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Nitric oxide is a mediator of tachykinin NK_3 receptor-induced relaxation in rat mesenteric artery

Aki Mizuta, [1]Yukio Takano, Kenji Honda, Ryo Saito, [2]Takafumi Matsumoto & Hiro-o Kamiya

Department of Pharmacology, Faculty of Pharmaceutical Sciences, Fukuoka University, Fukuoka 814-80, Japan

1 The mechanism of vasodilatation induced by tachykinin peptides was studied in isolated mesenteric arteries of rats.

2 Senktide, a selective NK_3 agonist, elicited potent endothelium-dependent relaxation of arteries precontracted with phenylephrine (10^{-5}M), but an NK_1 agonist did not.

3 A non-peptide NK_3 antagonist, SR 142801, inhibited senktide-induced relaxation. However, a non-peptide NK_1 antagonist, CP-96,345, and a peptide-based NK_2 antagonist, L-659,877, had no effect on senktide-induced relaxation.

4 N^{ω}-nitro-L-arginine (L-NOARG), a nitric oxide synthesis inhibitor, markedly attenuated the relaxant response to senktide.

5 These results suggest that the endothelium of rat mesenteric arteries possesses tachykinin NK_3 receptors, and that NK_3 agonist-induced vasodilatation is mediated by release of nitric oxide (NO) from the endothelium.

Keywords: Mesenteric artery; tachykinin; nitric oxide (NO); vasodilatation; NK_3 receptor; endothelium

Introduction

Tachykinin peptides have a common C-terminal amino acid sequence (Phe-X-Gly-Leu-Met-NH_2), and show a variety of pharmacological effects. Several lines of evidence indicate the existence of three major tachykinin receptors: NK_1, NK_2 and NK_3, which have highest affinities for substance P (SP), neurokinin A (NKA) and neurokinin B (NKB), respectively (see reviews by Erspamer, 1981; Nakanishi, 1987; Maggio, 1988; Takano & Kamiya, 1991; Otsuka & Yoshioka, 1993).

It is widely thought that the peripheral resistance of blood vessels is mainly regulated by sympathetic vasoconstrictor nerves, because various peripheral blood vessels isolated from mammals show a contractile response to noradrenaline which is released from vascular adrenergic nerves. Recently, calcitonin gene-related peptide (CGRP) was shown to be an important neuropeptide involved in the nerve-induced vasodilatation of rat mesenteric blood vessels (Kawasaki *et al.*, 1988; 1990).

On the other hand, SP has been shown to cause endothelium-dependent relaxation of precontracted arteries of the rabbit, dog and cat (Zawadzki *et al.*, 1981) and precontracted porcine coronary arteries (Regoli *et al.*, 1977; Cocks & Angus, 1983). Thus, SP dilates blood vessels mainly via the release of relaxing substances from the endothelium (Furchgott, 1983; Toda *et al.*, 1991; Stubbs *et al.*, 1992). In addition, binding studies have demonstrated that the endothelial cells of porcine aorta have NK_1 receptors which are coupled to G-protein (Saito *et al.*, 1990). Although there is much evidence for vasodilatation mediated by NK_1 receptors, little is known about NK_3 receptor-mediated relaxation of arteries.

In this study, we examined the mechanism of vasodilatation mediated by tachykinin peptides in isolated mesenteric arteries of rats, and obtained evidence that NK_3 agonist-induced vasodilatation is mediated by nitric oxide (NO) released from the endothelium of these arteries. We used senktide rather than NKB as an NK_3 agonist because the latter is essentially insoluble in water (Wormser *et al.*, 1986).

[1] Author for correspondence.
[2] Present address: Exploratory Research Lab., Dainippon Pharmaceut. Co., Ltd., Osaka 564, Japan.

Methods

Preparations

Male Wistar rats (200-250 g) were killed by decapitation and their mesenteric arteries were removed. The arteries were placed in chilled Krebs buffer solution (composition in mM: NaCl 118.7, KCl 4.7, $CaCl_2$ 1.8, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 24.8 and glucose 10.1; pH 7.4). They were then freed from adipose and connective tissues under a microscope, and cut into rings (0.8-1.2 mm o.d. and 2 mm long). Each ring was mounted on an L-shaped wire attached to a force-displacement transducer (NEC-Sanei, Tokyo, Japan) in a 5 ml organ bath (US-5, UFER, Kyoto, Japan). The organ bath was maintained at 37°C and constantly gassed with a mixture of 95% O_2 and 5% CO_2.

Relaxation response

The arterial rings were mounted in the organ bath and they were allowed to stabilize for 3 h. Then, the resting tension was adjusted to 1.0 g. The arterial rings were allowed to equilibrate for about 30 min in the normal medium, during which time the solution was replaced every 10 min. Before the start of experiments, the equilibration was confirmed by 50 mM K^+-precontraction responses. After the equilibration period, the relaxant responses of phenylephrine (1×10^{-5}M)-contracted rings to the tachykinin peptides were measured.

