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The long-lasting anti-anginal effects of CP-060*S* in a rat model of arginine vasopressin-induced myocardial ischaemia

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Abstract

The anti-anginal effect of CP-060*S*, a new cardioprotective agent that prevents myocardial Na^+-, Ca^{2+}-overload and has Ca^{2+}-channel blocking activity, was evaluated in a rat model of arginine^8-vasopressin (AVP)-induced cardiac ischaemia. Infusion of AVP (0.2 IU kg^{-1}) depressed the electrocardiogram (ECG) ST segment, an index of myocardial ischaemia. Vehicle, CP-060*S* and diltiazem were given orally 1, 2, 4, 8, 12 and 24 h before the administration of AVP. CP-060*S*, at 3 mg kg^{-1} and 10 mg kg^{-1}, suppressed AVP-induced ST-segment depression for 2 h and 12 h, respectively. In contrast, diltiazem, at 10 and 30 mg kg^{-1}, suppressed AVP-induced ST-segment depression for only 1 h. The persistent suppression of the AVP-induced ST-segment depression by CP-060*S* correlated with the time course of changes in its plasma concentration. The minimum effective concentration of CP-060*S* was estimated to be 30 ng mL^{-1} ($\cong$ 50 nM), consistent with its vasorelaxant potency in rat isolated aortic strips (concentration producing 50% relaxation of KCl contraction, IC50 = 32.6$\pm$8.3 nM). Intravenously administered CP-060*S*, at 300 μg kg^{-1} and diltiazem at 500 μg kg^{-1} showed similar haemodynamic changes, whereas CP-060*S*, at 300 μg kg^{-1}, significantly suppressed AVP-induced ST-segment depression and diltiazem, at 500 μg kg^{-1}, had no effect on AVP-induced ST-segment depression. In summary, orally administered CP-060*S* exerted a long-lasting anti-anginal effect proportionate to the time course of changes in its plasma concentration in a rat model of AVP-induced ischaemia.

Introduction

Angina pectoris is caused by myocardial ischaemia that results from a decrease in oxygen supply and an increase in oxygen demand (Opie 1991). Ca^{2+}-channel blockers are thought to improve the oxygen supply–demand imbalance by coronary vasodilatation and negative chronotropic and inotropic actions (Triggle 1990).

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, is a new cardioprotective agent that is able to block a non-inactivating Na^+ current without suppressing physiologic Na^+-channel activity, consequently preventing Ca^{2+} overload that is induced by Na^+ overload through the Na^+/Ca^{2+} exchanger in ischaemic cardiac myocytes (Tamura et al 1996; Fukazawa et al 1997). Thus, CP-060*S* can block Na^+-, Ca^{2+}-overload in ischaemic myocytes without blocking the voltage-dependent Na^+ current. CP-060*S* also inhibits L-type Ca^{2+} channels in cardiac and vascular smooth muscle cells (Tamura et al 1996; Fukazawa et al 1997; Ohya et al 1997; Suzuki et al 1998a; Sato et al 2000). CP-060*S*

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has a low affinity for other common ion channels and receptors, such as K^+ channels and arginine^8-vasopressin (AVP) receptors (unpublished data). By the combined effect of preventing the Na^+-, Ca^{2+}-overload and blocking Ca^{2+} channels, CP-060*S* reduces myocardial infarct size in dogs (Suzuki et al 1998b) and inhibits ischaemia- and reperfusion-induced arrhythmias in rats (Koga et al 1998). Moreover, in a model of myocardial pacing-induced ischaemia, which simulates stable effort angina, CP-060*S* also suppresses the pacing-induced ST-segment elevation in dogs by this dual action (Adachi et al 1999). On the other hand, as a result of its Ca^{2+}-channel blockade, CP-060*S* decreases myocardial oxygen consumption in dogs (Suzuki et al 1999) and suppresses the ST-segment changes in a rat model of methacholine-induced vasospastic angina, which simulates variant angina (Fukazawa et al 2001). However, the effects of orally administered CP-060*S* in models of experimental angina remain to be elucidated.

