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(+)-S-12967 and (−)-S-12968: 1,4-dihydropyridine stereoisomers with calcium channel agonistic and antagonistic properties in rat resistance arteries

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1 The actions of (+)-S-12967 and (−)-S-12968 two isomers of a new 1,4-dihydropyridine (DHP) derivative, were studied on 125 mM K^+-, Ca^{2+}- and noradrenaline-induced contractions in rat isolated mesenteric resistance arteries and compared to those of nifedipine.

2 The action of (+)-S-12967 and (−)-S-12968 was slow in onset in contrast to nifedipine. Both isomers had a dual contractile and relaxant action in arteries contracted with 125 mM K^+; however, the (−)-isomer was about 300 times more potent than the (+)-isomer. The response to 125 mM K^+, being depressed by 70%, recovered within 20 to 30 min for all DHP derivatives. All vessels were treated with 1×10^{-6} M phenoxybenzamine thus excluding the possibility that the contraction is mediated by activation of amine-receptors.

3 Both (+)-S-12967 and (−)-S-12968 at low concentrations potentiated responses induced by Ca^{2+} in arteries activated by 125 mM K^+ and inhibited the responses at higher concentrations. (+)-S-12967 and (−)-S-12968 had no contractile action in arteries kept in normal buffer. Nifedipine had only an inhibitory action on vessel responses to 125 mM K^+ and Ca^{2+}.

4 Both isomers and nifedipine depressed the maximal vessel response to noradrenaline by about 20% and 44%, respectively.

5 The results confirm that DHP calcium antagonists selectively inhibit vascular smooth muscle responses induced by high potassium and that the potency of 1,4-DHP isomers may vary considerably. Furthermore, since the agonistic/antagonistic properties on the calcium channel were shared by both stereoisomers of the 1,4-DHP molecule and apparently dependent on their concentration and the vascular smooth muscle membrane potential, it suggests that the agonistic action of 1,4-DHPs may be ascribed to functional characteristics of their binding site regulating the Ca^{2+}-channel.

Keywords: 1,4-dihydropyridine; isomers; calcium channel; noradrenaline; potassium; calcium; rat mesenteric resistance artery

Introduction

Transmembrane calcium influx via specific calcium channels plays a crucial role in the excitation-contraction coupling of cardiac and smooth muscle (Bolton, 1979). One of the most common criteria used to classify the different types of calcium channels has been their sensitivity to the blocking action of 1,4-dihydropyridines (DHPs), the most potent and selective calcium entry blockers (Fleckenstein, 1977), which seem to inhibit preferentially opening of the membrane potential-sensitive calcium-channels in the cell membrane (Cauvin *et al.*, 1983; Godfraind *et al.*, 1986; Janis *et al.*, 1987). Moreover, DHPs have become important pharmacological tools for studying voltage-sensitive calcium channels, especially since the discovery that some derivatives of this group, for example Bay K 8644 (Schram *et al.*, 1983) and CGP 28392 (Loutzenhiser *et al.*, 1984), can act directly as voltage-sensitive calcium channel activators. With these drugs it was further demonstrated that the calcium agonistic action of Bay K 8644 was associated with only one of its stereoisomers, suggesting that DHP derivatives may behave as calcium entry blockers or calcium entry activators depending on the stereochemistry (Franckowiak *et al.*, 1985; Hof *et al.*, 1985).

The present study evaluates the action of the two isomers (+)-S-12967 and (−)-S-12968 of a new 1,4-DHP derivative [2-(7 amino 2,5-dioxaheptyl)3-ethoxycarbonyl 4-(2,3-dichlorophenyl) 5-methoxycarbonyl 6-methyl 1,4-dihydropyridine] (Figure 1) on mechanical responses of vascular smooth muscle of rat mesenteric resistance arteries to potassium, calcium and noradrenaline and compares it with that of nifedipine, a symmetrical DHP molecule.

