Effects of S-312, a New Calcium Antagonist, on the Mechanical and Electrophysiological Responses of Isolated Cardiovascular Preparations

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Abstract—S-312, a new calcium antagonist with a bicyclic dihydrothienopyridine structure, potently relaxed the helical strips of various isolated rabbit arteries pre-contracted with high K^+ -depolarization, serotonin (5-HT) and U46619 (thromboxane A_2 analogue), and it competitively inhibited Ca^{++} -induced contractions in depolarized basilar and femoral arteries. These effects of S-312 were more potent than nifedipine and almost comparable to or slightly more potent than those of nicardipine. In comparison with nifedipine and nicardipine, the calcium antagonistic effect and the relaxant effect on 5-HT-induced contractions of S-312 were most prominent in the basilar artery. The potent vasodilating action of S-312 in the high K^+ -depolarized basilar artery was not easily reversed by washing. S-312 did not affect Ca^{++} -induced contraction in the skinned fiber of guinea pig taenia caecum. The negative inotropic effect of S-312 in isolated guinea pig left atria was much less potent than those of nifedipine and nicardipine. S-312 above 10^{-7} M preferentially increased AV nodal conduction time in Langendorff-perfused isolated rabbit hearts; and above 3×10^{-8} M, it mainly decreased the maximum upstroke velocity of the action potential in isolated rabbit sinus node preparations. In summary, the present results indicate that S-312 is a potent new calcium antagonist possessing vasculoselectivity, especially for cerebral vessels.

Several dihydropyridine calcium antagonists including nifedipine have been widely used to treat various cardiovascular disorders such as hypertension, angina pectoris, myocardial infarction and cerebrovascular disorders (1, 2). Recently, several dihydropyridine derivatives have been developed which have a long lasting effect or relatively selective action in some vascular tissues. Nicardipine, which improves cerebrovascular circulation, has been clinically used in the treatment of various cerebrovascular disorders (3, 4). It has been recently shown that nimodipine selectively inhibits spasms of isolated cerebral arteries induced by spasmogenic agonists (5, 6), and clinical evaluations are in progress for the treatment of ischemic complications in the brain (7, 8).

The calcium antagonistic effect of S-312, methyl 4,7-dihydro-3-isobutyl-6-methyl-4-(3-nitrophenyl)thieno[2,3-*b*]pyridine-5-carboxylate (Fig. 1), was reported by Adachi et al. (9). Marked inhibitory effects of S-312 on specific [3H]-nitrendipine (NTD) binding in rat cerebral cortex membranes, long lasting coronary dilation without any negative inotropic effect in the isolated guinea pig heart, and

Fig. 1. Chemical structure of S-312.

acute antihypertensive effect in conscious SHR by i.p. administration have been observed. These pharmacological effects of S-312 indicated its potent and selective vasodilating property in comparison with its cardiac actions. However, the mode of action of S-312 on the vascular smooth muscle and cardiac muscle preparations has not been clarified yet.

In the present study, the mechanical and electrophysiological effects of S-312 on the isolated rabbit arteries, skinned fiber of guinea pig taenia caecum and cardiac preparations of rabbit or guinea pig were investigated. The mode of action and tissue specificity of S-312 were also evaluated and compared with those of nifedipine and nicardipine.

Materials and Methods

1. Preparation of rabbit arterial strips and tension measurement

Adult male Japanese white rabbits, weighing 1.9–2.7 kg, were stunned on the head and exsanguinated. Femoral arteries and proximal portions of the mesenteric arteries were quickly dissected. The right coronary artery and circumflex branch of the left coronary artery, basilar artery and interlobar branches of the renal artery were carefully isolated from the heart, brain and kidneys, respectively. After removal of connective tissue, isolated arteries were helically cut into strips (1–2 cm long). The strip was mounted in a 20-ml muscle bath filled with Krebs-Henseleit solution (37°C) which was aerated with a mixture of 95% O₂ and 5% CO₂. The lower end of a strip was attached to a stainless steel rod, and the upper end was connected to a force transducer (Nihon Kohden) for isometric tension measurement. The resting tension was adjusted to 0.5 g. The composition of the solution was as follows: 130.9 mM NaCl, 5.9 mM KCl, 2.5 mM CaCl₂, 11.9 mM NaHCO₃, 1.2 mM NaH₂PO₄, 1.2 mM MgSO₄ and 11.0 mM glucose (pH 7.3). Before the start of each experiment, the strips were allowed to equilibrate in the muscle bath for 60–90 min and then contracted repeatedly with 50 mM K⁺ to obtain constant responses.

