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Pharmacological profile of semotiadil fumarate, a novel calcium antagonist, in rat experimental angina model

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1 The aim of the present study was to determine whether antianginal efficacy of semotiadil fumarate (SD-3211), a structurally novel calcium antagonist, is distinct from those of diltiazem, nifedipine and nisoldipine.

2 First, the duration of the inhibitory effects of semotiadil was compared with that of other Ca^{2+} antagonists in rat experimental angina evoked by vasopressin. Semotiadil (10 mg kg^{-1}, p.o.) was effective for at least 9 h in the anginal model and those effects of semotiadil were longer-lasting than those of diltiazem (30 mg kg^{-1}, p.o.), nifedipine (10 mg kg^{-1}, p.o.), and nisoldipine (3 mg kg^{-1}, p.o.).

3 Second, the selectivity of actions of these Ca^{2+} antagonists for the coronary arteries and myocardium was evaluated in rat isolated perfused hearts. Diltiazem (10^{-6} M) reduced cardiac contractility without inhibiting the elevation of perfusion pressure evoked by acetylcholine. Semotiadil (10^{-7} M) significantly suppressed cardiac contractility and inhibited the coronary response to acetylcholine. In contrast, nifedipine (3×10^{-9}–3×10^{-8} M) and nisoldipine (3×10^{-10}–10^{-8} M) did not reduce cardiac contractility at concentrations which significantly inhibited the increase in perfusion pressure to acetylcholine.

4 The selectivity of semotiadil for coronary artery and myocardium is intermediate between diltiazem and dihydropyridines tested in the present study.

5 These findings suggest that semotiadil has an advantage of diltiazem, nifedipine, and nisoldipine in the treatment of angina with regard to long-lasting action and selectivity for coronary artery and myocardium.

Keywords: Calcium antagonist; semotiadil; ST-segment; tissue selectivity; angina pectoris

Introduction

Calcium channel antagonists are classified into the following three groups based on the chemical structure, which show different pharmacological and therapeutic properties; (a) 1,4-dihydropyridines, (b) benzothiazepine and (c) phenylalkylamines (Kendall *et al.*, 1987; Opie, 1988). The 1,4-dihydropyridines such as nifedipine and nisoldipine have higher selectivity for blood vessels than cardiac tissues, whereas diltiazem, a benzothiazepine, and phenylalkylamine such as verapamil show lower selectivity for vascular tissues (Taira, 1987; Quartaroli *et al.*, 1991; Triggle, 1991). For these reasons, the dihydropyridines are likely to cause reflex tachycardia in patients with hypertension and angina (Pederson, 1980; Mitchell *et al.*, 1993). Diltiazem and verapamil can induce cardiac suppression including bradycardia and atrioventricular block (Henry, 1980; Mitchell *et al.*, 1982). Furthermore, lack of long-lasting actions of these Ca^{2+} antagonists is unfavourable in the management of patients with angina. Thus, the pharmacological profile of these dihydropyridines and diltiazem still remains to be improved. Semotiadil fumarate (SD-3211), (+)-(R)-2-[5-methoxy-2-[3-[methyl[2-[3,4(methylenedioxy)phenoxy]ethyl]-amino]propoxy]phenyl]-4-methyl-2H-1,4-benzothiazine-3(4H)-one hydrogen fumarate, is a novel Ca^{2+} antagonist with a distinctive chemical structure from dihydropyridines, diltiazem and verapamil (Fujita *et al.*, 1990). The pharmacological profile of semotiadil is also different from those of the earlier Ca^{2+} antagonists. Indeed, the binding site of semotiadil to Ca^{2+} channel differs from those of dihydropyridines, diltiazem and verapamil since semotiadil has a negative allosteric interaction with those of Ca^{2+} antagonists in displacement studies. (Nakayama *et al.*, 1992; 1994). The actions of semotiadil are more selective for cardiac tissues than those of dihydropyridines, and are more selective for vessels than that of diltiazem (Miyawaki *et al.*, 1990; 1991; Nishimura *et al.*, 1990; Takada *et al.*, 1991b). Furthermore, semotiadil shows long-lasting antihypertensive effects in spontaneously hypertensive rats (SHR) and renal hypertensive dogs (Kageyama *et al.*, 1991; Takada *et al.*, 1991a; Kanda & Hashimoto, 1993). Thus, the earlier studies suggest that semotiadil is a new class of Ca^{2+} antagonist. However, the pharmacological profile of semotiadil is not well-established in experimental angina models.

The present study was therefore designed to determine whether antianginal effects of semotiadil are distinct from nifedipine, nisoldipine and diltiazem.

