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Effects of semotiadil fumarate, a novel Ca^{2+} antagonist, on cytosolic Ca^{2+} level and force of contraction in porcine coronary arteries

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1 The mechanisms of action of semotiadil fumarate, a novel Ca^{2+} antagonist, were examined by measuring the cytosolic Ca^{2+} level ($[Ca^{2+}]_i$) and force of contraction in porcine coronary arteries, and by determining [^{3}H]-pyrilamine binding to bovine cerebellar membranes.

2 Semotiadil or verapamil (0.1 and 1 μM) inhibited both the high KCl-induced increases in $[Ca^{2+}]_i$ and force in a concentration-dependent manner.

3 Histamine (30 μM) produced transient increases followed by sustained increases in $[Ca^{2+}]_i$ and force, which were inhibited by semotiadil and verapamil (1 and 10 μM). The agents were different in that semotiadil reduced the maximum $[Ca^{2+}]_i$ and force responses to histamine, but not pD_2 values, whereas verapamil did reduce the pD_2 values for histamine, but not the maximum responses.

4 Verapamil (10 μM), but not semotiadil, inhibited histamine-induced increases in $[Ca^{2+}]_i$ and force in Ca^{2+}-free solution. Neither semotiadil nor verapamil affected the increases in $[Ca^{2+}]_i$ and force induced by caffeine. Semotiadil even at the higher concentration (10 μM) did not displace specific binding of [^{3}H]-pyrilamine to bovine cerebellar membranes.

5 These results suggest that semotiadil inhibits both KCl- and histamine-induced contractions mainly by blocking voltage-dependent L-type Ca^{2+} channels.

Keywords: Semotiadil; verapamil; Ca^{2+} antagonists; fura-2; cytosolic Ca^{2+} level; force of contraction; porcine coronary arteries

Introduction

Semotiadil fumarate, (SD-3211, (+)-(**R**)-2-[5-methoxy-2-[3-[methyl[2-[3,4-(methylenedioxy)phenoxy]ethyl]amino]propoxy] phenyl]-4-methyl-2*H*-1,4-benzothiazine-3(4H)-one hydrogen fumarate) (chemical structure shown in Figure 1), was reported to be an orally active Ca^{2+} antagonist with antihypertensive (Kageyama *et al.*, 1991b; Takada *et al.*, 1991) and antianginal activities (Mori *et al.*, 1990a; 1991). Semotiadil has a quite different chemical structure from dihydropyridine Ca^{2+} antagonists but like the dihydropyridines, it is vasoselective compared with other non-dihydropyridine Ca^{2+} antagonists such as verapamil and diltiazem (Yoneyama *et al.*, 1990; Nishimura *et al.*, 1990; Miyawaki *et al.*, 1991). Semotiadil has been shown to decrease the plateau of the fast action potential without affecting the maximum upstroke velocity and to depress the slow action potential induced by isoprenaline in guinea-pig isolated papillary muscles (Miyawaki *et al.*, 1990). Semotiadil has also been shown to inhibit, in a heterotropic and allosteric manner, the binding of [^{3}H]-PN200-110, a dihydropyridine, [^{3}H]-verapamil and [^{3}H]-diltiazem in porcine coronary arterial membranes (Nakayama *et al.*, 1992). These results suggested that semotiadil dilated arteries by inhibiting Ca^{2+} channels by binding to a site other than those occupied by Ca^{2+} antagonists currently available. However, the exact mechanisms underlying semotiadil-induced arterial dilatation are still unclear.

In the present study, we examined the effects of semotiadil on high KCl- or histamine-induced increases in the cytosolic Ca^{2+} level ($[Ca^{2+}]_i$) and force by simultaneous measurement of both variables to clarify further its vasodilator mechanisms in comparison with a classical Ca^{2+} antagonist verapamil. To measure $[Ca^{2+}]_i$, porcine coronary arterial rings were loaded with fura-2, an indicator of $[Ca^{2+}]_i$ (Grynkiewicz *et al.*, 1985). In addition, the effect of semotiadil on histamine H_1 receptors was examined by the [^{3}H]-pyrilamine binding assay.

Methods

Tissue preparation

Fresh porcine hearts were obtained from a slaughter house and transported on ice to the laboratory. Small coronary arteries (1-2 mm in outer diameter, second and third branches of the anterior descending artery) were excised and cleaned of excess fat and connective tissues in physiological salt solution (PSS) under a binocular microscope. The arteries were cut into rings 1 mm in width and everted with the luminal side outwards. The endothelium was removed by rubbing gently the luminal surface with a pair of forceps. Removal of endothelium was verified by the absence of relaxation after applications of acetylcholine (1 μM) or bradykinin (0.1 μM).

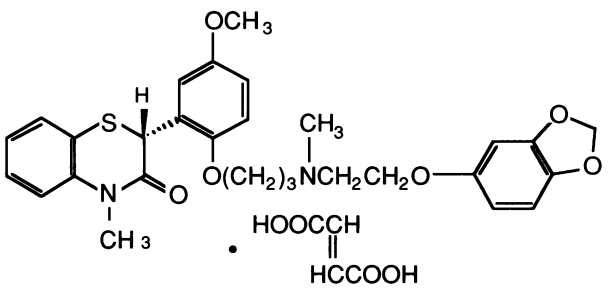

Figure 1 The chemical structure of semotiadil fumarate.