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Methods

Dissection and mounting

Male Wistar rats (body wt 300–350 g) were killed by cervical dislocation and bled. The superior mesenteric artery in the proximal part of the jejunum was removed and placed in cold oxygenated (5% CO_2 in O_2) physiological salt solution (PSS, see below). Two ring segments (1–2 mm long) were dissected out from the second or third branch of the superior mesenteric artery, threaded on two stainless steel wires (40 μm diameter) and mounted on a double myograph which allowed direct determination of the isometric wall tension while the internal circumference was controlled (Mulvany & Halpern, 1977; Mulvany & Nyborg, 1980). After an equilibration period of 30 min in PSS at 37°C the vessels were normalized, that is, the internal circumference, L_1, was set to 90% of L_{100} where L_{100} is the internal circumference the vessels would have *in situ* under a transmural pressure of 13.3 kPa (100 mmHg)

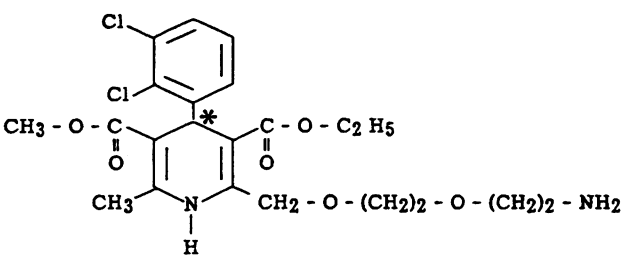

Figure 1 Chemical structure of (+)-S-12967 and (−)-S-12968, [2-(7 amino 2,5-dioxaheptyl) 3-ethoxycarbonyl 4-(2,3-dichlorophenyl) 5-methoxycarbonyl 6-methyl 1,4-dihydropyridine]. *indicates asymmetric C-atom in the dihydropyridine ring.

(Mulvany & Halpern, 1977). The vessels were finally activated three times with K-PSS (see below).

Protocol

In the first set of experiments the time-course of the effects of (+)-S-12967 and (−)-S-12968 was determined and compared with those of nifedipine. The vessels were contracted with 125 mM K-PSS and once the response had stabilized after 30 min a single concentration of either of the two isomers or nifedipine was added to the bath. The action of the compounds was studied for 120 min, the maximum time the vessels could maintain a constant contraction to 125 mM K^+ (K-PSS). The recovery of the K-PSS-induced responses was studied over the next 2 h after the vessels were washed thoroughly in drug-free PSS by activating the vessels with K-PSS (5 min) every 15 min.

The effects of (+)-S-12967, (−)-S-12968 and nifedipine were further studied on potassium-activated calcium concentration-response characteristics. The vessels were incubated in Ca^{2+}-free PSS (see below) for 5 min. The vessels were then washed twice with Ca^{2+}-free K-PSS (see below) for 10 min before Ca^{2+} was added to the tissue bath again. The vessels were then incubated with different concentrations of the isomers for 2 h and nifedipine for 30 min, respectively, in Ca^{2+}-free K-PSS before they were exposed to increasing concentrations of $CaCl_2$ again.

The vessels were initially incubated with 1 μM phenoxybenzamine for 10 min and then washed for 30 min, in order to block α-adrenoceptors and avoid the effects of the neuronally released noradrenaline (NA) by K-PSS depolarization in the experiments with vessels exposed to K-PSS.

Finally, the effects of (+)-S-12967, (−)-S-12968 and nifedipine on NA-induced contractions were studied. Two NA concentration-response curves were obtained, the first serving as control. The vessels were incubated either 2 h (isomers) or 30 min (nifedipine), respectively, before the NA concentration-response curve was repeated in the presence of the calcium antagonist. Because of the difficulty in washing out the isomers from the tissue bath, the vessels were exposed only to a single concentration of the drugs.

Solutions and drugs

The vessels were dissected and kept relaxed in PSS with the following composition (mM): NaCl 119, $NaHCO_3$ 25, KCl 4.7, KH_2PO_4 1.18, $MgSO_4$ 1.17, $CaCl_2$ 1.5, EDTA 0.026 and glucose 11.

K-PSS was similar to PSS except that NaCl was exchanged with KCl on an equimolar basis.

Ca^{2+}-free PSS and Ca^{2+}-free K-PSS were similar to PSS and K-PSS, respectively, except that $CaCl_2$ was omitted and replaced with 1×10^{-4} M EGTA. EGTA was omitted when $CaCl_2$ was readministered.

Drugs used were phenoxybenzamine (Smith Kline & French), (−)-noradrenaline HCl (Sigma); nifedipine (Bayer AG); (+)-S-12967 and (−)-S-12968 tartrate (Servier). Stock solutions of nifedipine and phenoxybenzamine were made in ethanol and then diluted in distilled water. Nifedipine solution was protected from light and experiments were carried out in darkness. (+)-S-12967 and (−)-S-12968 were water-soluble and light-insensitive.