Methods

ST-segment changes caused by vasopressin

Animals Male Donryu rats weighing 280–450 g were used in these experiments. The animals were anaesthetized with sodium pentobarbitone (50 mg kg^{-1}, i.p.); cannulae were inserted into the trachea and right jugular vein for spontaneous respiration and injection of arginine vasopressin (AVP, 0.25 iu kg^{-1}), respectively. Electrocardiograms (lead II) were obtained with an electrocardiograph (Cardiofax ECG6503, Nihon Kohden, Tokyo) and changes in ST-segment to AVP were analyzed with an ECG processor (Version 2.0, Softron, Tokyo). The mean voltage of the electrocardiogram for 13 ms from the peak of the S-wave was defined as the value of the ST-segment.

Experimental protocol The duration of the inhibitory effects of the following dose of Ca^{2+} antagonists were examined on the responses to AVP 1, 3, 6, and 9 h after the administration of the compounds. Semotiadil (1, 3, 10 mg kg^{-1}), diltiazem (3, 10, 30 mg kg^{-1}), nifedipine (1, 3, 10 mg kg^{-1}), nisoldipine (0.3,

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1, 3 mg kg^{-1}), and vehicle (0.5% methylcellulose, 5 ml kg^{-1}) were given orally to the animals. In the cases of semotiadil (10 mg kg^{-1}) and diltiazem (30 mg kg^{-1}), the inhibitory effects of the compounds were also examined 24 h after administration.

Acetylcholine-induced coronary vasocontraction

Preparation of isolated, perfused hearts The hearts were removed from heparinized (250 iu heparin, i.p.) Sprague-Dawley rats weighing 280–430 g under sodium pentobarbitone-anaesthesia (50 mg kg^{-1}, i.p.) and were arrested immediately in ice-cold saline. The hearts were subjected to Langendorff perfusion with Krebs-Henseleit bicarbonate solution (composition, mM: NaCl 118, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 25 and glucose 10). The solution was adjusted to pH 7.4 by gassing with a mixture of 95% O_2 + 5% CO_2, and maintained at 37°C. The hearts were perfused at a constant flow rate of 9.5 ml min^{-1} with a peristaltic pump (SJ-1220, Atto, Tokyo, Japan) and paced at 300 beats min^{-1} via silver-electrodes attached to the auricles with an electric stimulator (SEN-3201, Nihon Kohden, Tokyo, Japan). Perfusion pressure was recorded at the aortic orifice with a pressure transducer (DX-360, Nihon Kohden). Developed tension was monitored with a force-displacement transducer (TB-612T, Nihon Kohden). Resting tension of 1 g was applied upon starting the experiments.

Experimental protocol After 15 min of equilibration, acetylcholine (0.3 μg) was injected into the perfusate adjacent to the aorta and the changes in perfusion pressure were recorded. When the increase in perfusion pressure to acetylcholine was less than 5 mmHg, the hearts were excluded from the experiments. After 5 min of equilibration, semotiadil (3×10^{-8}–3×10^{-7} M), diltiazem (10^{-7}–3×10^{-6} M), nifedipine (10^{-9}–3×10^{-7} M), nisoldipine (10^{-10}–10^{-7} M) or vehicle (0.01% dimethyl sulphoxide) was infused into the perfusate. The effects of the compounds on developed tension were recorded 10 min after the start of the infusion. Then, the changes in perfusion pressure to acetylcholine (0.03–1.0 μg) were studied.

Statistical analysis

Data are expressed as mean ± s.e.mean. Statistical comparisons were performed by means of Kruskal-Wallis test followed by

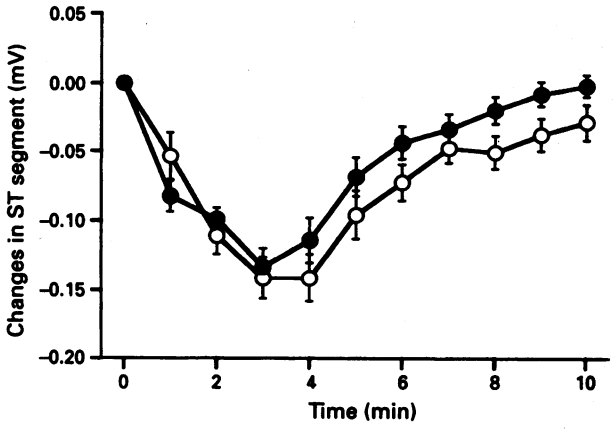

Figure 1 Time-course of the changes in ST-segment produced by intravenous injection of Arg-vasopressin 1 h after the oral administration of saline (○, $n=18$) and vehicle (0.5% methylcellulose solution; ●, $n=19$) in anaesthetized rats. Each value represents mean ± s.e.mean.