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Measurements of fluorescence and force of contraction

Fluorescence and force of contraction were measured simultaneously by the method described previously (Yanagisawa *et al.*, 1989; Mori *et al.*, 1990b; Kageyama *et al.*, 1991a). Briefly, coronary arterial rings were incubated in PSS containing 10 μM fura-2 acetoxymethyl ester (AM) and 0.1% w/v Pluronic F-127, a non-cytotoxic detergent, for 5–7 h at room temperature. After the fura-2 loading, the rings were rinsed with PSS for more than 1 h to remove excess dye on the tissue surface. They were mounted between two tungsten hooks, one of which was connected to a force displacement transducer (Shinko UL2GR, Minebea Co., Ltd., Nagano, Japan) under a resting tension of about 5 mN. The rings were placed horizontally in a 0.4 ml organ bath, which was attached to the stage of an inverted microscope (TMD-8, Nikon, Tokyo, Japan) and perfused at a rate of 4 ml min^{-1} with PSS warmed at 37°C. An equilibration period of at least 30 min was allowed before each protocol was started.

Fluorescence was measured by a fluorimeter equipped with a dual wavelength excitation device (CAM-200 or CAM-220, Japan Spectroscopic, Tokyo, Japan) connected to the microscope. Smooth muscle cells in the medial layers of rings were illuminated for 2 s every 10 s alternately with wavelengths of 340 and 380 nm by chopping at a frequency of 100 Hz. Fluorescence at excitation with 340 nm (F_{340}) and 380 nm (F_{380}) through a 500 nm filter was detected by a photomultiplier. After each experiment was completed, autofluorescence derived from tissues was obtained by adding a detergent, Triton X-100, and $MnCl_2$ and this was subtracted from each of the photosignals. A ratio of corrected F_{340} and F_{380} was calculated and used as an index of $[Ca^{2+}]_i$. Force, F_{340}, F_{380} and their ratio were continuously recorded on the thermal pen recorder (Recti-Horiz 8K, NEC-San-ei, Tokyo, Japan). At the beginning of each experiment, tissues were perfused with PSS containing 90 mM KCl (90 mM KCl-PSS) for 10 min. Changes in $[Ca^{2+}]_i$ and force induced by each agent are expressed as a percentage of those induced by the 90 mM KCl-PSS at 10 min.

Binding assay

The method of Chang *et al.* (1979) was used for the binding assay, carried out by Nova Pharmaceuticals (Baltimore, MD, U.S.A.) as contract work. Briefly, the bovine cerebellum were homogenized with a Brinkmann Kinematica Polytron in ice-cold 50 mM Na-KPO_4 (pH 7.5). The homogenate was centrifuged at 50,000 *g* for 10 min, and then the pellets were resuspended in the same volume of fresh buffer. After the suspension was centrifuged again, the final pellets were resuspended to a concentration of 37.5 mg wet tissue weight ml^{-1}. The membrane fraction (15 mg wet tissue weight/each tube) was incubated with [^{3}H]-pyrilamine in 50 mM NaH_2PO_4-K_2HPO_4 buffer (pH 7.5) at 25°C for 30 min. The reaction was terminated by rapid vacuum filtration of the reaction contents through glass fibre filters with a Brandell 48 well Cell Harvester. The filtration was followed by 10 to 15 ml washes per well. Radioactivity trapped on the filters was determined by liquid scintillation counting (LS3801, Bechman Instruments Inc., Fullerton, CA, U.S.A.) Non-specific binding was determined in the presence of 10 μM triprolidine.

Drugs and solutions

PSS contained (in mM): NaCl 140, KCl 5, $CaCl_2$ 2.5, $MgCl_2$ 2.5, glucose 11.1 and 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid 3 (HEPES, pH 7.4). The PSS containing high concentrations of KCl was made by substituting NaCl with equimolar KCl. Ca^{2+}-free PSS was made by removing $CaCl_2$ from normal PSS and by adding 1 mM ethyleneglycol-*bis*(b-aminoethylether)-*N*,*N*,*N*',*N*'-tetraacetic acid (EGTA).

The following drugs were used: semotiadil fumarate (Santen Pharmaceutical Co., Ltd., Osaka, Japan), (±)-verapamil hydrochloride and triprolidine (Sigma Chemical, Co., St. Louis, MO, U.S.A.), bradykinin (Peptide Institute Osaka, Japan), acetylcholine chloride, histamine dihydrochloride, caffeine, dimethyl sulphoxide (DMSO), Triton X-100 (Wako Pure Chemical Co., Osaka, Japan), fura-2 AM, Pluronic F-127 and HEPES (Dojin Chemical Co., Kumamoto, Japan), [^{3}H]-pyrilamine (New England Nuclear Co., Boston, MA, U.S.A.). Semotiadil was dissolved in DMSO at a concentration of 10 mM. Triprolidine was also dissolved in DMSO. Verapamil, acetylcholine, bradykinin and histamine were dissolved in distilled water at a concentration of 10 mM. Fura-2 AM and Pluronic F-127 were dissolved in DMSO at 1 mM and 25% (w/v), respectively. These stock solutions were diluted to the desired concentrations with PSS. Caffeine was dissolved in PSS at a concentration of 25 mM.