Statistics

Vessel responses are expressed as either active wall tension, $N\,m^{-1}$ (force per vessel wall length) or as a percentage of the maximum contraction in the control concentration-response curves. In the time course experiments the effects of the drugs are expressed as a percentage of the K-PSS response at time 0, i.e., just prior to the addition of the drugs. The inhibitory potency of the drugs was estimated by determining the EC_{50}[M]-concentration, i.e. the concentration of the antagonist causing a 50% inhibition of the 125 mM K^+-induced response after 2 h of incubation with the isomers. EC_{50}[M] for nifedipine was determined in separate experiments where the vessels were exposed to increasing concentrations of nifedipine. EC_{50}[M]s were estimated by fitting plots of responses, R, vs. concentration, A[M], on a log-scale to the equation $R = R_{max} \times A[M]^n/(A[M]^n + EC_{50}[M]^n)$, where R_{max} is maximal tissue response to drug, using the commercially available GraphPAD programme. In each concentration-response experiment, sensitivities to the agonists are expressed in terms of pD_2 values where $pD_2 = -\log([EC_{50}[M])$. Differences between means were compared by Student's t test for paired observations. Significance level was for both tests set at $P < 0.05$. Values are given as mean ± s.e.mean.

Results

Both (+)-S-12967 and (−)-S-12968 had a contractile and a relaxant effect on the mesenteric resistance vessels depolarized with 125 mM K-PSS (Figure 2) in a narrow concentration range between 3×10^{-10} M and 3×10^{-9} M for (−)-S-12968 and between 1×10^{-7} M and 6×10^{-7} M for (+)-S-12967. The (−)-isomer was about 300 times more potent than the (+)-isomer. At the lowest concentrations used, (+)-S-12968 (3×10^{-10} M) and (−)-S-12967 (1×10^{-7} M) elicited contractions, of 70 ± 13% ($n = 5$) and 80 ± 11% ($n = 4$), respectively, above the K-PSS-induced response. Nifedipine, 1×10^{-6} M, totally abolished the contraction induced by both isomers ($n = 4$). The effect of the isomers became relaxations at slightly higher concentrations. The inhibitions caused by (−)-S-12968

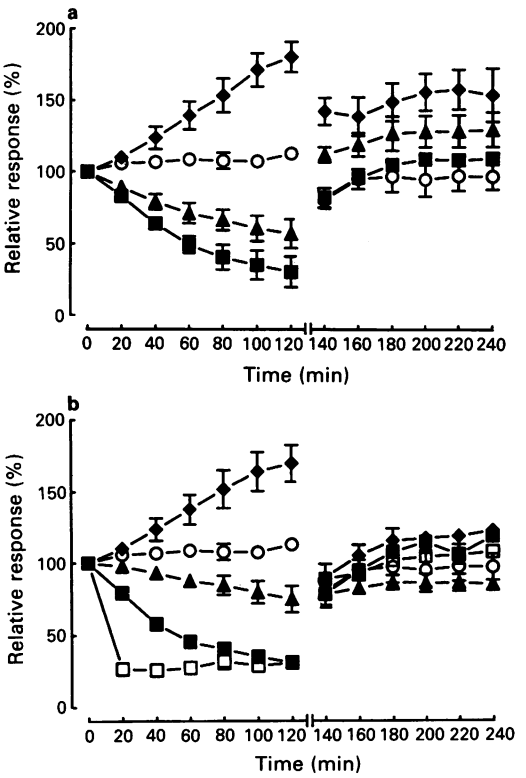

Figure 2 Time-course of the action of (+)-S-12967 (a), (−)-S-12968 (b) and nifedipine (lowest curve) on rat isolated mesenteric resistance arteries contracted with 125 mM K^+ (○, represent control) and recovery of vessel responses to 125 mM K^+ after wash out of the drugs. Wash out is indicated by a gap in the X-axes. Concentrations of antagonists: in (a): (◆) 1×10^{-7} M; (▲) 3×10^{-7} M and (■) 6×10^{-7} M; in (b) (◆) 3×10^{-10} M; (▲) 1×10^{-9} M; (■) 3×10^{-9} M and (□) nifedipine, 1×10^{-9} M. Each point represents mean of 4–8 vessels and vertical bars show ± s.e.mean. Responses are given as percentage of initial vessel tone to 125 mM K^+ just prior to addition of drug at time zero.