2. Effect on isolated rabbit arteries

2.1. Calcium antagonistic effect in depolarized rabbit arteries: Isolated femoral and

basilar arterial strips were washed with Ca⁺⁺-free, K⁺ (50 mM)-depolarizing solution, in which the NaCl concentration was reduced to 80.9 mM and NaH₂PO₄ was not used to avoid precipitation of Ca-salts. This treatment produced a transient contraction, which vanished completely by washing four times with Ca⁺⁺-free, K⁺-depolarizing solution. After 65 min exposure to Ca⁺⁺-free, K⁺-depolarizing solution, the cumulative dose-response curve for Ca⁺⁺ was determined. This set of experiments was performed three times. Between each set of experiments, the strips were washed with normal solution for 30 min. No significant difference was observed between the second and third dose-response curves for Ca⁺⁺. In the second set of experiments, the control dose-response curve for Ca⁺⁺ was determined, and calcium antagonist was added 45 min before the third dose-response curve.

2.2. Effects on high K⁺, 5-HT- and U46619-induced contractions of rabbit arteries: In order to evaluate the relaxant activity, S-312, nifedipine or nicardipine was added cumulatively to the muscle bath during sustained contraction of various arteries induced by high K⁺ (50 mM), 5-HT (3×10⁻⁶–2×10⁻⁵ M) or U46619 (10⁻⁸ M). At the end of each series of experiments, papaverine (10⁻⁴ M) was applied to produce the maximum relaxation (10).

2.3. Effect on NE-induced contraction of rabbit femoral artery: Cumulative administration of NE was repeated to obtain steady dose-response curves for NE. Then the effect of pretreatment of the calcium antagonist for 60 min on the NE dose-response curve was examined.

2.4. Effect on Ca⁺⁺-induced contraction of NE-exposed rabbit femoral artery: Isolated femoral arterial strips were washed with Ca⁺⁺-free solution containing 0.1 mM EGTA for 20 min and then exposed to 3×10⁻⁶ M NE. These procedures were repeated four times. NE-induced contraction in Ca⁺⁺-free solution vanished almost completely with these four repetitions. Dose-response curves for Ca⁺⁺ in the presence of NE were then determined in drug-treated (60 min) and non-treated strips which were prepared from the same animal.

2.5. Duration of vasodilating activity of

calcium antagonist: Isolated rabbit basilar arterial strips were contracted with high K^+ (50 mM) and relaxed with calcium antagonist. When the strips relaxed to reach a new steady level, they were washed out with drug-free high K^+ solution and allowed to recover the contraction. In these experiments, we examined the effects of the calcium antagonist at two different concentrations which induced half maximum and submaximum relaxation in the high K^+ -depolarized basilar artery.

3. Effect on the contraction of isolated skinned guinea pig taenia caecum

A strip of taenia caecum, 0.05–0.1 mm wide and 2–3 mm long, was freshly isolated from male guinea pigs weighing 500–700 g. Preparations were mounted in a small chamber filled with relaxing solution containing 130 mM K-propionate, 20 mM Tris-maleate, 4 mM $MgCl_2$, 4 mM ATP and 2 mM EGTA, and then aerated with 100% O_2 at 20°C (pH 6.8). The preparations were treated with 50–100 μ g/ml saponin for 20–30 min. The relaxant effect of the drugs was examined in the strips which were partially precontracted with 10^{-6} M Ca^{++} solution containing 2 mM EGTA. The apparent binding constant of EGTA for Ca^{++} at 20°C, pH 6.8, was considered to be 10^6 M $^{-1}$ (11).

4. Effect on the isolated guinea pig or rabbit cardiac preparations

4.1. Inotropic effect in guinea pig left atria: Left atria were isolated from guinea pigs and suspended in a 20-ml muscle bath filled with Krebs-Ringer solution (30°C) which was aerated with a mixture of 95% O_2 and 5% CO_2 . The composition of the Krebs-Ringer solution was as follows: 120.3 mM NaCl, 4.0 mM KCl, 1.2 mM $CaCl_2$, 1.3 mM $MgSO_4$, 1.2 mM NaH_2PO_4 , 25.2 mM $NaHCO_3$ and 5.8 mM glucose (pH 7.4). Resting tension was adjusted to 0.2 g. Electrical stimulation was applied with a pair of bipolar silver electrodes by rectangular pulses of 0.5 msec duration at twice the threshold intensity and with a frequency of 1.0 Hz. The developed tension was recorded by procedures similar to those described in section 1. Under these conditions, the preparation was treated with a single concentration of drug for 60 min.