In this study, we compared the anti-anginal effect of orally administered CP-060*S* with that of diltiazem, a Ca^{2+}-channel blocker, in a model of AVP-induced myocardial ischaemia. To consider the probable mechanism of CP-060*S* in this angina model, CP-060S and diltiazem were administered intravenously.

Materials and Methods

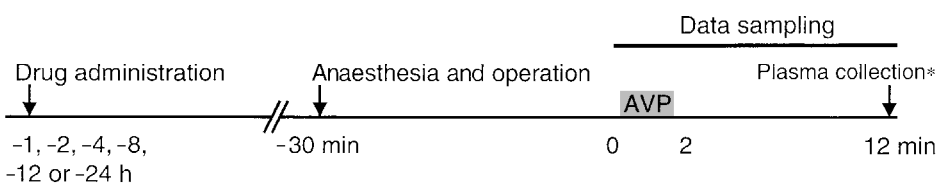
The animals used in this experiment were treated in accordance with Chugai Pharmaceutical's ethical guidelines of animal care, handling and termination.

Rat model of AVP-induced ischaemia

Male Donryu rats (140–270 g, Charles River Japan) were anaesthetized with sodium pentobarbital (60 mg kg^{-1} i.p.). A polyethylene catheter connected to a pressure transducer (DX-300, Nihon Kohden, Tokyo, Japan) was inserted into the right femoral artery to measure arterial blood pressure. Electrocardiograms (ECG) were obtained in standard limb lead II with an electrocardiograph (ECG-8250, Nihon Kohden, Tokyo, Japan). The amplitude of the ST segment, mean arterial blood pressure (MBP) and heart rate (HR) were analysed with an ECG processor (SBP-8, Softoron, Tokyo, Japan), which automatically recorded data every 30 s. The average of 9 sequential beatings of the ECG ST-j point was defined as that of the ST segment. The rate–pressure product (RPP = HR × MBP/100) was calculated as an index of myocardial oxygen consumption. In the oral administration study, rats were fasted overnight. The protocol of the experiment is shown in Figure 1. After each drug was administered, AVP (0.2 IU kg^{-1}) was infused into the femoral vein for 2 min with an infusion pump (FP-W-100, Toyo-Kogyo, Tokyo, Japan). In the intravenous administration study, each drug was injected into the left femoral vein. AVP was infused 5 min after administration of the drugs.

Vasorelaxant experiment in rat aortic strips

Thoracic aortas were removed from male Sprague Dawley rats (360–500 g, Charles River Japan), dissected free from surrounding connective tissue and cut into ring segments about 2–3 mm long. Each strip was mounted



Drug:	Vehicle (distilled water)	1, 2, 4, 8, 12 or 24 h	n = 6
	CP-060*S*		
	1 mg kg^{-1}	1 or 2 h	n = 6 – 7
	3 mg kg^{-1}	1, 2, 4 or 8 h	n = 6 – 8
	10 mg kg^{-1}	1, 2, 4, 8, 12 or 24 h	n = 6 – 8
	Diltiazem		
	10 mg kg^{-1}	1 or 2 h	n = 6
	30 mg kg^{-1}	1, 2, 4 or 8 h	n = 6

Figure 1 Protocol of oral administration study in rats. AVP, arginine vasopressin. *Plasma was collected in the CP-060*S* groups only (n = 2–3).

for isometric tension recording in an organ bath filled with 10 mL of Krebs-Henseleit solution (mM: NaCl, 119.0; KCl, 4.8; $CaCl_2$, 2.5; $MgSO_4$, 1.2; KH_2PO_4, 1.2; $NaHCO_3$, 24.8; glucose, 10.0) bubbled with 95% O_2–5% CO_2 and maintained at pH 7.4 at 37°C. The strips were given a stretched tension of 2 g. Isomeric tension changes were monitored using an isomeric transducer (TB-611T, Nihon Kohden, Tokyo, Japan). After KCl (30 mM)-induced contraction was obtained, CP-060*S* or diltiazem was added in a step-wise manner into the organ bath, and concentration–relaxation curves were obtained.