(3×10^{-9} M) and (+)-S-12967 (6×10^{-7} M) were $69 \pm 4\%$ ($n = 4$) and $70 \pm 11\%$ ($n = 4$), respectively, of the K^+-induced responses. No change was observed with the two isomers in normal PSS at the range of concentrations mentioned above. On the contrary, nifedipine did not elicit any contractile effect in the vessels depolarized with 125 mM K^+ when tested at low concentrations (1×10^{-15} M–1×10^{-12} M) (results not shown), but relaxed them at higher concentrations (10^{-11} M–10^{-7} M). The relative inhibitory potencies of the three drugs were in the order: nifedipine >(−)-S-12968 >(+)-S-12967, the IC_{50} values being 3.8×10^{-11} M for nifedipine, 1.9×10^{-9} M for (−)-S-12968 and 1.4×10^{-7} M for (+)-S-12967.

Both the potentiation and the inhibition induced by the isomers were characterized by a slow onset, reaching its maximal effects in about 2 h, whereas the maximal inhibition elicited by a single dose of nifedipine (1 nM) was reached in the first 20 min and remained constant thereafter throughout the 2 h drug-contact time. Recovery of the 125 mM K-induced responses was obtained within 20–30 min after the drug washout for both nifedipine and both isomers; however, the K^+-induced contractions which developed in the arteries treated with (−)-S-12968 and (+)-S-12967, especially those with the (+)-isomer, were significantly increased compared with the controls during the 2 h washout period.

Figure 3 shows the effects of (−)-S-12968 and (+)-S-12967 and nifedipine on the calcium concentration-response characteristics of mesenteric resistance arteries activated with 125 mM K-PSS. (−)-S-12968 (3×10^{-10} M) and (+)-S-12967 (1×10^{-7} M) induced a significant leftward shift of the concentration-response curves but no change in the maximal responses (Table 1). Higher concentrations of the two isomers reduced in a non-competitive concentration-dependent manner both the sensitivity and maximal contraction (Figure 3, Table 1). Nifedipine, tested in a wider range of concentrations (1×10^{-10} M–1×10^{-7} M), did not potentiate the calcium-induced contractions but decreased both sensitivity and maximal tension gradually.

Table 1 Effect of (+)-S-12967, (−)-S-12968 and nifedipine on sensitivity and maximal response to Ca^{2+} in 125 mM K^+ activated rat mesenteric resistance arteries

	(+)-S-12967	
Concentration, (M)	pD_2	ΔT_{max}
Control	3.08 ± 0.04	2.91 ± 0.08 (12)
0.1 μM	3.55 ± 0.08[b]	3.10 ± 0.21[NS] (4)
0.3 μM	2.10 ± 0.29[b]	1.93 ± 0.38[b] (4)
0.6 μM	0.42 ± 0.11[a]	0.36 ± 0.08[a] (4)
	(−)-S-12968	
Concentration, (M)	pD_2	ΔT_{max}
Control	2.93 ± 0.08	2.70 ± 0.16 (13)
0.3 nM	3.70 ± 0.09[b]	2.85 ± 0.32[NS] (5)
1 nM	2.18 ± 0.13[c]	2.00 ± 0.26[c] (4)
3 nM	0.93 ± 0.11[a]	0.79 ± 0.12[b] (4)
	Nifedipine	
Concentration, (M)	pD_2	ΔT_{max}
Control	3.22 ± 0.05	2.14 ± 0.16 (19)
1 nM	2.71 ± 0.09[b]	1.92 ± 0.22[c] (7)
10 nM	1.78 ± 0.11[a]	1.22 ± 0.25[b] (8)
100 nM	1.07 ± 0.12[a]	0.38 ± 0.08[b] (4)

Values given as mean ± s.e.mean (number of vessels). Sensitivities are expressed as pD_2-values and ΔT_{max} is maximal tension developed by vessels to Ca^{2+}. Control values represent mean for all vessels used. [a] $P < 0.05$; [b] $P < 0.01$; and [c] $P < 0.001$ different from control value tested by Student's t test for paired data. NS is not significant.