4.2. Dromotropic effect in Langendorff-perfused rabbit heart: The isolated rabbit heart

was perfused with Krebs-Ringer solution ($32 \pm 1^\circ C$) equilibrated with 95% O_2 and 5% CO_2 at a constant perfusion rate of about 10 ml/min. The His bundle electrogram was recorded by bipolar silver wires placed on the area of the His bundle. The preparation was stimulated with a pair of bipolar silver electrodes on the pacemaker area of the right atrium under the following conditions: a basic stimulus interval, about 480 msec; intensity, 2x threshold; duration, 2 msec.

Atrial conduction time (SA), atrioventricular nodal conduction time (AH) and His-Purkinje conduction time (HV) were measured with the His bundle electrogram: SA interval, from the stimulus artifact to the earliest onset of atrial excitation (A wave); AH interval, from the A wave to the His signal; HV interval, from the His signal to the earliest onset of ventricular excitation.

4.3. Electrophysiological effects in rabbit sinus node preparations: A sinus node specimen (2x2 mm) was cut perpendicular to the crista terminalis of rabbit heart. The preparation of the sinus node was superfused continuously with Krebs-Ringer solution saturated with 95% O_2 and 5% CO_2 at 33°C. Transmembrane action potentials of the isolated sinus node preparations were recorded using standard glass microelectrodes filled with 3 M KCl. The maximum upstroke velocity of the action potential ($\dot{V}_{max}$) was obtained by electronic differentiation. The records of the transmembrane potential and $\dot{V}_{max}$ were displayed on a Tektronic 5113 dual beam storage oscilloscope.

5. Data analysis and drugs

Experimental results are expressed as the mean $\pm$ S.E. Statistical analysis were performed using Tukey's method after one-way analysis of variance (12) and Student's *t*-test. Drugs employed in these experiments were S-312 (Shionogi), nifedipine (Teikoku Kagaku), nicardipine (Yamanouchi), saponin (ICN), trifluoperazine (Sigma), serotonin creatinine sulfate (Merck), U46619 (Cayman Chemi), ($\pm$)-norepinephrine hydrochloride, and papaverine hydrochloride. S-312, nifedipine and nicardipine were dissolved in 90% dimethylsulfoxide (DMSO) and 10% castor oil (HCO-50) and diluted with distilled water before use. Nifedipine solution was prepared

just before its application. To examine the calcium antagonistic effects of nifedipine in the isolated arteries, experiments were performed under a sodium-light in a dark room. Other experiments with nifedipine were done under indoor-lighting; however, the muscle bath was covered by aluminum foil or black tape to protect the drug from degradation by light.

Results

1. Effect on isolated rabbit arteries

1.1. Calcium antagonistic effect in depolarized rabbit arteries: S-312 showed competitive antagonism against Ca^{++} -induced contraction, producing a rightward displacement of the dose-response curve for Ca^{++} in rabbit femoral and basilar arteries (Fig. 2). Parallelism between control and dose-response curves with S-312 was indicated by parametric parallel line testing (13). Similar results were obtained with nifedipine and nicardipine (Fig. 2). pA_2 values of S-312, nifedipine and nicardipine were determined by Schild plots (14). The Schild plot for these

calcium antagonists gave a regression line with a slope of 1.03–1.21 and a correlation coefficient above 0.991 (Fig. 3). The pA_2 values of S-312, nifedipine and nicardipine in the femoral artery were 10.045, 9.343 and 10.310, respectively. The values in the basilar artery were 10.420, 9.455 and 10.303, respectively. The calcium antagonistic effect of S-312 in the basilar artery was clearly more potent than that of the femoral artery. However, such a significant difference in the potencies between the two arteries was not clear with nifedipine and nicardipine. In the isolated basilar artery, the calcium antagonistic effect of S-312 is approximately 9.2 or 1.3 times more potent than that of nifedipine or nicardipine, respectively.