Tachykinin peptides were added to the bath cumulatively to determine the concentration-response curve of each ring. Tachykinin antagonists were added 10 min before the agonist. The pA_2 values were obtained from a Schild plot (Arunlakshana & Schild, 1959). Relaxation responses to peptides were expressed relative to the contractile response to phenylephrine (1×10^{-5}M). To exclude tachyphylaxis to tachykinin peptides, the peptides were administered again after equilibration for 1-2 h.

To remove the endothelium, basilar aorta near the branch to the mesenteric artery was connected with a syringe, and the other side was fastened, then 5 ml of Krebs solution was injected rapidly 4-5 times. At the end of each experiment, the presence of the endothelium was confirmed by the abolition or marked suppression of the relaxation produced by acetylcholine (ACh; 1×10^{-7}M).

The relaxation responses of guinea-pig aorta were measured as described previously (Saito *et al.*, 1991).

Drugs

SP and NKA were obtained from the Peptide Institute (Osaka, Japan), NKB and senktide (Suc-[Asp^6, MePhe^8]-substance P 6–11) from Peninsula Laboratories (California, U.S.A.). N$^\omega$-nitro-L-arginine and indomethacin were obtained from Sigma (St. Louis, U.S.A.). ACh was from Daiichi Pharmaceutical Co. (Tokyo, Japan), nitroglycerin (GTN) from Nippon-Kayaku Co. (Tokyo, Japan), phenylephrine from Wako (Osaka, Japan), and atropine from Merck (Darmstadt, Germany). CP-96,345 ((2S, 3S)-*cis*-2-(diphenylmethyl)-N-[(2-methoxyphenyl)-methyl]-l-azabicyclo [2.2.2] octan-3-amine) was a gift from Pfizer (Groton, U.S.A.). L-659,877 (cyclo (Leu-Met-Gln-Trp-Phe-Gly)) was a gift from Merck (Essex, U.K.). SR 142801 ((S)-(N)-(1-(3-(1-benzoyl-3-(3,4-dichlorophenyl) piperidin-3-yl)-4-phenylpiperidin-4-yl)-N-methylacetamide) was a gift from Sanofi (Cedex, France).

Statistics

Statistical analysis of data was performed by the two-tailed Student's *t* test or Dunnett's test.

Results

Figure 1 shows typical recordings of relaxant responses by tachykinin peptides of rat mesenteric arteries and guinea-pig aorta. The selective NK_3 receptor agonist senktide caused relaxation of rat isolated mesenteric arteries pre-contracted by phenylephrine (1×10^{-5} M). ACh (1×10^{-7} M) also elicited relaxation of endothelium-intact rings. In endothelium-denuded arteries, senktide and ACh did not induce a relaxant response although GTN (1×10^{-5} M) induced marked relaxation (Figure 1a). The endothelium-dependent relaxation induced by senktide was not inhibited by atropine (1×10^{-6} M) or indomethacin (1×10^{-5} M) (data not shown). The NK_1 agonist SP did not have any effect in rat mesenteric arteries (Figure 1a), although it caused a marked relaxation of guinea-pig aorta (Figure 1b).

The relaxation induced by senktide was dose-dependent, with an EC_{50} value of 1.52×10^{-8} M, calculated by Hill's equation. The endogenous NK_3 agonist NKB, also caused relaxation having a similar maximal effect to that of senktide (Figure 2). As shown in Figure 3, the non-peptide NK_3 antagonist SR 142801 (3×10^{-7}–3×10^{-6} M) produced a dose-dependent rightward shift of the concentration-response curves of senktide-induced relaxation. A Schild plot of the results gave a straight line slope (slope; 0.96), indicating competitive antagonism. The pA_2 value was 6.87. However, the NK_1 antagonist CP-96,345 (1×10^{-6} M) and the NK_2 antagonist L-659,877 (1×10^{-6} M) did not (data not shown).

Figure 4 shows the effect of the NO synthesis inhibitor N$^\omega$-nitro-L-arginine (L-NOARG) on vasodilatation induced by senktide in rat mesenteric arteries. In arterial rings with intact endothelium, L-NOARG inhibited senktide-induced relaxation concentration-dependently (1×10^{-6} M – 1×10^{-4} M).