Analysis of concentration-response curves and statistical analysis

Each value represents the mean ± s.e. Concentration-response curves for the effects of KCl and histamine on $[Ca^{2+}]_i$ and force in the absence or presence of semotiadil or verapamil were computer-fitted to a logistic equation:

$$E = M \times A^P/(A^P + K^P)$$

where E is the normalized response, M is the maximum response. A is the concentration of KCl or histamine, K is

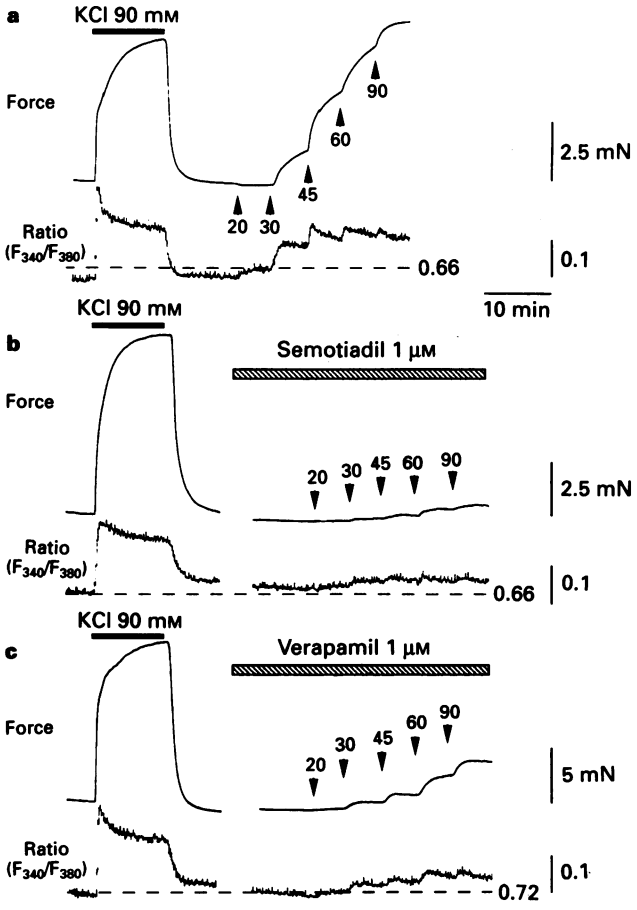

Figure 2 Typical recordings of changes in $[Ca^{2+}]_i$ (indicated by fluorescence ratio) and force induced by increasing concentrations of KCl-PSS in the absence (upper traces) or presence of semotiadil (middle traces) or verapamil (lower traces) in porcine coronary arteries loaded with fura-2. Semotiadil or verapamil was applied 20 min before cumulative applications of KCl-PSS. In this protocol, the changes in fluorescence ratio and force of contraction induced by perfusion of 90 mM KCl-PSS for 10 min were 0.35 ± 0.03 and 7.7 ± 0.6 mN, respectively ($n = 31$).

the EC_{50} of KCl or histamine and is the slope parameter. The EC_{50} of histamine was expressed as the pD_2 ($-\log EC_{50}$) value. Statistical significance was determined by a one-way analysis of variance followed by Dunnett's multiple comparison test, or by Kruskal-Wallis's test followed by Dunnett-type multiple comparison test after Bartlett's test. If $P<0.05$, the value was considered statistically significant.

Results

Effects of semotiadil or verapamil on KCl-induced contraction

Both semotiadil and verapamil decreased slightly basal $[Ca^{2+}]_i$ and force. Figures 2 and 3 show the effects of semotiadil and verapamil (0.1 and 1 μM) on changes in $[Ca^{2+}]_i$ and force induced by cumulatively increasing concentrations of KCl-PSS in porcine coronary arteries. Perfusion of 90 mM KCl-PSS rapidly increased $[Ca^{2+}]_i$ and force. $[Ca^{2+}]_i$ reached a peak within 2 min and then decreased gradually during the perfusion. Force reached a peak 10 min after the start of the perfusion. Following the 90 mM KCl-PSS perfusion, in the absence of each vasodilator, cumulative applications of KCl-PSS from 5 to 90 mM resulted in concentration-dependent increases in $[Ca^{2+}]_i$ and force. After applications of semotiadil or verapamil (0.1 and 1 μM) for 20 min, both of the increases in $[Ca^{2+}]_i$ and force induced by KCl-PSS were attenuated in a concentration-dependent manner. Both agents reduced the maximum KCl-induced increases in $[Ca^{2+}]_i$ and force, and increased the EC_{50} values for KCl (Table 1).