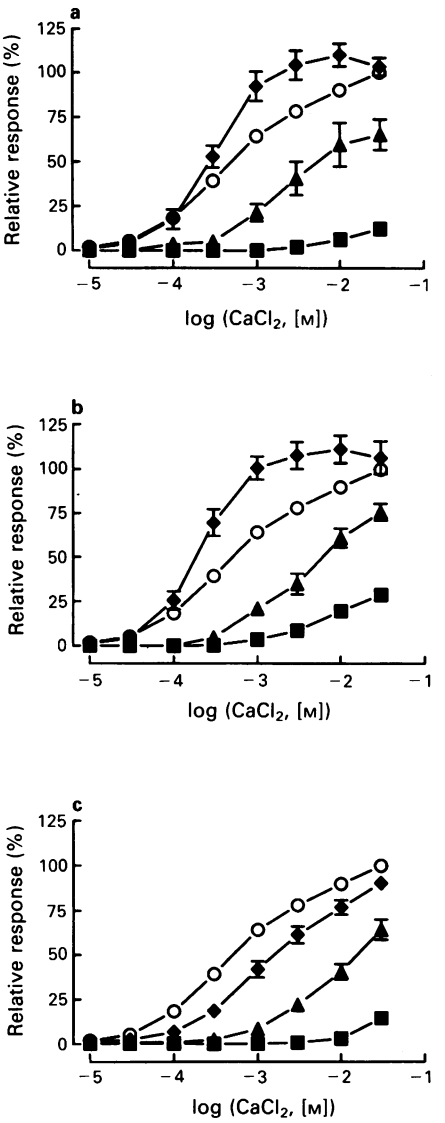

Figure 3 Effect of (a) (+)-S-12967, (b) (−)-S-12968 and (c) nifedipine on Ca^{2+} concentration-response characteristics of isolated mesenteric resistance arteries activated with 125 mM K^+ in Ca^{2+}-free PSS. Responses are calculated with reference to maximal response of the control concentration-response curve (○). Points represent means of 4–19 vessels and vertical bars show ± s.e.mean. Concentration of antagonists are: in (a) (◆) 1×10^{-7} M; (▲) 3×10^{-7} M and (■) 6×10^{-7} M; in (b): (◆) 3×10^{-10} M; (▲) 1×10^{-9} M and (■) 3×10^{-9} M; and in (c): (◆) 1×10^{-9} M; (▲) 1×10^{-8} M and (■) 1×10^{-7} M.

The effects of (−)-S-12968, (+)-S-12967 and nifedipine on NA contraction-response characteristics of the mesenteric resistance arteries are illustrated in Figure 4. Sensitivity and maximal contractions to NA of the arteries are given in Table 2. Responses to NA were much less affected by the isomers than those induced by calcium. The maximal responses still present at the highest concentrations of (−)-S-12968 (1×10^{-8} M) and (+)-S-12967 (1×10^{-6} M) were $80 \pm 1\%$ ($n = 4$) and $80 \pm 1\%$ ($n = 4$) of the controls, respectively. There was a small decrease in sensitivity to NA at these concentrations and also a slight but signficant increase in NA sensitivity in arteries treated with 3×10^{-7} M (+)-S-12967. Nifedipine affected the contractile responses to NA more than (−)-S-12968 and (+)-S-12967. When used in the same range of concentrations as that for the calcium curves (1×10^{-9} M–1×10^{-7} M), nifedipine caused a gradual decrease in both sensitivity and maximal tension development to NA. The maximal response to NA at the highest concentration of