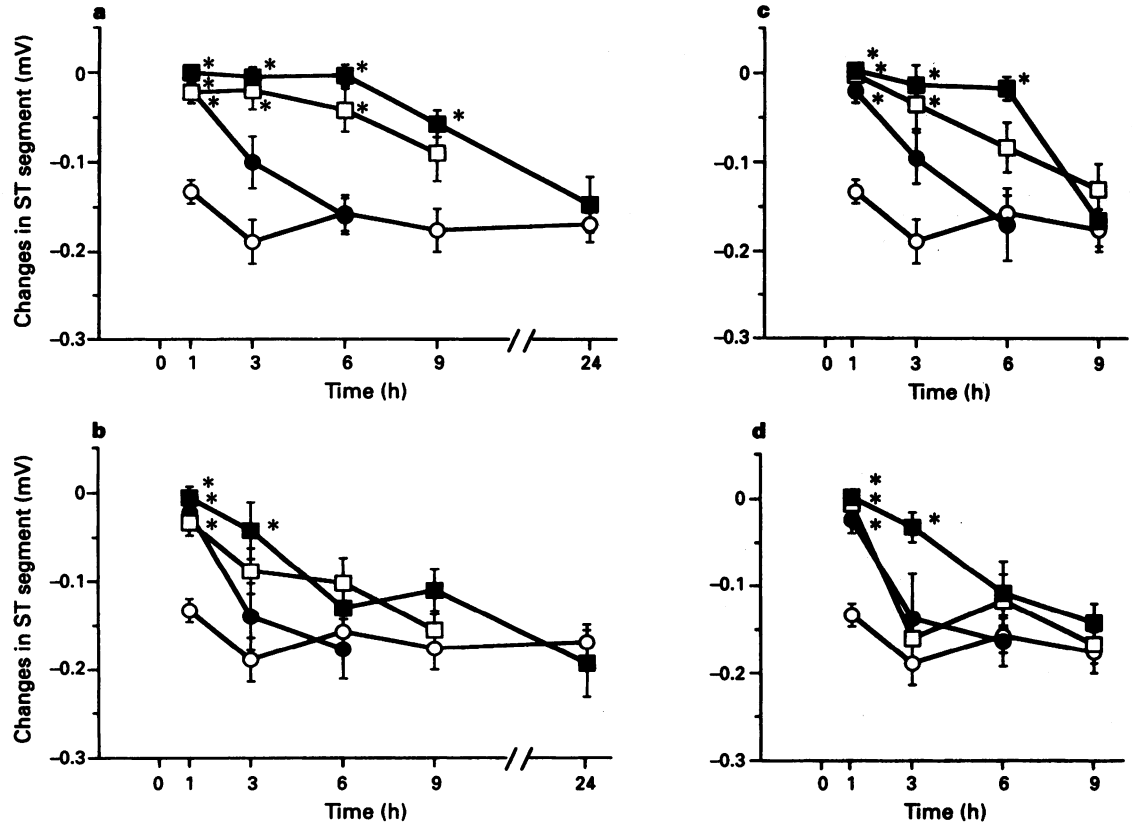

Figure 2 Inhibitory effects of orally administered (a) semotiadil (1 mg kg^{-1}; ●, 3 mg kg^{-1}; □ and 10 mg kg^{-1}; ■), (b) diltiazem (3 mg kg^{-1}; ●, 10 mg kg^{-1}; □ and 30 mg kg^{-1}; ■), (c) nifedipine (1 mg kg^{-1}; ●, 3 mg kg^{-1}; □ and 10 mg kg^{-1}; ■), and (d) nisoldipine (0.3 mg kg^{-1}; ●, 1 mg kg^{-1}; □ and 3 mg kg^{-1}; ■) on Arg-vasopressin (AVP)-induced ST-segment depression in rats. Each point represents mean ± s.e.mean of the data from 6 rats. *$P<0.05$ compared with vehicle-treated group (0.5% methylcellulose; ○).

Table 1 Effects of Ca^{2+} antagonists on developed tension after 10 min infusion

	Semotiadil		*Diltiazem*		*Nifedipine*		*Nisoldipine*	
	Baseline	*10 min*	*Baseline*	*10 min*	*Baseline*	*10 min*	*Baseline*	*10 min*
Concentration (M)	*Developed tension* (g)							
Saline	1.93±0.10	2.13±0.07						
Vehicle	1.89±0.10	2.01±0.11						
10^{-10}							1.77±0.15	1.76±0.16
3×10^{-10}							2.18±0.12	2.14±0.09
10^{-9}					2.02±0.11	2.11±0.12	2.18±0.10	2.04±0.10
3×10^{-9}					1.67±0.11	1.62±0.11	1.83±0.16	1.87±0.13
10^{-8}					1.66±0.17	1.49±0.19	1.59±0.15	1.59±0.15
3×10^{-8}	2.00±0.13	1.82±0.10			1.84±0.05	1.62±0.08	1.71±0.14	1.29±0.14*
10^{-7}	1.72±0.26	1.16±0.14*	1.62±0.10	1.65±0.06*	1.95±0.10	0.94±0.08*	1.80±0.11	0.93±0.15*
3×10^{-7}	1.95±0.10	0.98±0.04*	1.73±0.11	1.70±0.11	1.83±0.09	0.52±0.04*		
10^{-6}			1.77±0.10	1.13±0.08*				
3×10^{-6}			1.82±0.11	0.80±0.14*				

Each value represents the mean±s.e.mean of six to seven experiments. *$P<0.05$ compared to vehicle-treated group (10 min).