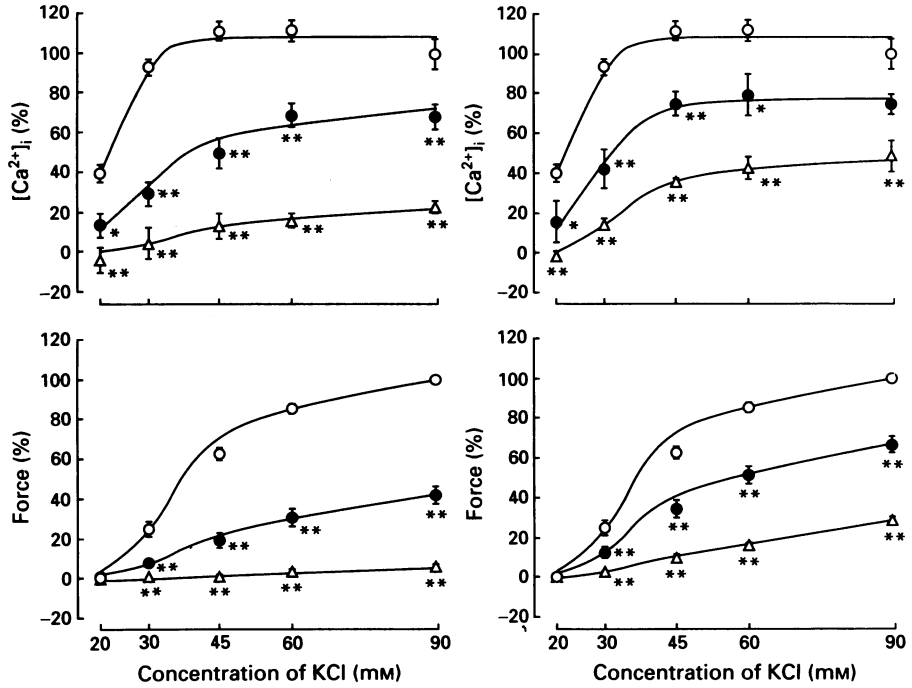

Figure 3 Effects of semotiadil (left panels) and verapamil (right panels) on increases in $[Ca^{2+}]_i$ and force induced by cumulative applications of KCl-PSS in porcine coronary arteries loaded with fura-2. Semotiadil or verapamil was applied 20 min before cumulative applications of KCl-PSS. (○) Control; (●) 0.1 μM semotiadil or 0.1 μM verapamil; (△) 1 μM semotiadil or 1 μM verapamil. Changes in $[Ca^{2+}]_i$ and force induced by each concentration of KCl are expressed as a percentage of those induced by the 90 mM KCl-PSS for 10 min. Concentration-response curves were computer-fitted using mean values of the data. $*P<0.05$; $**P<0.01$, compared with control. Each value represents the mean ± s.e.mean of 5 to 9 different experiments.

Table 1 The maximum responses and pD_2 values for the KCl- and histamine-induced increases in $[Ca^{2+}]_i$ and force in the absence or presence of vasodilators

		$[Ca^{2+}]_i$		Force	
KCl	n	*Maximum response* (%)	EC_{50} (mM)	*Maximum response* (%)	EC_{50} (mM)
Control	(5)	108.0 ± 5.7	21.7 ± 0.6	102.0 ± 1.9	40.4 ± 1.3
Semotiadil 0.1 μM	(9)	76.9 ± 7.1*	34.9 ± 4.0	49.2 ± 5.5*	52.5 ± 4.5
1 μM	(5)	24.3 ± 3.8**	44.8 ± 6.8**	8.4 ± 1.6**	69.5 ± 10.6**
Verapamil 0.1 μM	(5)	77.9 ± 7.2*	27.6 ± 2.4	72.1 ± 5.2	47.1 ± 3.5
1 μM	(7)	50.1 ± 8.2**	37.1 ± 2.5*	35.9 ± 4.0**	59.2 ± 4.5*
Histamine	n	*Maximum response* (%)	pD_2 (-logM)	*Maximum response* (%)	pD_2 (-logM)
Control	(7)	72.5 ± 7.4	5.83 ± 0.06	88.5 ± 14.5	5.46 ± 0.12
Semotiadil 1 μM	(7)	37.9 ± 8.1*	5.82 ± 0.42	57.7 ± 13.0	5.37 ± 0.14
10 μM	(5)	30.1 ± 9.2*	5.16 ± 0.29	34.7 ± 9.9	5.42 ± 0.18
Verapamil 1 μM	(6)	69.0 ± 15.9	5.48 ± 0.23	91.3 ± 28.9	5.44 ± 0.16
10 μM	(5)	57.1 ± 6.7	4.79 ± 0.18*	61.4 ± 6.6	4.77 ± 0.09**

Each value represents the mean ± s.e.mean. The numbers of experiments (n) are shown in parentheses. $*P<0.05$; $**P<0.01$, statistically significant compared with control in the absence of vasodilators.