1.2. Effects on high K^+ -, 5-HT- and U46619-induced contractions of rabbit arteries: Sustained contractions in strips of basilar, coronary, mesenteric, renal and femoral arteries with 50 mM K^+ were dose-dependently relaxed by the cumulative addition of S-312 at concentrations ranging from 3×10^{-11} to 10^{-8} M (Fig. 4). These ar-

Fig. 2. Effects of S-312, nifedipine and nicardipine on concentration-response curves for Ca^{++} in the isolated rabbit basilar and femoral arteries. Each point represents the mean $\pm$ S.E. of 15–16 preparations in control curves ($\circ$) and 5–6 preparations in curves with calcium antagonists. The concentrations of calcium antagonists were 5×10^{-11} M ($\bullet$), 1.5×10^{-10} M ($\triangle$), 5×10^{-10} M ($\blacktriangle$), 1.5×10^{-9} M ($\square$) and 5×10^{-9} M ($\blacksquare$). The maximal contraction in the control curve was taken as 100%.

Fig. 3. The Schild plot for S-312, nifedipine and nicardipine antagonism on the Ca^{++} -induced contraction in rabbit basilar and femoral arteries. Regression lines were fitted by the method of least squares. The pA_2 values and slope for the antagonism by calcium antagonists were given in the Results.

Fig. 4. Concentration-relaxation curves for S-312 in the isolated rabbit basilar, coronary, mesenteric, renal and femoral arterial strips precontracted with 50 mM K^+ depolarization. Relaxation induced by 10^{-4} M papaverine was taken as 100%. Mean developed tensions with $\pm$ S.E. (mg) in basilar, coronary, mesenteric, renal and femoral arteries induced with 50 mM K^+ were 483 ± 74 ($n=5$), 493 ± 75 ($n=7$), 202 ± 23 ($n=5$), 1343 ± 190 ($n=5$) and 2510 ± 90 ($n=6$), respectively.

terial strips were relaxed with S-312 almost completely at 10^{-9} M to 10^{-8} M.

S-312 also relaxed the strips of basilar, coronary, mesenteric and femoral arteries precontracted with 5-HT in a dose-related manner (Fig. 5). In the strips of basilar, coronary and mesenteric arteries, almost complete relaxations were observed at 10^{-7} M, while the relaxations were incomplete in the

Fig. 5. Concentration-relaxation curves for S-312 in rabbit basilar, coronary, mesenteric and femoral arterial strips precontracted with 3×10^{-6} to 2×10^{-5} M 5-HT. Relaxation induced by 10^{-4} M papaverine was taken as 100%. Mean developed tensions with $\pm$ S.E. (mg, $n=5$) in basilar, coronary, mesenteric and femoral arteries induced with 5-HT were 382 ± 75 , 431 ± 109 , 223 ± 34 and 730 ± 53 , respectively.

femoral artery even at the highest concentration tested (10^{-6} M). Similar relaxations were observed for nifedipine and nicardipine in strips precontracted with high K^+ or 5-HT.

ED_{50} values of these relaxant effects for each drug are given in Table 1. Here, the ED_{50} values were obtained by fitting the dose-response curves to 4-parameter logistic curves (15). As seen in the Table, these relaxant effects of S-312 on the high K^+ - and 5-HT-induced contractions were more potent than those of nifedipine, and they were almost comparable to those of nicardipine. The relaxant effect of S-312 on high K^+ -induced contraction was more pronounced in mesenteric, basilar and coronary arteries than those in the other arteries. The relaxant effect of S-312 on 5-HT-induced contraction was most prominent in the basilar artery, and its potencies decreased in the order of basilar > coronary > mesenteric > femoral arteries. Such a selective action of S-312 on the basilar artery was remarkable compared with nifedipine and nicardipine. Relaxant effects of nifedipine and nicardipine were more prominent in the coronary artery than in the basilar artery.

In isolated rabbit coronary arterial strips, sustained contractions were induced by 10^{-8}

Table 1. Negative logarithm of ED50 values of calcium antagonists for high K⁺, 5-HT and U46619-induced contraction in isolated rabbit arteries

		Artery				
		Basilar	Coronary	Mesenteric	Renal	Femoral
K ⁺	S-312	9.65±0.06 ^a	9.65±0.09 ^a	9.70±0.04 ^a	9.39±0.02 ^{a,b}	9.06±0.03 ^a
	Nifedipine	8.75±0.11	8.96±0.02	8.65±0.05	8.47±0.02	8.25±0.06
	Nicardipine	9.40±0.05 ^a	9.56±0.05 ^a	9.46±0.08 ^a	9.33±0.04 ^a	9.18±0.05 ^a
5-HT	S-312	9.67±0.11 ^{a,b}	9.41±0.20	9.04±0.22 ^a	—	8.36±0.23 ^a
	Nifedipine	8.34±0.07	8.44±0.10	8.06±0.15	—	7.04±0.44
	Nicardipine	9.35±0.05 ^a	9.71±0.11 ^a	8.60±0.31	—	7.70±0.32
U46619	S-312	—	9.19±0.09 ^a	—	—	—
	Nifedipine	—	8.36±0.08	—	—	—
	Nicardipine	—	9.04±0.11 ^a	—	—	—