Mann-Whitney U test. P values less than 0.05 were considered to indicate statistically significant differences between groups.

Drugs

Semotiadil and acetylcholine chloride were synthesized at Daiichi Pharmaceutical Co. (Tokyo, Japan). Nisoldipine was obtained from Bayer (Germany); diltiazem, nifedipine and Arg-vasopressin from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Test drugs were suspended in 0.5% methylcellulose for the oral administration experiments. In Langendorff perfusion, test drugs were dissolved in a small amount of DMSO and then diluted with distilled water to a final concentration of 1% DMSO.

Results

ST-segment changes evoked by vasopressin

The maximal depression in ST-segment was observed 3 min after AVP administration, and the voltage of the segment returned to the baseline within 10 min (Figure 1). The vehicle solution (0.5% methylcellulose) did not affect the depression of ST-segment induced by AVP. All tested drugs inhibited the changes in ST-segment in a dose-dependent manner. The minimum doses of the Ca^{2+} antagonists that completely inhibited the AVP-induced ST-segment depression 1 h after oral administration were as follows: semotiadil 1 mg kg^{-1}; diltiazem 3 mg kg^{-1}; nifedipine 1 mg kg^{-1} and nisoldipine 0.3 mg kg^{-1}. However, the Ca^{2+} antagonists at these minimum doses did not significantly inhibit the responses to AVP 3, 6 and 9 h after the administration (Figure 2).

Duration of action of Ca^{2+} antagonists

Semotiadil at 3 mg kg^{-1}, p.o. significantly inhibited the AVP-induced ST-segment depression 6 h after the administration (Figure 2). Furthermore, semotiadil at 10 mg kg^{-1} reduced the changes in ST-segment to AVP even 9 h after administration, but this effect was not evident at 24 h. The inhibitory effects of the other Ca^{2+} antagonists were less persistent than that of semotiadil. The effects of diltiazem and nisoldipine did not last for 6 h even at the highest doses (diltiazem; 30 mg kg^{-1}, nisoldipine; 3 mg kg^{-1}) and nifedipine (10 mg kg^{-1}) failed to inhibit the response to AVP at 9 h.

ACh-induced coronary vasocontraction

Effects of Ca^{2+} antagonists on developed tension of the perfused hearts reached steady-state within 10 min after the start of drug infusion. Baselines of the developed tension were not significantly different between the groups. Semotiadil (3×10^{-8}–3×10^{-7} M), diltiazem (10^{-7}–3×10^{-6} M), nifedipine (10^{-9}–3×10^{-7} M) and nisoldipine (10^{-10}–10^{-7} M) all decreased the developed tension in a concentration-dependent manner (Table 1).

ACh, in the range of 0.03 to 1.0 g, dose-dependently increased the perfusion pressure due to coronary vasocontraction. The Ca^{2+} antagonists inhibited the increase in perfusion