Effects of histamine on $[Ca^{2+}]_i$ and force

Figure 4 shows typical recordings of the effects of histamine (30 μM) on $[Ca^{2+}]_i$ and force in porcine coronary arteries. On perfusion of histamine, $[Ca^{2+}]_i$ and force increased rapidly and reached a peak around 1 min after the application and force reached a peak within 3 min (the first phase). $[Ca^{2+}]_i$ and force decreased gradually to a level higher than the resting level 10 min after the perfusion of histamine (the second phase). Perfusion of Ca^{2+}-free 5 mM KCl-PSS containing 1 mM EGTA for 10 min decreased the resting $[Ca^{2+}]_i$, whereas force was decreased very slightly. When histamine (30 μM) was applied in the absence of extracellular Ca^{2+}, $[Ca^{2+}]_i$ and force increased transiently and $[Ca^{2+}]_i$ or force reached a peak around 1 min or within 2 min after the application, respectively. Then $[Ca^{2+}]_i$ and force returned to the decreased resting level within 3 min. Thus, removal of extracellular Ca^{2+} markedly reduced the first phase of the histamine-induced increases in $[Ca^{2+}]_i$ and force, and it abolished the second phase of the increase in $[Ca^{2+}]_i$.

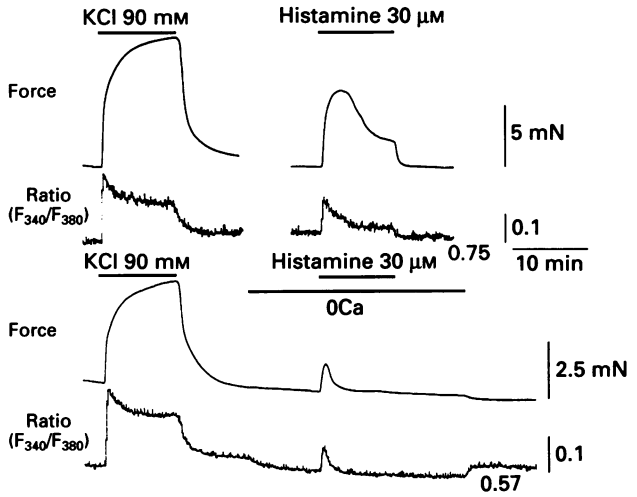

Figure 4 Typical recordings of the effects of histamine (30 μM) on $[Ca^{2+}]_i$ (indicated by fluorescence ratio) and force in the presence (upper traces) or absence of extracellular Ca^{2+} (lower traces) in porcine coronary arteries loaded with fura-2. Extracellular Ca^{2+} was removed by perfusion with Ca^{2+}-free PSS containing 1 mM EGTA 10 min before the application of histamine (0Ca).

Effects of semotiadil or verapamil on histamine-induced contraction

An application of semotiadil at 1 and 10 μM for 20 min before the addition of histamine inhibited both first and second phases of the increases in $[Ca^{2+}]_i$ and force induced by histamine (30 μM) in a concentration-dependent manner (Figure 5). The second phase of the increase in $[Ca^{2+}]_i$ was almost abolished by semotiadil at 1 μM. Verapamil (1 and 10 μM) also inhibited the histamine-induced increases in $[Ca^{2+}]_i$ and force, but its inhibitory effects were less potent than those of semotiadil.

Figure 6 shows the concentration-response curves for the first phase of histamine-induced increases in $[Ca^{2+}]_i$ and force in the absence or presence of semotiadil or verapamil. Semotiadil (1 and 10 μM) flattened the concentration-response curves for the increases in $[Ca^{2+}]_i$ and force induced by histamine (1 to 300 μM). On the other hand, verapamil at 1 μM did not affect the concentration-response curves for histamine. At the higher concentration (10 μM) verapamil shifted rightward the concentration-response curves for histamine. As shown in Table 1, the maximum responses of this histamine-induced increase in $[Ca^{2+}]_i$ were significantly reduced in the presence of 1 and 10 μM semotiadil, but were not affected by verapamil even at the higher concentration (10 μM). In contrast, pD_2 values of the histamine-induced increases in $[Ca^{2+}]_i$ and force were significantly reduced by verapamil at 10 μM, but not 1 μM, whereas they were not altered by semotiadil at either concentration.