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, was synthesized at Fuji Gotemba Research Laboratories, Chugai Pharmaceutical Co. (Shizuoka, Japan). AVP and diltiazem were purchased from Sigma (St Louis, MO). CP-060*S* and diltiazem were dissolved in distilled water for the oral administration study, dissolved in 10 mM HCl at 2 mg kg^{-1} and diluted with saline. AVP was dissolved in saline at a concentration of 0.2 IU mL^{-1}. In some rats given CP-060*S* orally, blood samples from the abdominal aortic artery were centrifuged and plasma stored at −20°C. The plasma concentration of CP-060*S* was measured with an HPLC system (LC-6A, Shimadzu, Kyoto, Japan). The detection limit of the CP-060*S* concentration in this system was 5 ng mL^{-1}. The plasma concentration of diltiazem was not measured.

Data analysis

Data are expressed as mean±s.e.m. except plasma concentration data (n = 2 or 3), which are expressed as mean±s.d. Statistical analysis was performed by one-way analysis of variance followed by Dunnett's test. Values of $P < 0.05$ were considered statistically significant.

Results

Model of AVP-induced ischaemia

In the oral administration study, the baseline (just before AVP infusion) ST-segment value was similar in all groups (data not shown). The baseline MBP value in the vehicle group was 97±7 mmHg at 1 h and 98±4 mmHg at 2 h. CP-060*S* at 10 mg kg^{-1} significantly decreased MBP at 1 and 2 h (84±4 mmHg and 87±3 mmHg, $P < 0.05$, respectively). Diltiazem at 30 mg kg^{-1} significantly decreased MBP at 1 h (86±4 mmHg, $P < 0.05$). There were no baseline haemodynamic changes with the other doses and at the other times in the CP-060*S* and diltiazem groups. Maximal ST-segment depression was observed at 1–2 min after AVP infusion, and the ST-segment value returned to baseline levels within 5 min. AVP slightly decreased HR and increased MBP. As a result, the RPP was increased. These parameters returned to baseline levels within 10 min (data not shown). Figure 2 summarizes the effects of CP-060*S* and diltiazem on AVP-induced ST-segment depression. The ST-segment depression induced by AVP after vehicle treatment was similar at all time points. CP-060*S* and diltiazem at 1 h after oral administration suppressed AVP-induced ST-segment depression in a dose-dependent manner. Orally administered CP-060*S*, at 3 mg kg^{-1} and 10 mg kg^{-1}, suppressed AVP-induced ST-segment depression for 2 h and 12 h, respectively (Figure 2A), whereas diltiazem at 10 and 30 mg kg^{-1} suppressed AVP-induced ST-segment depression for only 1 h (Figure 2B). Neither CP-060*S* nor diltiazem affected AVP-induced maximal changes in the RPP (data not shown).