Values are means±S.E. of five to seven preparations. ^a ^bSignificantly different from the nifedipine or nicardipine group at $P < 0.05$, respectively (Tukey's Method).

Fig. 6. Comparison of relaxant effects of S-312, nifedipine and nicardipine in rabbit coronary arterial strips precontracted with 10^{-8} U46619. Each point represents the mean±S.E. of five preparations. Relaxation induced by 10^{-4} M papaverine was taken as 100%. Mean developed tension induced with U46619 was 435 ± 29 mg ($n=15$).

M U46619, a stable thromboxane A₂ analogue (16, 17). S-312, nifedipine and nicardipine produced relaxation in coronary arterial strips precontracted with U46619 in a dose-related manner (Fig. 6), and the ED50 values of these relaxant effects are shown in Table 1. The relaxant effect of S-312 was more potent than that of nifedipine and slightly more potent than that of nicardipine.

No significant differences in the upper and lower limits were observed between calcium antagonists in the logistic curves, to which

the dose-response curves for calcium antagonist-induced relaxant effects on high K⁺, 5-HT- and U46619-induced contractions were fitted. However, some significant differences in the slopes, of the logistic curves were observed as follows (mean±S.E.): K⁺-induced contraction (coronary), nicardipine (3.41 ± 0.04) > nifedipine (2.53 ± 0.06) and S-312 (2.08 ± 0.19); K⁺-induced contraction (renal), nifedipine (1.82 ± 0.01) < S-312 (2.86 ± 0.24) and nicardipine (3.10 ± 0.19); 5-HT-induced contraction (coronary), S-312 (2.86 ± 0.33) > nifedipine (1.61 ± 0.11) and nicardipine (1.78 ± 0.08); U46619-induced contraction, S-312 (2.74 ± 0.17) > nifedipine (1.82 ± 0.19) and nicardipine (2.40 ± 0.18).

1.3. Effect on NE-induced contraction of rabbit femoral artery: S-312 shifted the NE dose-response curve in rabbit femoral artery not only to the right, but also depressed the maximal response (Fig. 7). A similar mode of action was observed for nifedipine and nicardipine. S-312, nifedipine and nicardipine thus inhibited the NE contraction in a non-competitive manner. However, these three drugs only partially inhibited the NE-induced contraction even at the higher concentration (10^{-6} M) tested. The mean % decrease ($n=5$) in the maximal NE-response caused by 10^{-7} M of each calcium antagonist was as follows: S-312, 23.6; nifedipine, 13.7; nicardipine, 21.7.

Fig. 7. Effects of S-312 on NE-induced contraction (left panel) and Ca^{++} -induced contraction of NE-exposed strips (right panel) in rabbit femoral artery. Maximal contraction induced by NE determined in the same strip exposed to normal solution was taken as 100% in each graph. Mean maximal developed tension induced by NE was 3.04 ± 0.14 g ($n=15$).

1.4. Effect on NE-induced contraction in Ca^{++} -free solution of rabbit femoral artery:

When NE was added repeatedly every 20 min under the Ca^{++} -free condition, NE-induced contraction decreased gradually. The mean contractions of the first, second, third and fourth NE-induced response were 58, 18, 3.0 and 1.8%, respectively, when the response in normal solution is taken as 100%. These contractions obtained in Ca^{++} -free solution were not affected by the addition of 10^{-9} M S-312 to the muscle bath.