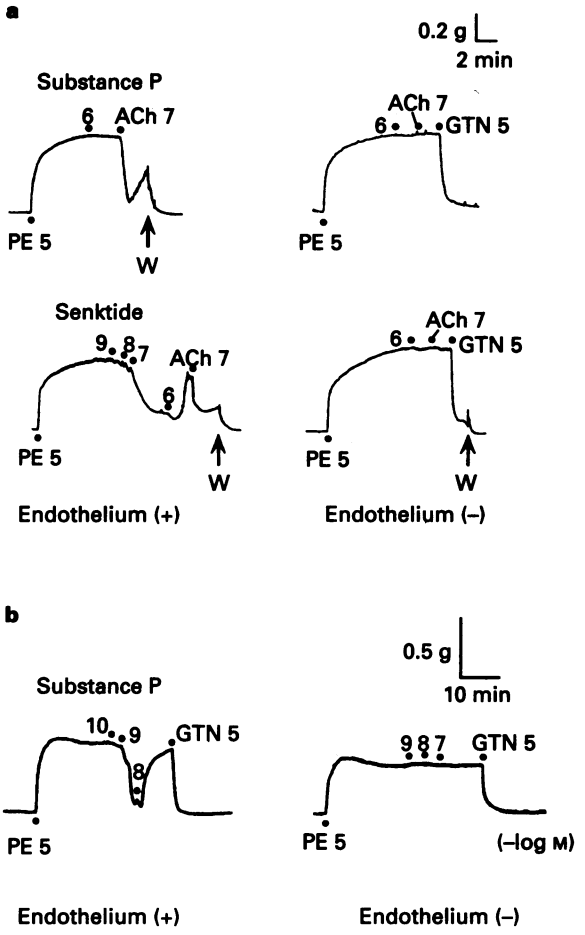

Figure 1 Typical recordings of relaxant responses by tachykinin peptides of rat mesenteric arteries (a), and guinea-pig aorta (b). Arteries with and without endothelium were precontracted by phenylephrine (1×10^{-5} M). PE, phenylephrine; ACh, acetylcholine; W, washing; GTN, nitroglycerin.

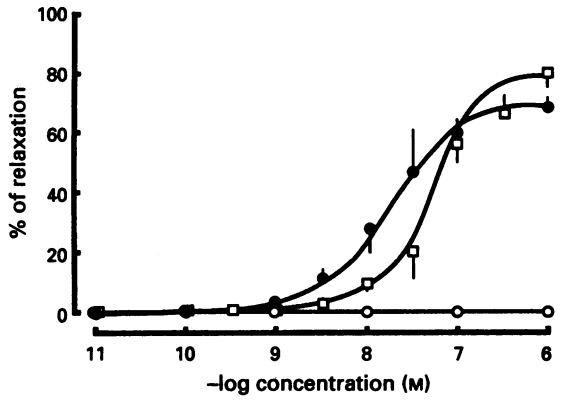

Figure 2 Cumulative concentration-dependent responses to substance P (○), senktide (●) and neurokinin B (□) of mesenteric arteries of rats with intact endothelium. Mesenteric arteries were precontracted with phenylephrine (1×10^{-5} M). Relaxation is expressed as a percentage of the initial contraction. Values are means ± s.e.mean in at least 4 experiments.

Discussion

The present results demonstrated that a tachykinin NK_3 agonist, senktide, caused potent endothelium-dependent relaxation of rat mesenteric artery. Recently, the selective, non-peptide NK_3 receptor antagonist SR 142801 was developed (Emonds-Alt *et al.*, 1994). SR 142801 dose-dependently inhibited the senktide-induced relaxation ($pA_2 = 6.87$), suggesting that functional NK_3 receptors in rat mesenteric artery are present, although we have no direct evidence for them, such as receptor binding sites or expression of NK_3 receptor mRNA. The pA_2 value of SR142801 in rat mesenteric artery is lower than the pA_2 value obtained in guinea-pig ileum (Emonds-Alt *et al.*, 1994). In the central nervous system, NKB is suggested to be a neurotransmitter in the central regulation of water balance and cardiovascular activity (Takano *et al.*, 1990; Nakayama *et al.*, 1992; Saigo *et al.*, 1993). In addition, high NKB-like immunoreactivity is found in the hypothalamus and nucleus tractus solitarius (Nagashima *et al.*, 1989).

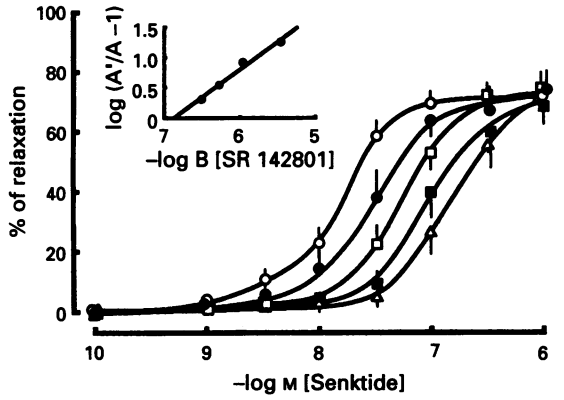

Figure 3 Concentration-response curves for senktide-induced vasodilatation in rat mesenteric arteries in the absence (control) and in the presence of SR 142801: control (○), SR 142801 3×10^{-7}M (●), 5×10^{-7}M (□), 1×10^{-6}M (■), 3×10^{-6}M (△). Schild plot for the determination of the pA_2 value of SR 142801. The regression line was calculated by a computer programme for linear regression (Insert). Relaxation is expressed as a percentage of the initial contraction. Values are means±s.e.mean for 3–6 experiments.