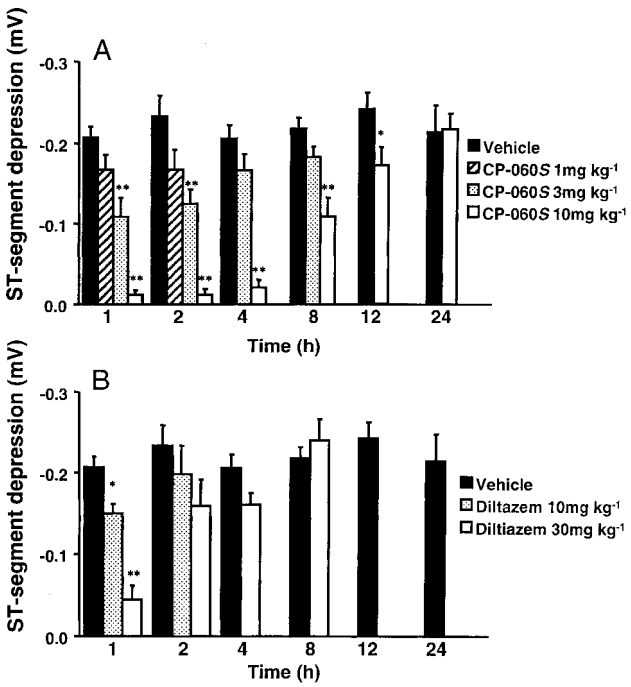


Figure 2 Effects of orally administered CP-060*S* (A) and diltiazem (B) on arginine vasopressin (AVP)-induced ST-segment depression in rats. AVP was infused into the femoral vein at a given time point after oral administration of each drug. Data are expressed as mean±s.e.m. (n = 6–8); $*P < 0.05$, $**P < 0.01$ vs vehicle group.

Table 1 Pre-drug values of ST segment and haemodynamic variables in rats.

Drug	Dose (μg kg^{-1})	ST (1/100 mV)	MBP (mmHg)	HR (beats min^{-1})	RPP	n (mmHg×beats min^{-1}×1/100)
Vehicle		6.8±2.2	105±4	391±15	414±30	7
CP-060*S*	100	3.0±2.2	94±6	380±9	356±25	6
	300	11.4±1.9	110±4	393±8	433±21	6
Diltiazem	300	10.0±1.6	107±5	409±12	439±20	6
	500	12.5±1.0	105±5	392±7	412±22	6
	1000	8.3±2.1	106±4	400±12	424±18	6

Each value represents mean±s.e.m. ST, ST segment; MBP, mean arterial blood pressure; HR, heart rate; RPP, rate–pressure product.

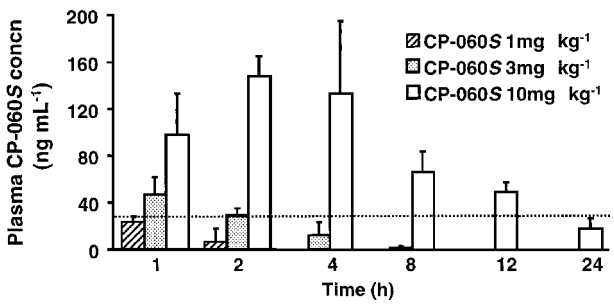


Figure 3 Time course of changes in plasma CP-060*S* concentration after oral administration of CP-060*S* to rats. The dashed line indicates the minimum effective plasma concentration. Data are expressed as mean±s.d. (n = 2–3).

Table 2 Maximal changes in haemodynamic variables after intravenous drug injection in rats.

Drug	Maximal% change			
	Dose (μg kg^{-1})	MBP	HR	RPP
Vehicle		−3±2	−2±1	−4±3
CP-060*S*	100	−17±3**	−3±1	−16±2*
	300	−35±2**	−6±1	−34±3**
Diltiazem	300	−23±2**	−5±2	−25±2**
	500	−37±2**	−6±2	−40±3**
	1000	−48±2**	−9±3**	−52±3**

Each value represents mean±s.e.m. MBP, mean arterial blood pressure; HR, heart rate; RPP, rate–pressure product. *$P < 0.05$, **$P < 0.01$ vs. vehicle group.

Figure 3 shows the time course of the changes in plasma CP-060*S* concentration after oral administration. The persistent suppression by CP-060*S* of the AVP-induced ST-segment depression was coincident with the time course of changes in its plasma concentration. According to the significant suppressive effects of CP-060*S* on AVP-induced ST-segment depression, the minimum effective concentration of CP-060*S* was estimated to be approximately 30 ng mL^{-1} ($\cong$ 50 nM).