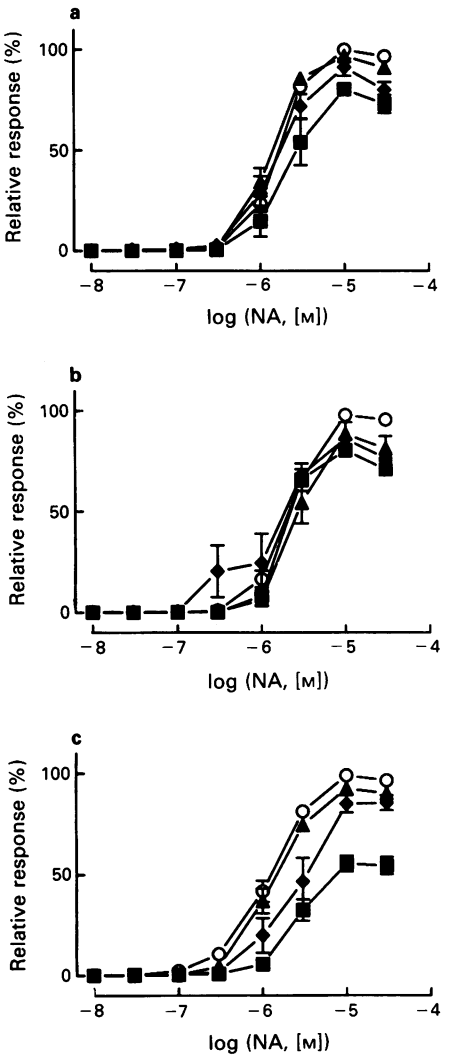

Figure 4 Effect of (a) (+)-S-12967, (b) (−)-S-12968 and (c) nifedipine on noradrenaline (NA) concentration-response characteristics of isolated mesenteric resistance arteries. Responses are calculated with reference to maximal response of the control concentration-response curve (○). Points represent means of 4–20 vessels and vertical bars show ± s.e.mean. Concentration of antagonists are: in (a): (◆) 1×10^{-7} M; (▲) 3×10^{-7} M and (■) 1×10^{-6} M; in (b): (◆) 3×10^{-10} M; (▲) 1×10^{-9} M and (■) 1×10^{-8} M; in (c): (◆) 1×10^{-9} M; (▲) 1×10^{-8} M and (■) 1×10^{-7} M.

nifedipine was 56 ± 4% ($n = 6$) of the control response and the pD_2 was 14 fold reduced.

Discussion

We have examined in the present study the effects of the two isomers of a new 1,4-DHP derivative on rat mesenteric resistance vessels, and the following observations can be made from these investigations. First, significant stereoselectivity of action has been detected, the (−)-isomer being about 300 times as potent as the (+)-isomer; there is no stereoselectivity, however, concerning opposing biological activities, since both isomers shared dual agonistic/antagonistic properties depending on only the concentration of drug used. Second, the isomers may be included in the new group of 1,4-DHPs with slow onset and long-lasting action, such as amlodipine (Burges *et al.*, 1985), lacidipine (Carpi *et al.*, 1986) and OPC-13340 (Nakayama *et al.*, 1990). Third, both isomers were much more effective on potassium and calcium-induced contractions than on those elicited by NA, thus indicating a selective action on the voltage-dependent as opposed to the receptor-operated calcium channels.

Table 2 Effect of (+)-S-12967, (−)-S-12968 and nifedipine on sensitivity and maximal response to noradrenaline (NA) in rat mesenteric resistance arteries

	(+)-S-12967	
Concentration, (M)	pD_2	ΔT_{max}
Control	5.77 ± 0.04	3.44 ± 0.27 (14)
0.1 μM	5.72 ± 0.08^{NS}	2.80 ± 0.47^{NS} (6)
0.3 μM	5.86 ± 0.05^{c}	3.35 ± 0.27^{b} (4)
1 μM	5.46 ± 0.09^{b}	3.22 ± 0.43^{b} (4)
	(−)-S-12968	
Concentration, (M)	pD_2	ΔT_{max}
Control	5.65 ± 0.05	3.45 ± 0.20 (20)
0.3 nM	5.92 ± 0.22^{NS}	2.67 ± 0.21^{a} (6)
1 nM	5.54 ± 0.02^{NS}	3.39 ± 0.51^{NS} (6)
10 nM	5.53 ± 0.03^{c}	3.41 ± 0.09^{a} (4)
	Nifedipine	
Concentration, (M)	pD_2	ΔT_{max}
Control	5.93 ± 0.01	2.76 ± 0.20 (18)
1 nM	5.54 ± 0.14^{c}	2.80 ± 0.34^{c} (8)
10 nM	5.83 ± 0.07^{b}	2.42 ± 0.54^{c} (4)
100 nM	4.87 ± 0.11^{b}	1.22 ± 0.24^{a} (6)

Values given as mean ± s.e.mean (number of vessels). Sensitivities are expressed as pD_2-values and ΔT_{max} is maximal tension developed by vessels to NA. Control values represent mean for all vessels used. $^{a}P < 0.05$; $^{b}P < 0.01$; and $^{c}P < 0.001$ different from control value tested by Student's t test for paired data. NS is not significant.