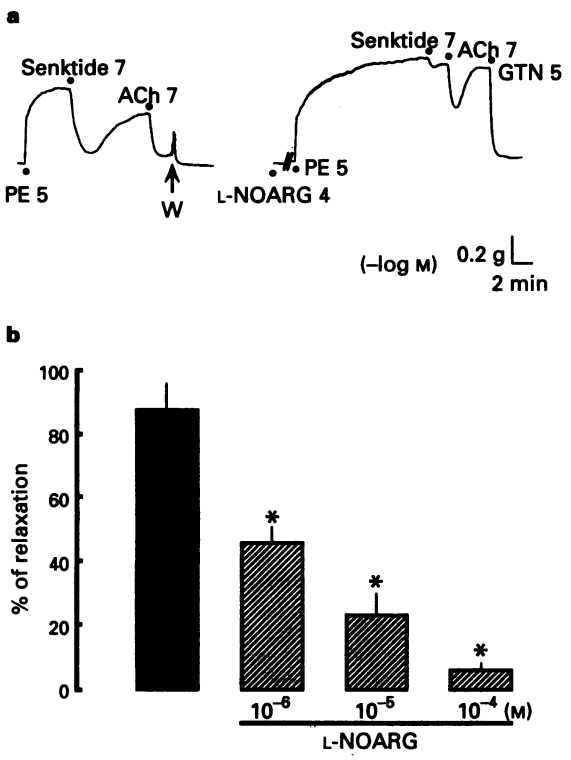

Figure 4 Effects of N^{ω}-nitro-L-arginine (L-NOARG) on vasodilatation induced by senktide (1×10^{-7}M) in rat mesenteric arteries. (a) Typical recordings of relaxant responses by senktide in the absence and presence of L-NOARG (1×10^{-4}M). Arteries were precontracted with phenylephrine (1×10^{-5}M). PE, phenylephrine; ACh, acetylcholine; W, washing; GTN, nitroglycerin. (b) Inhibitory effect of L-NOARG (1×10^{-6}–1×10^{-4}M) on vasodilatation caused by senktide. Values are means±s.e.mean for 4–6 experiments. *Significantly different from control at $P<0.01$.

Senktide-induced relaxation was endothelium-dependent and was inhibited by the NO synthesis inhibitor L-NOARG. These findings suggest that the NK_3 agonist senktide stimulates the release of NO from the endothelium, which activates the production of guanosine 3':5'-cyclic GMP (cyclic GMP) for muscle relaxation. The senktide-induced relaxation was not influenced by indomethacin or atropine, suggesting that the arachidonic cascade and cholinergic system are not involved. However, other relaxant factors, such as the ATP-activated K^+ channel or voltage-dependent K^+ channel remain to be elucidated.

Interestingly, SP did not cause vasodilatation of the rat mesenteric artery, although numerous isolated arteries are sensitive to SP. This finding is consistent with the observation that SP did not produce any relaxation of the rat perfused mesenteric artery (Li & Duckles, 1992). As SP has previously been reported to produce vasodilator responses in mesenteric arteries of rabbit or dog (Angus *et al.*, 1986; Stewart-Lee & Burnstock, 1989), it is suggested that the vasodilator responses to SP depend on the species or tissue.

Kawasaki *et al.* (1990) reported that CGRP is a potent neurotransmitter of the non-adrenergic non-cholinergic (NANC) vasodilator nerve in the perfused mesenteric vascular bed of rats. In addition, Claing *et al.* (1992) suggested that SP may be an important mediator of the response of veins, but not arteries, although Barja *et al.* (1983) showed that SP-immunoreactive fibres are present in rat mesenteric arteries. These results indicate that the NK_1 receptor in the mesenteric arteries does not play a role in vasomotor function. On the other hand, the NK_3 agonists senktide and NKB caused potent vasodilatation of mesenteric arteries, suggesting that NK_3 receptors are important in vasodilatation. However, it is still uncertain whether vasodilator nerves containing NKB are present and how NKB is involved in the functional regulation of the peripheral resistance of blood vessels.

The present results showed that the NK_3 agonist senktide caused vasodilatation mediated by NO in rat mesenteric arteries.

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