1.5. Effect on Ca^{++} -induced contraction of NE-exposed rabbit femoral artery:

Ca^{++} -induced contractions of NE-exposed femoral arterial strips (Fig. 7) were observed at a lower concentration of Ca^{++} than those of K^{+} -depolarized femoral arterial strips (Fig. 2). Similar high sensitivity to Ca^{++} has been reported for NE-exposed rat resistance vessels (18). S-312 inhibited this Ca^{++} -induced contraction even at a low concentration (10^{-9} M), producing a rightward parallel shift of the dose-response curve (Fig. 7). Parallelism between the control and dose-response curves with S-312 was indicated by parametric parallel line testing (13). Nifedipine and nicardipine also showed similar antagonism. The pA_2 values for S-312, nifedipine and nicardipine, which were determined by the method of van Rossum (19), were as follows (mean $\pm$ S.E., $n=5$): S-312, 9.71 ± 0.03 ; nife-

Fig. 8. Typical recordings of the relaxant response to S-312 and nifedipine, and of recontraction following drug removal from the isolated rabbit basilar arterial strip. The strip was precontracted with 50 mM K^{+} and relaxed with a submaximal concentration of S-312 (10^{-9} M) or nifedipine (5×10^{-9} M), and then washed out with drug-free 50 mM K^{+} solution.

dipine, 8.62 ± 0.06 ; nicardipine, 9.49 ± 0.04 .

1.6. Duration of vasodilating activity of calcium antagonist: In the drug-free high K^{+} solution, isolated rabbit basilar artery which had been relaxed with S-312 or nifedipine recontracted gradually almost to the initial level (Fig. 8). The time required for 50% recontraction was calculated as the index of the duration of the vasodilating action of the calcium antagonist (Table 2). The duration of vasodilating action caused by a high concentration of S-312 was significantly longer than that induced by a low concentration of S-312. Similar dose-dependency on the duration of the vasodilating action has been reported previously with nilvadipine (20). Both in high and low concentrations, S-312 caused more prolonged vasodilating action than those caused by nifedipine and nicardipine. The differences in the duration of the vasodilating action was considerable compared with that in the speed of relaxation, which was evaluated from the time required for 50% relaxation (Table 2).

2. Effects on contraction of isolated skinned guinea pig taenia caecum

In skinned muscle fibers of guinea pig taenia caecum, the contractile tensions produced with 10^{-4} or 10^{-5} M Ca^{++} almost corresponded to that with 140 mM K^{+} in intact muscles. After the contraction evoked by 10^{-6} M Ca^{++} had reached a steady level, the effect of S-312 at 10^{-8} or 10^{-4} M was observed.

Table 2. Times required for 50% relaxation and recontraction following addition and removal of calcium antagonist in depolarized (50 mM K⁺) rabbit basilar artery

Drug	Conc. (M)	Time (min)	
		Relaxation	Recontraction
S-312	2×10^{-10}	6.8 ± 0.8	29.7 ± 9.3
	10^{-9}	5.4 ± 1.0	93.3 ± 16.2
Nifedipine	10^{-9}	2.0 ± 0.2	2.1 ± 0.2
	5×10^{-9}	1.2 ± 0.1	2.7 ± 0.4
Nicardipine	2×10^{-10}	5.7 ± 0.4	8.3 ± 0.9
	10^{-9}	3.7 ± 0.2	16.9 ± 3.1

Values are means $\pm$ S.E. (n=5).

Fig. 9. Effects of S-312 and trifluoperazine on skinned fibers of guinea pig taenia caecum pre-contracted with Ca⁺⁺ at 10^{-6} M. The degree of the relaxant effect is shown as the percentage of the contraction induced by 10^{-6} M Ca⁺⁺, with the mean $\pm$ S.E. of five experiments.

Figure 9 shows the time course of contractile tension obtained after applications of S-312. S-312 did not affect the contraction evoked by 10^{-6} M Ca⁺⁺, while 10^{-4} M trifluoperazine, a calmodulin inhibitor (21, 22), caused marked relaxation (Fig. 9). These results indicate that the calcium receptors of contractile protein were not affected by S-312.

3. Effects on isolated guinea pig or rabbit cardiac preparations

3.1. Inotropic effect in guinea pig left atria:

The electrically driven contraction of the left atrium was dose-dependently inhibited by S-312 at concentrations ranging from 3×10^{-7} to 3×10^{-5} M (Fig. 10). These inhibitory concentrations were much higher than those required

Fig. 10. Negative inotropic effects of S-312, nifedipine and nicardipine in isolated guinea pig left atria which were electrically driven. Symbols represent the mean $\pm$ S.E. of four separate experiments. Mean developed tension induced by electrical stimulation was 435 ± 27 mg (n=32). The ED50 values with 95% confidence limits for S-312, nifedipine and nicardipine were 1.54 (0.70 – 5.88) $\times 10^{-5}$ M, 2.22 (1.65 – 3.08) $\times 10^{-7}$ M and 1.74 (1.30 – 2.23) $\times 10^{-6}$ M, respectively.

for calcium antagonistic and relaxant effects in the arterial preparations mentioned before. Nifedipine and nicardipine also showed similar negative inotropic effects, but their inhibitory potencies were significantly greater than that of S-312 (Fig. 10).