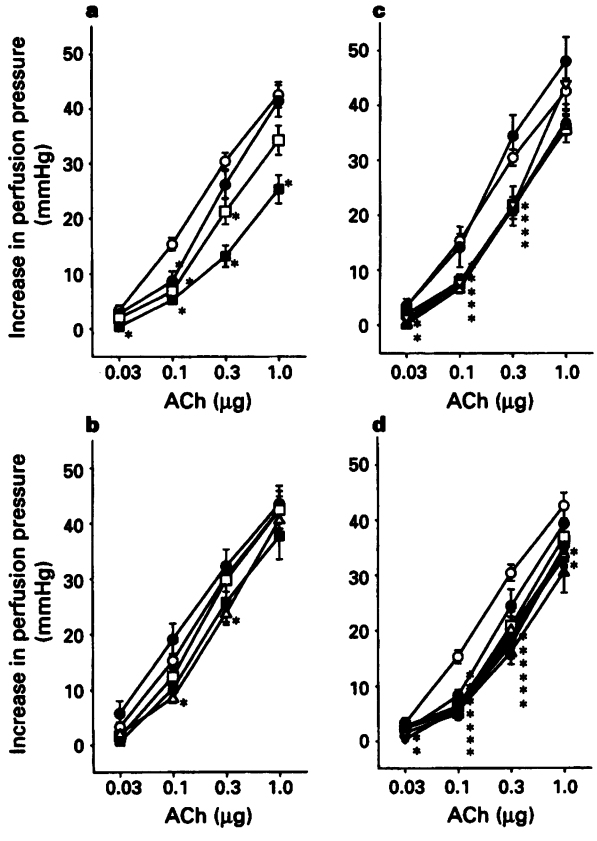

Figure 3 Inhibitory effects of (a) semotiadil (3×10^{-8} M; ●, 10^{-7} M; □ and 3×10^{-7} M; ■), (b) diltiazem (10^{-7} M; ●, 3×10^{-7} M; □, 10^{-6} M; ■ and 3×10^{-6} M; △), (c) nifedipine (10^{-9} M; ●, 3×10^{-9} M; □, 10^{-8} M; ■, 3×10^{-8} M; △, 10^{-7} M; ▲ and 3×10^{-7} M; ▽), and (d) nisoldipine (10^{-10} M; ●, 3×10^{-10} M; □, 10^{-9} M; ■, 3×10^{-9} M; △, 10^{-8} M; ▲, 3×10^{-8} M; ▽ and 10^{-7} M; ▼) on acetylcholine (ACh)-induced increase in perfusion pressure in rat isolated perfused hearts. The data are shown as mean±s.e.mean of six to seven experiments. *$P<0.05$ vs. vehicle (0.01% DMSO; ○).

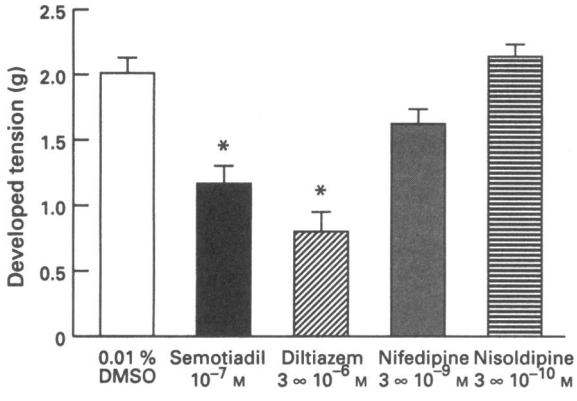

Figure 4 Effects of semotiadil (10^{-7} M), diltiazem (3×10^{-6} M), nifedipine (3×10^{-9} M) and nisoldipine (3×10^{-10} M) on the developed tension during 10 min infusion in the isolated perfused rat hearts. Each value represents mean ± s.e.mean of six preparations. $^*P < 0.05$ vs. vehicle (0.01% DMSO).

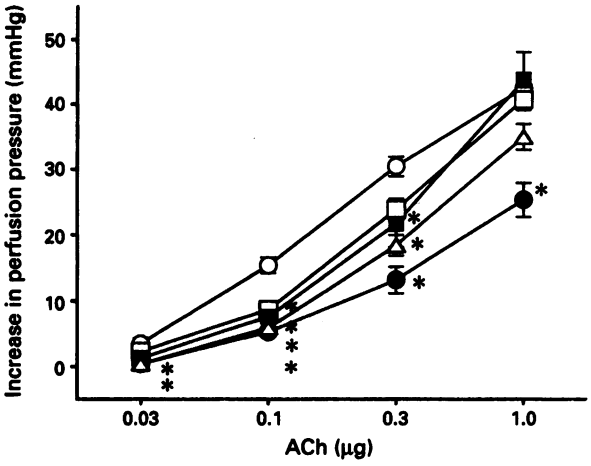

Figure 5 Inhibitory effects of the highest concentrations of semotiadil (3×10^{-7} M; ●), diltiazem (3×10^{-6} M; □), nifedipine (3×10^{-7} M; ■) and nisoldipine (10^{-7} M; △) on acetylcholine (ACh)-induced increase in perfusion pressure in the isolated perfused rat hearts. The data are shown as mean ± s.e.mean of six preparations. $^*P < 0.05$ vs. vehicle (0.0% DMSO; ○).

pressure in concentration-dependent manner (Figure 3). The potency of the compounds was evaluated using a submaximal dose of ACh (0.3 μg). The increase in perfusion pressure caused by 0.03 μg or 0.1 μg ACh was not sufficient to evaluate the inhibitory effects of the compounds, whereas the response to 1 μg ACh reached almost the maximum level an affect which dilitiazem and nifedipine could not inhibit significantly. The lowest concentrations of these Ca^{2+} antagonists that significantly inhibited the increase in perfusion pressure induced by 0.3 μg of ACh were as follows, dilitiazem 3×10^{-6} M, semotiadil 10^{-7} M, nifedipine 3×10^{-9} M and nisoldipine 3×10^{-10} M. The inhibitory effect of semotiadil on the coronary response to ACh was more potent than that of diltiazem, and less potent than the effects of nifedipine and nisoldipine.