In the intravenous administration study, the pre-drug ST-segment and haemodynamic data were similar in all groups (Table 1). CP-060*S* and diltiazem decreased MBP and the RPP in a dose-dependent manner (Table 2). The highest dose of diltiazem (1000 μg kg^{-1}) significantly decreased HR. The RPP returned to baseline levels just before AVP infusion (data not shown). AVP-induced maximal changes in the RPP were similar between groups (data not shown). Intravenously administered CP-060*S* at 300 μg kg^{-1} and diltiazem at 500 μg kg^{-1} caused similar haemodynamic changes (Table 2). Whereas CP-060*S* at 300 μg kg^{-1} significantly suppressed AVP-induced ST-segment depression (Figure 4), diltiazem at 500 μg kg^{-1} had no effect on AVP-induced ST-segment depression. A significant suppression of the ST-segment depression by diltiazem could be only achieved at 1000 μg kg^{-1}, which significantly decreased the RPP more potently than CP-060*S* 300 μg kg^{-1} ($P < 0.05$).

Vasorelaxant experiment in rat aortic strips

CP-060*S* and diltiazem induced concentration-dependent relaxation in rat aortic strips contracted with 30 mM KCl (Figure 5). The IC50 values (concentration producing 50% relaxation of KCl contraction) of CP-060*S* and diltiazem were 32.6±8.3 and 100.4±13.2 nM, respectively. These values were significantly different ($P < 0.001$).

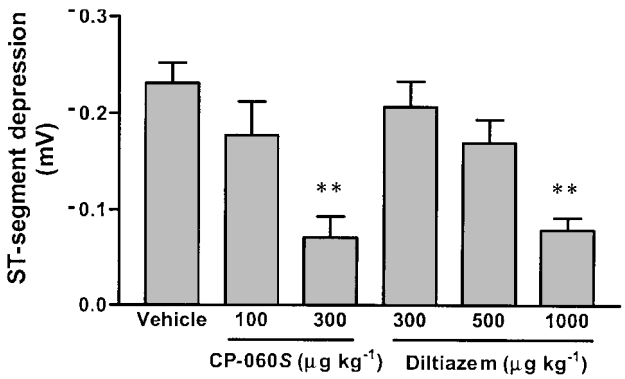


Figure 4 Effects of intravenously administered CP-060*S* and diltiazem on arginine vasopressin (AVP)-induced ST-segment depression in rats. AVP was infused into the femoral vein 10 min after intravenous administration of each drug. Data are expressed as mean±s.e.m. (n = 6–7); $^{**}P < 0.01$ vs vehicle group.

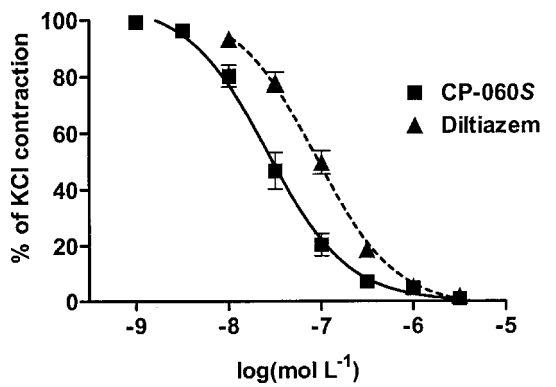


Figure 5 Vasorelaxant effects of CP-060*S* and diltiazem on 30 mM KCl-induced contraction in rat aortic strips. Data are expressed as mean±s.e.m. (n = 8).