As shown in Figure 7, semotiadil (1 and 10 μM) did not

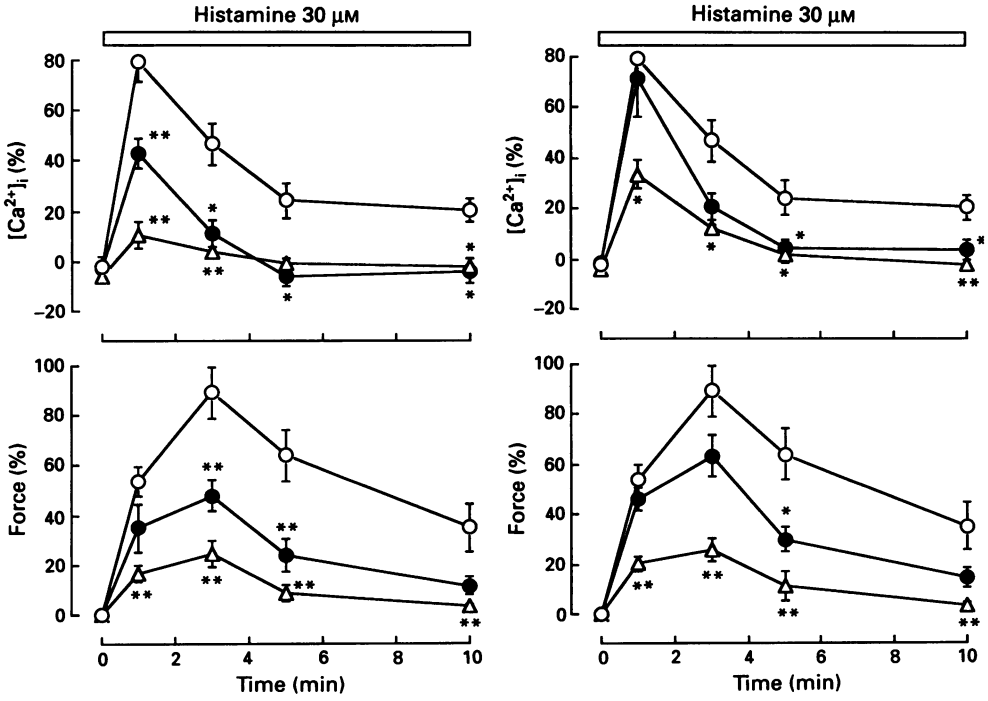

Figure 5 Effects of semotiadil (left panel) and verapamil (right panel) on increases in $[Ca^{2+}]_i$ and force induced by histamine (30 μM) in porcine coronary arteries loaded with fura-2. Semotiadil or verapamil was applied 20 min before the application of histamine. (○) Control; (●) 1 μM semotiadil or 1 μM verapamil; (△) 10 μM semotiadil or 10 μM verapamil. Changes in $[Ca^{2+}]_i$ and force induced by histamine are expressed as a percentage of those induced by 90 mM KCl-PSS for 10 min. $^*P<0.05$; $^{**}P<0.01$, compared with control. Each value represents the mean ± s.e.mean of 4 to 6 different experiments.

change the transient increases in $[Ca^{2+}]_i$ and force induced by histamine in Ca^{2+}-free PSS containing 1 mM EGTA. In contrast, verapamil at 10 μM inhibited significantly the $[Ca^{2+}]_i$ and contractile responses to histamine ($P<0.05$), but at 1 μM it did not affect them. Neither semotiadil nor verapamil (0.1 to 10 μM) had a statistically significant effect on the increases in $[Ca^{2+}]_i$ and force induced by 25 mM caffeine (data not shown).

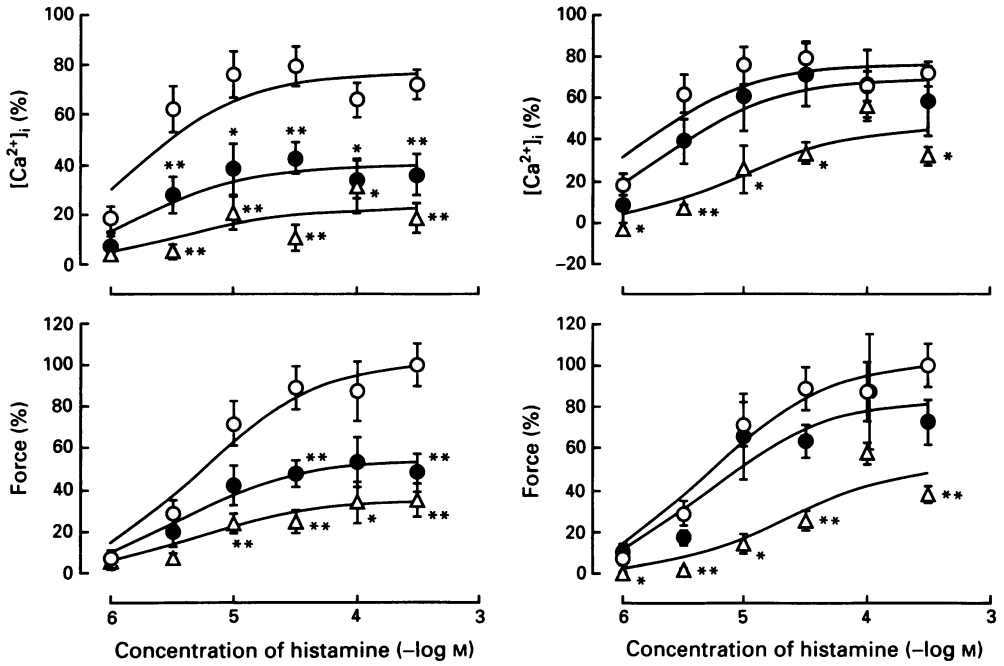

Figure 6 Concentration-response curves for the first phase of the response to histamine on $[Ca^{2+}]_i$ and force in the absence (○) or presence of 1 μM semotiadil or 1 μM verapamil (●) and 10 μM semotiadil or 10 μM verapamil (△) in porcine coronary arteries loaded with fura-2. Semotiadil or verapamil was applied 20 min before the application of each concentration of histamine. The histamine-induced increases in $[Ca^{2+}]_i$ and force in the first phase are defined as those observed at 1 and 3 min after the application of histamine, respectively. Changes in $[Ca^{2+}]_i$ and force induced by histamine are expressed as a percentage of those induced by 90 mM KCl-PSS for 10 min. The concentration-response curves were computer-fitted using mean values of the data. $*P<0.05$; $**P<0.01$, compared with control. Each value represents the mean ± s.e.mean of 4 to 7 different experiments.