3.2. Dromotropic effects in Langendorff-perfused rabbit heart: Figure 11 shows the percentage changes of the SA, AH and HV intervals after 30-min exposure to S-312. S-312 above 10^{-7} M produced a dose-dependent increase in the AH interval, but did not affect the SA and HV intervals even at the

highest concentration (3×10^{-7} M) tested. These results indicated that S-312 preferentially delayed the conduction through the atrioventricular node.

3.3. Electrophysiological effects in rabbit sinus node preparations: Each impalement of the microelectrode in the preparation yielded a typical pacemaker potential with a smooth rising phase as reported previously (23). The action potential records obtained before and 30 min after application of S-312 are shown in Fig. 12, and changes in the various parameters of the action potentials are summarized in Table 3.

Although no effect was observed in any

Fig. 11. Effects of S-312 on SA, AH and HV intervals of His bundle electrogram in the Langendorff-perfused isolated rabbit heart. Mean control values with $\pm$ S.E. of SA, AH and HV intervals (msec, $n=5$) were 21.4 ± 2.4 , 44.4 ± 3.9 and 36.5 ± 3.2 , respectively. *Significantly different from the initial value at $P < 0.05$.

action potential parameters at 10^{-8} M, S-312 above 3×10^{-8} M caused decreases in $\dot{V}_{max}$ and the action potential amplitude (AMP) in a dose-related manner, and significant changes were observed above 10^{-7} M of S-312. S-312 also significantly decreased the maximum diastolic potential (MDP) at 3×10^{-7} M, and tended to decrease the action potential duration at 50% repolarization (APD_{50}) and increased the spontaneous cycle length (SCL).

Fig. 12. Effects of S-312 on the transmembrane action potential of spontaneously beating rabbit sinus node preparations. The upper and lower tracing in each section show the membrane action potential and its differentiated upstroke spike. A: control, B: 10^{-7} M of S-312.

Table 3. Effects of S-312 on the transmembrane action potential of isolated rabbit sinus node preparations

Parameter	Control	S-312 (M)			
		10^{-8}	3×10^{-8}	10^{-7}	3×10^{-7}
AMP (mV)	75.1 ± 2.6	73.3 ± 3.5	68.0 ± 3.6	$58.0 \pm 3.2^*$	$45.2 \pm 3.8^*$
MDP (mV)	70.2 ± 2.7	69.4 ± 3.4	69.1 ± 2.2	64.0 ± 1.0	$54.8 \pm 4.9^*$
$\dot{V}_{max}$ (V/sec)	8.84 ± 0.88	8.89 ± 1.13	6.65 ± 0.58	$4.94 \pm 0.36^*$	$3.73 \pm 1.14^*$
APD_{50} (msec)	94.9 ± 5.7	92.9 ± 6.2	92.2 ± 7.1	86.2 ± 7.3	82.2 ± 9.2
SCL (msec)	359 ± 10	374 ± 8	383 ± 13	404 ± 28	399 ± 40

Values are means $\pm$ S.E. ($n=5$). Abbreviations: AMP, action potential amplitude; MDP, maximum diastolic potential; $\dot{V}_{max}$, maximum upstroke velocity; APD_{50} , action potential duration at 50% repolarization; SCL, spontaneous cycle length. *Significantly different from the control at $P < 0.05$ (Student's *t*-test).

Discussion

The present study shows that S-312, a new dihydrothienopyridine derivative, markedly relaxed the contractions of isolated rabbit arteries evoked by high K^+ , 5-HT and U46619, and inhibited Ca^{++} -induced contraction in depolarized preparations. These actions are qualitatively similar to those of nifedipine and nicardipine.