Diltiazem (10^{-6} M) significantly suppressed cardiac contractility without affecting the coronary response to ACh. Semotiadil (10^{-7} M) inhibited both cardiac contractility and the coronary response. Nifedipine (3×10^{-9}–3×10^{-8} M) and nisoldipine (3×10^{-10}–10^{-8} M) inhibited the coronary response to ACh without affecting cardiac contractility. At the lowest concentrations of the Ca^{2+} antagonists that significantly inhibited the coronary response to ACh, the suppressive effect of semotiadil on cardiac contractility was intermediate between that of diltiazem and those of nifedipine and nisoldipine (Figure 4). In contrast, when the highest doses of these Ca^{2+} antagonists were given to the perfused hearts, semotiadil was the most potent inhibitor of the increase in coronary perfusion pressure to ACh (Figure 5).

Discussion

To elucidate the beneficial effects of semotiadil in angina in comparison to diltiazem, nifedipine and nisoldipine, the present studies focused on the duration of actions of the compounds after oral administration and selectivity of the actions for cardiac contractility and coronary vasodilatation.

The depression of the ST-segment to AVP indicates a subendocardiac ischaemia (Hiramatsu *et al.*, 1970; Hatano *et al.*, 1980; Kita *et al.*, 1994). In this experimental model of angina, Ca^{2+} antagonists such as nifedipine, verapamil and diltiazem significantly inhibit the changes in ST-segment evoked by AVP, whereas these changes are not affected by β-blocker propranolol (Karasawa *et al.*, 1988; Uchida *et al.*, 1993). Hence, an increase in oxygen supply due to coronary vasodilatation or antispasmodic action is crucial to inhibit these ST-segment changes. Thus, the AVP-induced ST-segment change is a useful model for evaluating the efficacy of the compounds in vasospastic angina (Tsuboi *et al.*, 1980; Kottegoda *et al.*, 1982). The selection of three oral doses of each Ca^{2+} antagonist was based on the minimum doses that completely inhibited the AVP-induced depression of ST-segment 1 h after oral administration of the compounds in the preliminary studies. In the present study, these minimum effective doses and three and ten fold higher doses were used to evaluate the duration of action of semotiadil in the angina model. Antianginal effects of semotiadil at a dose of 10 mg kg^{-1} lasted for at least 9 h, which was longer than those of other Ca^{2+} antagonists tested. These results in the angina model are consistent with the earlier studies showing that hypotensive effects of semotiadil (10 mg kg^{-1} and 30 mg kg^{-1}, p.o.) last for 10 and 18 h, respectively, in conscious SHR (Kanda & Hashimoto, 1993). In the isolated depolarized aorta of the rabbit the dilator effect of semotiadil on the vessels is not easily reversed compared to dilitiazem by washing out the compounds from the bathing medium (Nishimura *et al.*, 1990). Therefore, a tight-binding of semotiadil to the vascular tissue may explain the long-lasting activity of the compound *in vivo*. Indeed, highly lipophilic Ca^{2+} antagonists like semotiadil show a slow onset and a long duration (Nakayama *et al.*, 1992). Furthermore, Nakayama *et al.* (1992) suggested a possibility that the long lasting action of semotiadil in Ca^{2+} antagonism is due to slow dissociation of the compound from the receptor site. However, the present experiments do not permit further speculation concerning the precise mechanism of the long action of semotiadil.

ACh given locally into the coronary artery causes an increase in coronary perfusion pressure in the isolated, donor-perfused hearts of rats, monkeys and pigs (Sakai, 1980; 1981). The intracoronary injection of ACh also induces coronary spasm and cardiac attack in patients with variant angina (Yasue *et al.*, 1986; Okumura *et al.*, 1988). ACh was used as a coronary spasmogen in the present experiments. To clarify the selectivity of action of semotiadil for coronary artery and myocardium, the spasmolytic and cardiosuppressive effects of semotiadil were compared with those of diltiazem, nifedipine and nisoldipine in isolated perfused hearts. Since the perfusate was conducted into the coronary vasculature at a constant flow rate, the changes in perfusion pressure to ACh indicated coronary vasocontraction in the present study. All Ca^{2+} antagonists induced cardiac arrest at the concentrations three times as much as the highest concentrations used in these experiments. The suppressive effect of semotiadil on cardiac tissues was intermediate between that of diltiazem and those of nifedipine and nisoldipine at the concentrations which showed an equal inhibition of coronary vasocontraction. Thus, semotiadil is located between diltiazem and dihydropyridines like nifedipine and nisoldipine with regard to the tissue-selectivity for coronary artery and myocardium. The differences among semo-

tiadil, diltiazem and typical dihydropyridines in the tissue selectivity may have a relevance to the different binding sites of these Ca^{2+} antagonists to Ca^{2+} channel (Nakayama *et al.*, 1992; 1994). In addition, the maximal inhibition by semotiadil at highest concentration (3×10^{-7} M) was most potent among the compounds in the response to ACh. However, the precise mechanism of these profiles of semotiadil remains unclear in the present study. Semotiadil is expected to prevent angina attack by its reduction of cardiac work and inhibition of the decrease in coronary blood flow.