Dual calcium channel activator/inhibitor properties have previously been reported for some 1,4-DHP derivatives. Thus, nifedipine, nicardipine, nimodipine and nitrendipine have been shown to display an agonistic activity at low concentrations (Schram *et al.*, 1983; Hess *et al.*, 1984; Thomas *et al.*, 1984; Schwartz *et al.*, 1984; Dubé *et al.*, 1985; Frank, 1990). On the other hand, the antagonistic activity of Bay K 8644 at high concentrations (Thomas *et al.*, 1984; Schwartz *et al.*, 1984; Dubé *et al.*, 1985) has been attributed, in part, to the racemic mixture of Bay K 8644 in which one of the isomers exhibits agonist activity and the other has the opposite effect on the membrane potential-operated calcium channel (Hof *et al.*, 1985). However, this explanation does not seem to be sufficient to explain the mechanism of action of all 1,4-DHP calcium channels activators and inhibitors, since the action of these drugs can be changed from antagonistic to agonistic and *vice versa* not only by modifications in the molecular conformation, but also by changes in the concentration (Thomas *et al.*, 1984; Franckowiak *et al.*, 1985; McDonald *et al.*, 1987) and membrane potential (Hess *et al.*, 1984; Kokubun *et al.*, 1986). Thus, in the study by Franckowiak *et al.* (1985), the agonistic isomer (−)-Bay K 8644 had a partial antagonistic effect at high concentrations on guinea-pig aortic rings and isolated perfused heart; in addition, the calcium channel-activating effect of the (+)-(S)-enantiomer of 202-791 became a blocking effect at holding membrane potentials postive to −20 mV (Kokubun *et al.*, 1986).

Our experiments with the new 1,4-DHP isomers in rat mesenteric vascular smooth muscle demonstrate that the ability to act as an agonist or an antagonist on the calcium channel resides in the same molecule and is determined by the concentration of the drug rather than by its molecular conformation or isomeric properties at least when the vessels are kept in K-PSS. Similar results have been recently reported for another 1,4-DHP derivative, DHMP (Kaplita *et al.*, 1990), which behaves as a weak mixed calcium agonist/antagonist on guinea-pig ileum at low and high concentrations, respectively. However, the authors attribute these properties to the sym-

metry of DHMP molecule. In addition, the action of both (−)-S-12968 and (+)-S-12967 seems to be voltage-dependent, since they exhibited their agonistic effect only in arteries depolarized with 125 mM K-PSS, but not in the basal resting state. This agrees, in part, with that reported for the two isomers of another 1,4-dihydropyridine derivative, isradipine (PN 200 110, Morel & Godfraind, 1987). In this case, membrane depolarization increased both the blocking effect on KCl contractions and the binding affinity of the two isomers. The authors explain this according to the 'modulated receptor theory' (Godfraind, 1986) in which the binding of a drug to a site within the calcium channel is modulated by the state of activation of the channel which again is regulated by the membrane potential. We must note that a definitive proof of regulation of membrane potential-operated calcium-channels by the isomers studied here can only be derived from patch- or whole cell clamp experiments but the complete relaxation of vessels contracted with low agonistic concentrations of both isomers on top of the K-PSS-induced response with a high concentration of nifedipine supports the view that both isomers activate these calcium-channels in the mesenteric vascular smooth muscle. Furthermore the very high concentration of phenoxybenzamine used would eliminate all amine receptor and cholinoceptor systems in the vessels.

Our results strongly confirm the stereoselectivity reported for several pairs of isomers of 1,4-DHPs, the (−)-enantiomer being 2–3 orders of magnitude more potent than the (+)-enantiomer, as has been demonstrated for Bay K 8644 (Franckowiak *et al.*, 1985), S-202 791 (Hof *et al.*, 1985) and amlodipine (Rigby *et al.*, 1988). The potency and the difference between the isomers studied here is however greater than that published by Randle *et al.* (1990) using the same isomers in binding studies on heart and vascular smooth muscle. An agonistic activity of the isomers was not observed on the inward Ca^{2+} current in guinea-pig isolated myocytes (Randle *et al.*, 1990).