In K^+ -depolarized femoral and basilar arteries, the inhibitory effect of S-312 on the Ca^{++} -induced contraction was overcome by raising the Ca^{++} concentration of the bathing solution, and a competitive antagonism was found. Such a calcium antagonistic effect on vascular smooth muscles of well-known calcium antagonists has been previously reported (24–26), and those effects with nifedipine and nicardipine were also reconfirmed in our experiments. S-312 caused dose-dependent relaxation in high K^+ -induced sustained contractions. Direct vasocontractions produced by K^+ -depolarization are mainly due to increased Ca^{++} influx through the depolarized cell membrane (27, 28). In addition, S-312 inhibited specific [3H]-NTD binding in rat cerebral cortex membrane (9). NTD has been shown to block the voltage-dependent Ca^{++} channel in vascular smooth muscle cells (29). Therefore, the calcium antagonistic and relaxant effects of S-312 in depolarized rabbit arteries observed in the present study would be due to a blockade of the transmembrane Ca^{++} influx through the voltage-dependent Ca^{++} channel. These effects of S-312 on K^+ - and Ca^{++} -induced contractions were much greater than those of nifedipine and were comparable to or slightly more potent than those of nicardipine. It is assumed, therefore, that the blocking potencies on the voltage-dependent Ca^{++} channel in vascular smooth muscles are in the order of $S-312 \geq \text{nicardipine} > \text{nifedipine}$. The present study also showed that the calcium antagonistic effect of S-312 was more pronounced in the basilar artery than in the femoral artery.

Differing from the inhibitory effect of S-312 in depolarized preparations, S-312 inhibited the NE-induced contraction only partially even at the highest concentration

tested, and non-competitive antagonism was found. NE is considered to bring about vascular contractions both by increasing the transmembrane Ca^{++} influx and by releasing Ca^{++} from the intracellular binding site (28, 30), which can be triggered by inositol trisphosphate (31, 32). A low concentration of S-312 markedly inhibited the Ca^{++} -induced contraction in NE-exposed femoral strips. However, NE-induced contraction in the Ca^{++} -free condition was not affected. These results indicate that S-312 can block the NE-operated Ca^{++} influx from extracellular fluid across the plasma membrane, not the release of intracellularly bound Ca^{++} . These properties of S-312 could explain its partial inhibition of NE-contraction in normal solution. In addition, no effect of S-312 on the contraction evoked by Ca^{++} in skinned fiber of isolated taenia caecum indicates that the calmodulin and contractile protein systems were not affected directly by S-312.

5-HT and thromboxane A_2 have been suggested as possible mediators of cerebral and coronary vasospasms (33–35). S-312 inhibited the 5-HT-induced contraction of isolated rabbit basilar artery and the U46619-induced contraction in the coronary artery more potently than nifedipine and nicardipine. Therefore S-312 may effectively prevent cerebral and coronary vasospasms caused by some circulatory disorders.

In high K^+ -depolarized femoral arteries, the duration of the vasodilating action of S-312, which was evaluated in the wash-out experiment, was longer than those of nifedipine and nicardipine. These results show that S-312 is not easily removed from the voltage-dependent Ca^{++} channel by washing. This property might explain the long lasting increase by S-312 of coronary perfusion flow in the isolated guinea pig heart (9) and also supports its long lasting vasodilating effects *in vivo* as has been discussed by other investigators (20, 26).

In isolated guinea pig atria, the negative inotropic effect of S-312 was observed at a higher concentration (above 3×10^{-7} M), and this effect was considerably weaker compared with those of nifedipine and nicardipine. Also, high concentrations (above 3×10^{-8} or 10^{-7} M) of S-312 increased the AH interval of the

His bundle electrogram in the Langendorff-perfused isolated rabbit heart and depressed the $\dot{V}_{\max}$ and AMP of the action potential in isolated rabbit sinus node preparations. These electrophysiological effects on the myocardium should be mainly due to a blockade of transmembrane Ca^{++} influx through the cardiac slow channel (36, 37). In the Langendorff-perfused isolated rabbit heart, SA and HV intervals were not affected by S-312, which indicates that S-312 does not block the fast sodium channels.

These studies thus show that S-312 seems to have higher selectivity for vascular smooth muscles, especially for cerebrovascular smooth muscles, and this selectivity is apparently greater than those of nifedipine and nicardipine. The precise explanation for such tissue and regional selectivity of drug action is not known, but this selectivity is interesting and has been studied by several investigators from the viewpoints of voltage-dependency and others (2, 29, 38).

Recently, several Ca^{++} antagonists have been widely used for the treatment of many cardiovascular disorders (2). Marked prophylactic effects of S-312 on the occurrence of stroke and significant increase of life span in SHRSP were already reported by our group (39). The potent and vasculo-selective, especially to cerebral vessels, action of S-312, and its long-lasting action may be useful in the treatment of various cerebrovascular disorders.

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