In conclusion, semotiadil has long-acting and moderate effects on both coronary arteries and myocardium, which would favour the treatment of patients with angina pectoris.

References

FUJITA, M., ITO, S., OTA, A., KATO, N., YAMAMOTO, K., KAWASHIMA, Y., YAMAUCHI, H. & IWAO, J. (1990). Synthesis and Ca^{2+} antagonistic activity of 2-[2-[(aminoalkyl)oxy]-5-methoxyphenyl]-3,4-dihydro-4-methyl-3-oxo-2H-1,4-benzothiazines. *J. Med. Chem.*, **33**, 1898–1905.

HATANO, N., NAKATSUJI, K., NOSE, I. & SHIMIZU, M. (1980). Effects of antianginal agents on electrocardiographic change induced with vasopressin in rats. *Pharmacometrics*, **19**, 311–317.

HENRY, P.D. (1980). Comparative pharmacology of calcium antagonists: nifedipine, verapamil and diltiazem. *Am. J. Cardiol.*, **46**, 1047–1058.

HIRAMATSU, Y., IZUMI, A., TEZUKA, T. & KUROSAWA, Y. (1970). Antagonizing substances obtained from whale heart extract to vasopressin induced myocardial hypoxia. *Jpn. J. Pharmacol.*, **20**, 313–324.

KAGEYAMA, M., NISHIMURA, K., TAKADA, T., MIYAWAKI, N. & YAMAUCHI, H. (1991). SD-3211, a novel benzothiazine calcium antagonist, alone and in combination with a beta-adrenoceptor antagonist, produces antihypertensive effects without affecting heart rate and atrioventricular conduction in conscious renal hypertensive dogs. *J. Cardiovasc. Pharmacol.*, **17**, 102–107.

KANDA, A. & HASHIMOTO, H. (1993). Effects of semotiadil fumarate, a novel calcium antagonist, on blood pressure and heart rate in conscious spontaneously hypertensive rats. *Jpn. J. Pharmacol.*, **63**, 121–124.

KARASAWA, A., KUBO, K., SHUTO, K., OKA, T. & NAKAMIZO, N. (1988). Antianginal effects of the new calcium antagonist benidipine hydrochloride in anesthetized rats and spontaneously hypertensive rats. *Arzneimittel-Forsch.*, **38**, 1702–1707.

KENDALL, H.E. & LUSCOMBE, D.K. (1987). Calcium channel blocking drugs. In *Progress in Medicinal Chemistry*, Vol. 24. ed. Ellis, G.P. & West, G.B. pp. 249–297. Amsterdam: Elsevier Science Publishers.

KITA, Y., OZAKI, R., SAKAI, S., SUGIMOTO, T., HIRASAWA, Y., OHTSUKA, M., SENOH, H., YOSHIDA, K. & MAEDA, K. (1994). Antianginal effects of FK409, a new spontaneous NO releaser. *Br. J. Pharmacol.*, **113**, 1137–1140.

KOTTEGODA, S.R., ADAIKAN, P.G. & KARIM, S.M. (1982). Reversal of vasopressin-induced coronary vasoconstriction by a PGE1 analogue (one 1206) in primates. *Prostaglandins Leukot. Med.*, **8**, 343–348.

MITCHELL, J., FRISHMANN, W. & HEIMANN, M. (1993). Nisoldipine: a new dihydropyridine calcium-channel blocker. *J. Clin. Pharmacol.*, **33**, 46–52.

MITCHELL, L.B., SCHROEDER, J.S. & MASON, J.W. (1982). Comparative clinical electrophysiological effects of diltiazem, verapamil and nifedipine: a review. *Am. J. Cardiol.*, **49**, 629–634.

MIYAWAKI, N., FURUTA, T., SHIGEI, T., YAMAUCHI, H. & ISO, T. (1990). Electrophysiological properties of SD-3211, a novel putative Ca^{++} antagonist, in isolated guinea pig and rabbit hearts. *J. Cardiovasc. Pharmacol.*, **16**, 769–775.

MIYAWAKI, N., FURUTA, T., SHIGEI, T., YAMAUCHI, H. & ISO, T. (1991). Cardiovascular characterization of SD-3211, a novel benzothiazine calcium channel blocker, in isolated rabbit hearts. *Life Sci.*, **48**, 1903–1909.