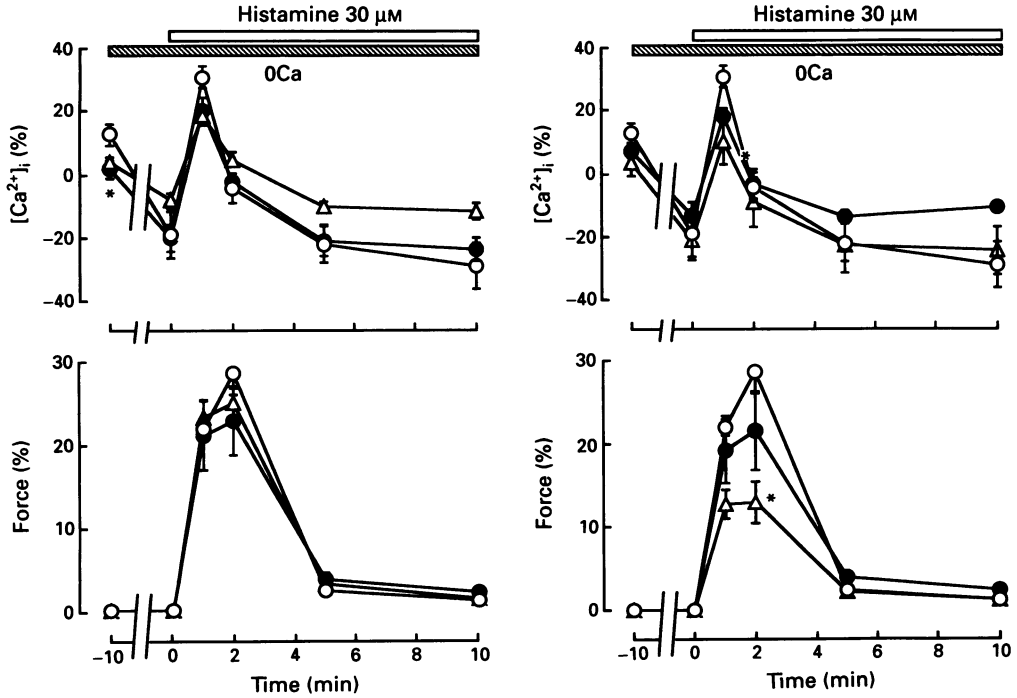

Figure 7 Effects of semotiadil (left panel) and verapamil (right panel) on increases in $[Ca^{2+}]_i$ and force induced by histamine (30 μM) in the absence of extracellular Ca^{2+} in porcine coronary arteries loaded with fura-2. Extracellular Ca^{2+} was removed by perfusion with Ca^{2+}-free PSS containing 1 mM EGTA before the application of histamine (0Ca). (○) Control; (●) 1 μM semotiadil or 1 μM verapamil; (△) 10 μM semotiadil or 10 μM verapamil. Changes in $[Ca^{2+}]_i$ and force induced by histamine are expressed as percentage of those induced by the 90 mM KCl-PSS for 10 min. $*P<0.05$, compared with control. Each value represents the mean ± s.e.mean of 4 to 5 different experiments.

Effect of semotiadil on [^{3}H]-pyrilamine binding

Verapamil has been demonstrated to bind to histamine H_1 receptors in membrane fractions from porcine aorta with a dissociation constant (K_i) of 7.1 ± 1.9 μM (Matsumoto *et al.*, 1989). Therefore, we tried to clarify whether or not semotiadil inhibits the histamine-induced contraction by interacting with histamine H_1 receptors. The specific binding of [^{3}H]-pyrilamine to bovine cerebellar membrane was saturable, and non-specific binding was only 20% of total binding. The binding affinity (K_d value) was 1.3 nM, and receptor density (B_{max}) was 6.2 pmol g^{-1} wet weight of tissue. Triprolidine (1 pM to 300 nM) displaced the specific [^{3}H]-pyrilamine binding with the K_i value of 1.0 nM, and the displacement at 100 nM was complete. Semotiadil even at 10 μM had little effect on the specific [^{3}H]-pyrilamine binding, and the inhibition by semotiadil was only 10.9% ($n = 2$).

Discussion

In the present study, semotiadil and verapamil each inhibited both the KCl-induced increases in $[Ca^{2+}]_i$ and force in porcine coronary arteries. They also inhibited the histamine-induced increases in $[Ca^{2+}]_i$ and force. However, in the absence of extracellular Ca^{2+}, semotiadil had no effect on the $[Ca^{2+}]_i$ and force responses to histamine, whereas verapamil inhibited them. These results suggest that in coronary arteries contracted with KCl, the vasodilator mechanisms of semotiadil and verapamil are the same, but semotiadil differs from verapamil in the mechanisms for inhibition of the histamine-induced contraction.

