. This is supported by the finding that R56865 decreased the incidence of arrhythmia during myocardial ischaemia, but without delaying time to appearance, in anaesthetized rats (Garner *et al*., 1990).

The deleterious effects of calcium overload in myocardial cells following ischaemia and reperfusion involve not only arrhythmias, but also myocardial stunning and/or necrosis (Hearse & Bolli, 1992; Opie, 1992). Considering its two fold nature, CP-060*S* may potentially protect hearts from these kinds of damage. Further investigation on this potential is now in progress.

In conclusion, CP-060*S* was shown to inhibit both ischaemia- and reperfusion-induced arrhythmias. The combination of calcium overload inhibition and calcium channel blocking effect of CP-060*S* seems to contribute to these inhibitory effects. Combined intervention via a calcium overload inhibitor together with a calcium channel blocker might turn out to be a new, appropriate intervention in myocardial ischaemia diseases, and CP-060*S* has been shown to achieve this as one sole drug.

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