NAKAYAMA, K., MORIMOTO, K., NOZAWA, Y. & FUKUTA, Y. (1994). Allosteric interaction of semotiadil fumarate, a novel benzothiazine, with 1,4-dihydropyridines, phenylalkylamines, and 1,5-benzothiazepines at the Ca^{2+}-channel antagonist binding sites in canine skeletal muscle membranes. *J. Cardiovasc. Pharmacol.*, **23**, 731–740.

NAKAYAMA, K., MORIMOTO, K., NOZAWA, Y. & TANAKA, Y. (1992). Calcium antagonistic and binding properties of semotiadil (SD-3211), a benzothiazine derivative assessed in cerebral and coronary arteries. *J. Cardiovasc. Pharmacol.*, **20**, 380–391.

NISHIMURA, K., MIYAWAKI, N., YAMAUCHI, H. & ISO, T. (1990). Tissue selectivity of the novel calcium antagonist sesamodil fumarate in isolated smooth muscles and cardiac muscles. *Arzneimittel-Forsch.*, **40**, 244–248.

OKUMURA, K., YASUE, H., MATSUYAMA, K., GOTO, K., MIYAGI, H., OGAWA, H. & MATSUYAMA, K. (1988). Sensitivity and specificity of intracoronary injection of acetylcholine for the induction of coronary artery spasm. *J. Am. Coll. Cardiol.*, **12**, 883–888.

OPIE, L.H. (1988). Calcium channel antagonists part II: Use and comparative properties of the three prototypical calcium antagonists in ischemic heart disease, including recommendations based on an analysis of 41 trials. *Cardiovasc. Drugs Ther.*, **1**, 461–491.

PEDERSON, O.L. (1983). Calcium blockade in arterial hypertension. *Hypertension*, **5**, Suppl. 2, 74–79.

QUARTAROLI, M., GAMBINI, F., TARTER, G., MICHELI, D., TRIST, D.G. & GAVIRAGHI, G. (1991). The hemodynamic effects of Lacidipine in anesthetized dogs: comparison with nitrendipine, amlodipine, verapamil, and diltiazem. *J. Cardiovasc. Pharmacol.*, **18**, 326–336.

SAKAI, K. (1980). Coronary vasoconstriction by locally administered acetylcholine, carbachol and bethanechol in isolated, donor-perfused, rat hearts. *Br. J. Pharmacol.*, **68**, 625–632.

SAKAI, K. (1981). Vasoconstriction produced by intracoronary cholinomimetic drugs in isolated donor-perfused hearts of rhesus monkeys: comparison with pig, dog, and rabbit hearts. *J. Cardiovasc. Pharmacol.*, **3**, 500–509.

TAIRA, N. (1987). Differences in cardiovascular profile among calcium antagonists. *Am. J. Cardiol.*, **59**, 24B–29B.

TAKADA, T., MIYAWAKI, N., KAGEYAMA, M., MATSUNO, K., ISHIDA, N., YAMAUCHI, H. & ISO, T. (1991a). Antihypertensive effect of a novel calcium antagonist, SD-3211, in experimental hypertensive rats. *J. Cardiovasc. Pharmacol.*, **18**, 855–862.

TAKADA, T., MIYAWAKI, N., NISHIMURA, K., NAKATA, K., MATSUNO, K., ISHIDA, N., YAMAUCHI, H. & ISO, T. (1991b). Cardiohemodynamic effects of a novel calcium antagonist, SD-3211, in the dog. *Arch. Int. Pharmacodyn.*, **309**, 75–87.

TRIGGLE, D.J. (1991). Calcium-channel drugs: structure-function relationships and selectivity of action. *J. Cardiovasc. Pharmacol.*, **18**, (Supplement 10), S1–S6.

TSUBOI, T., HATANO, N., NAKATSUJI, K., FUJITANI, B., YOSHIDA, K., SHIMIZU, M., KAWASAKI, A., SAKATA, M. & TSUBOSHIMA, M. (1980). Pharmacological evaluation of OP 1206 a prostaglandin E1 derivative, as an antianginal agent. *Arch. Int. Pharmacodyn.*, **247**, 89–102.

UCHIDA, W., SHIBASAKI, K., ASANO, M. & TAKENAKA, T. (1993). Antianginal effects of YM-16151-4 in various experimental angina models. *J. Cardiovasc. Pharmacol.*, **21**, 701–708.

YASUE, H., HORIO, Y., NAKAMURA, N., FUJII, H., IMOTO, N., SONODA, R., KUGIYAMA, K., OBATA, K., MORIKAMI, Y. & KIMURA, T. (1986). Induction of coronary artery spasm by acetylcholine in patients with variant angina: possible role of the parasympathetic nervous system in the pathogenesis of coronary artery spasm. *Circulation*, **74**, 955–963.

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