As extracellular KCl concentration was increased, force of contraction increased even after $[Ca^{2+}]_i$ had reached a peak level. If membrane depolarization with KCl increases an apparent sensitivity of contractile elements to cytosolic Ca^{2+}, a contraction should develop without a further increase in $[Ca^{2+}]_i$. Our recent studies have demonstrated that the apparent Ca^{2+} sensitivity is increased by membrane depolarization with KCl (Yanagisawa & Okada, 1994) and is decreased by hyperpolarization with K^+ channel openers (Okada *et al.*, 1993). These results suggest that membrane potential is one of the regulators of the apparent Ca^{2+} sensitivity. Thus, one possible explanation of the present results seems to be increased Ca^{2+} sensitivity of the contractile elements by KCl.

Semotiadil like verapamil inhibited increases in $[Ca^{2+}]_i$ as well as force induced by elevation of extracellular KCl concentrations. In a similar way, in canine basilar arteries contracted with KCl, semotiadil also decreased both $[Ca^{2+}]_i$ and force (Nakayama *et al.*, 1992). Such a simultaneous inhibition of the KCl-induced increases in $[Ca^{2+}]_i$ and force is a common characteristic of Ca^{2+} antagonists (Sato *et al.*, 1988; Yanagisawa *et al.*, 1989). Recently, the inhibition by semotiadil of voltage-dependent Ca^{2+} currents has been shown in the smooth muscle cells of rabbit portal vein (Teramoto, 1993). Thus, as seen with verapamil, the inhibition by semotiadil of the KCl-induced contraction may be due to blockade of voltage-dependent L-type Ca^{2+} channels in porcine coronary arteries.

Histamine is a strong spasmogen which contracts atherosclerotic porcine (Shimokawa *et al.*, 1983) and human coronary arteries (Kalsner & Richards, 1984). When histamine is applied in the presence of extracellular Ca^{2+}, $[Ca^{2+}]_i$ rapidly increases, reaches a maximum level (the first phase), and then decreases to a level higher than the resting level (the second phase, Rembold & Murphy, 1988; Mori *et al.*, 1990c). The histamine-induced change in $[Ca^{2+}]_i$ precedes the increase in force as seen with other agonists such as noradrenaline (Sato *et al.*, 1988) and carbachol (Himpens & Somlyo, 1988). In the present study, histamine also produced biphasic changes in $[Ca^{2+}]_i$ and force. The first phase of the $[Ca^{2+}]_i$ and contractile responses to histamine was greatly reduced, but still obtainable in the absence of extracellular Ca^{2+}. This means that the first phase is partially mediated by the release of Ca^{2+} from intracellular stores. Actually, histamine has been shown to produce inositol 1,4,5-trisphosphate (Donaldson & Hill, 1985), which releases Ca^{2+} from intracellular stores (Berridge & Irvine, 1984). The second phase of the histamine-induced increase in $[Ca^{2+}]_i$ was abolished and that of force was greatly reduced by removing extracellular Ca^{2+}. Therefore, histamine may stimulate both Ca^{2+} release and influx in the first phase, and only Ca^{2+} influx in the second phases.

To clarify the pathways of Ca^{2+} influx in the first and second phases, we examined the effects of verapamil on the histamine-induced increase in $[Ca^{2+}]_i$. We observed that verapamil inhibited, in a concentration-dependent manner, both the first and second phases of $[Ca^{2+}]_i$ and force responses to histamine. In an earlier study using porcine coronary arteries (Hirano *et al.*, 1990), diltiazem also attenuated these responses to histamine. Furthermore, it has been reported that the stimulation of histamine H_1 receptors increases L-type Ca^{2+} currents in rabbit coronary artery cells (Ishikawa *et al.*, 1993). Therefore, Ca^{2+} may enter into the cytosol through voltage-dependent L-type Ca^{2+} channels in the first and second phases.

We found that verapamil caused rightward shifts in the concentration-response curves for the first phase of histamine responses: the pD_2 value for histamine was reduced by verapamil, whereas the maximum response was not affected. Furthermore, verapamil at 10 μM attenuated the $[Ca^{2+}]_i$ and force responses to histamine in Ca^{2+}-free PSS, but not those to caffeine, suggesting that it does not act directly on intracellular Ca^{2+} stores. Therefore, verapamil at 10 μM may antagonize histamine at histamine H_1-receptors. This notion is directly supported by the earlier study indicating that verapamil displaces the [^{3}H]-mepyramine binding to porcine aortic membranes with a K_i of 7.1 μM (Matsumoto *et al.*, 1989). In contrast, the concentration-response curves for histamine responses were non-competitively inhibited by semotiadil (1 and 10 μM), which reduced the maximum responses without affecting the pD_2 values. Semotiadil did not affect the $[Ca^{2+}]_i$ and force responses to histamine in Ca^{2+}-free PSS and those to caffeine, and the binding of [^{3}H]-pyrilamine. These results suggest that semotiadil inhibits the histamine-induced contraction mainly by blocking Ca^{2+} influx through voltage-dependent L-type Ca^{2+} channels, whereas verapamil acts by both Ca^{2+} channel blockade and H_1-receptor antagonism.

In conclusion, semotiadil like verapamil may inhibit both KCl-induced and histamine-induced contractions by blocking voltage-dependent L-type Ca^{2+} channels in porcine coronary arteries. Semotiadil may be different from verapamil in that it does not bind to histamine H_1-receptors.

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