One of the main differences found in the present study between the two isomers and nifedipine, the prototype of the 1,4-DHP family, is their slow onset in action. This property has also been described for amlodipine in rat aorta and portal vein (Burges *et al.*, 1987) and pig and human coronary arteries (Matlib *et al.*, 1988), and for lacidipine in rabbit ear artery (Michelli *et al.*, 1990). This pharmacological feature of amlodipine has been attributed to its low lipid solubility due to the presence of the side chain in the 2-position of the DHP ring carrying a basic amino group which gives the molecule a pKa of 8.6, thus rendering the molecule more than 90% ionized at physiological pH (Burges *et al.*, 1989). This is also the case for (+)-S-12967 and (−)-S-12968 (pKa 8.6), and it seems that this chemical feature is responsible for marked differences in physicochemical, pharmacological and pharmacokinetic properties of this new group of slow-acting DHPs, since binding experiments demonstrate that amlodipine displays slow association and dissociation rates with the calcium channel receptor. Furthermore, the haemodynamic responses of amlodipine in animals are also characterized by a gradual onset of action and by long plasma half-life (Burges *et al.*, 1989).

It has also been reported that the effect of this new type of 1,4-DHPs was reversed very slowly after wash-out *in vitro* (Carpi *et al.*, 1986; Burges *et al.*, 1987; Michelli *et al.*, 1990). In our experiments, 125 mM K^+-induced contractions reached pre-drug levels 20–30 min after wash-out of the two isomers as well as nifedipine. This was expected for nifedipine according to its kinetics of action. However, the quick recovery of the vessel responses after the long-lasting action of the isomers could be explained by the contractile effect of these drugs at low concentrations, assuming that some drug is still present in the cell membrane.

There is now considerable evidence suggesting that there are at least two types of calcium channels in the smooth muscle cells (Bolton, 1979; Cauvin *et al.*, 1983; Janis *et al.*, 1987): potential-operated channels (POCs) and receptor-operated channels (ROCs) which are activated by membrane depolarization and receptor occupation by agonists, respectively. One of the most common criteria used to establish this distinction has been that POCs are selectively blocked by organic calcium antagonists, whereas ROCs are much more resistant to this blocking effect. In addition, the extracellular calcium-dependence and release of calcium from intracellular pools induced by several agonists, has also to be taken in account when determining the presence of ROCs in vascular smooth muscle.

The results of the present study clearly demonstrate that (+)-S-12967 and (−)-S-12968 are much more effective in blocking contractions of vessels activated by potassium than by NA. This selectivity of calcium antagonists has been reported in several vascular beds (Schuman *et al.*, 1975; Kondo *et al.*, 1980; Godfraind *et al.*, 1986), and specifically for some new 1,4-DHP derivatives such as felodipine in rat resistance mesenteric vessels (Nyborg & Mulvany, 1984) and amlodipine in rat aorta (Matlib *et al.*, 1988). In addition, the electrophysiological study by Mulvany *et al.* (1982) demonstrates that NA (10 μM) induces a significant depolarization in rat mesenteric arteries; therefore, the possibility cannot be excluded that at least a part of the noradrenaline contraction in these arteries is due to the influx of calcium through POCs, since it is known that NA responses in rat resistance mesenteric arteries are mostly dependent on the influx of extracellular calcium (Mulvany & Nyborg, 1980; Högestätt, 1984; Nyborg & Mulvany, 1984). However, the action of (+)-S-12967 and (−)-S-12967 seems to be rather selective on POCs, since NA contractions are very little affected even by higher concentrations than those which almost totally block calcium contractions in potassium-activated vessels. The effect of nifedipine compared with that of (+)-S-12967 and (−)-S-12968, appears to be less selective since both sensitivity and maximal responses to NA are greatly reduced at the same concentration range as that used to depress the calcium-induced responses.

In summary, the present study demonstrates that (+)-S-12967 and (−)-S-12968 behave as slow-acting calcium channel modulators in rat resistance mesenteric arteries in contrast to nifedipine; the action of the isomers is stereoselective, but they both share agonistic/antagonistic properties on the calcium channel. Nifedipine had only an inhibitory action on the mesenteric resistance arteries. In addition, the two isomers selectively act on contractions induced by potassium as opposed to those induced by NA.

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