Discussion

AVP-induced ST-segment depression has been considered to represent myocardial ischaemia, which is an imbalance between the supply and demand of myocardial oxygen caused by coronary vasoconstriction and afterload elevation (Hiramatsu et al 1970). Vasodilating drugs such as Ca^{2+}-channel blockers, K^+-channel openers and nitric oxide donors have been reported to suppress AVP-induced ST-segment depression (Karasawa et al 1988; Uchida et al 1993; Kita et al 1994; Mori et al 1995; Hirasawa et al 1996; Hirata et al 1998). Therefore, the rat model of AVP-induced myocardial ischaemia is considered to be useful in evaluating the anti-anginal effects of vasodilating drugs. In this study, orally administered CP-060*S* suppressed AVP-induced ST-segment depression more persistently than diltiazem. This persistent suppression by CP-060*S* was closely correlated with the time course of changes in its plasma concentration. Whereas diltiazem has been reported to be eliminated rapidly from the blood of dogs and rats (Piepho et al 1982; Nakamura et al 1987), this difference in duration of action between CP-060*S* and diltiazem seems to be dependent on their pharmacokinetic profiles. The long-lasting anti-anginal effect of CP-060*S* may be beneficial in the treatment of angina pectoris. The effective plasma concentration of CP-060*S* in the AVP-induced angina model was estimated to be more than 30 ng mL^{-1} ($\cong$ 50 nM), which was consistent with its vasorelaxant potency in isolated rat aortic strips (IC50, 32.6±8.3 nM). High doses of CP-060*S* and diltiazem decreased baseline MBP values. These reductions in afterload may be one of the causes of suppressing AVP-induced ST-segment depression for attenuating myocardial ischaemia. However, both drugs, at doses and times that did not reduce afterload, also suppressed AVP-induced ST-segment depression. In addition, neither affected AVP-induced maximal changes in the RPP, an index of myocardial oxygen consumption. These results suggest that CP-060*S* and diltiazem suppress AVP-induced ST-segment depression by the inhibition of AVP-induced coronary artery constriction (i.e. a Ca^{2+}-channel blocking action).

In the intravenous administration study, CP-060*S* at 300 μg kg^{-1} significantly suppressed AVP-induced ST-segment depression. Although diltiazem at 500 μg kg^{-1} exhibited a decrease in the RPP equal to that of CP-060*S* at 300 μg kg^{-1}, diltiazem at 500 μg kg^{-1} did not significantly suppress the AVP-induced ST-segment depression. We reported previously that the Ca^{2+}-channel blocking profile of CP-060*S* in the cardiovascular system in-vivo is qualitatively similar to that of diltiazem (Suzuki et al 1999). Therefore, this discrepancy seems irresolvable, unless CP-060*S* has an additional action that diltiazem does not. CP-060*S* not only has Ca^{2+}-channel blocking activity, but it also prevents Na^+-, Ca^{2+}-overload due to inhibition of the non-inactivating Na^+ current (Tamura et al 1996; Fukazawa et al 1997), which has been proposed to be one of the pathways for Na^+-overload in the ischaemic heart (Ver Donck et al 1993). In fact, CP-060*S* blocked ischaemia-induced myocardial Na^+ accumulation measured by ^{23}Na NMR in the rat Langendorf-perfused heart (Fukuda et al, personal communication). CP-060*S* has this Na^+-, Ca^{2+}-overload blocking effect at the same concentrations as its Ca^{2+}-channel blocking effect (Tamura et al 1996). In a model of myocardial pacing-induced ischaemia, which simulates stable effort angina, CP-060*S* also suppresses the pacing-induced ST-segment elevation in dogs by the

dual action of preventing Na^+-, Ca^{2+}-overload and Ca^{2+}-channel blockade (Adachi et al 1999). Moreover, a Na^+/H^+ exchange inhibitor has been reported to suppress AVP-induced ST-segment depression (Yamamoto et al 2000). Na^+/H^+ exchange has been proposed as another pathway for Na^+-overload (Ver Donck et al 1993). In the clinical GUARDIAN study, the Na^+/H^+ exchange inhibitor cariporide had a favourable effect on patients undergoing coronary artery bypass grafting (Theroux et al 2000). Therefore, the Na^+-, Ca^{2+}-overload blocking effect may contribute to the suppression of the AVP-induced ST-segment elevation. Further studies are required to clarify the mechanisms responsible for the potent anti-anginal effects of CP-060*S* in this model.

In summary, in correlation with the time course of its plasma concentration, orally administered CP-060*S* suppressed AVP-induced ST-segment depression, an index of myocardial ischaemia, more persistently than diltiazem as a result of Ca^{2+}-channel blockade. The minimum effective concentration of CP-060*S* was consistent with its vasorelaxant potency in-vitro. Moreover, the Na^+-, Ca^{2+}-overload-preventing activity of CP-060*S* may also contribute to the anti-anginal effect against coronary vasoconstriction.

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