ELSEVIER

European Journal of Pharmacology 283 (1995) 233–240

ejp

Tachykinin NK_1 receptors mediate both vasoconstrictor and vasodilator responses in the rabbit isolated jugular vein

Riccardo Patacchini *, Carlo Alberto Maggi

Pharmacology Department, Research Laboratories, A. Menarini Pharmaceuticals, Via Sette Santi 3, 50131 Florence, Italy

Received 2 March 1995; revised 6 June 1995; accepted 13 June 1995

Abstract

We have characterized the receptor(s) mediating contraction and relaxation produced by tachykinins in the rabbit isolated jugular vein. The tachykinin NK_1 receptor-selective agonists septide and [Pro^9]substance P produced concentration-dependent contractions which were potentiated by either the removal of the vascular endothelium (E_{max} = +106% and +72%, respectively) or by pretreatment with L-nitroarginine (100 μM; 60 min before) (E_{max} = +123% and +71%, respectively). The tachykinin NK_1 receptor-selective antagonist, (±)-CP-96,345 ([2-(diphenylmethyl)-*N*-[(2-methoxyphenyl)-methyl]-1-azabicyclo[2,2,2]octan-3-amine]) (10–300 nM) competitively antagonized septide (pK_B = 9.0) with 10-fold greater potency than [Pro^9]substance P (pK_B = 8.0). In preparations with intact endothelium both septide and [Pro^9]substance P (from 0.1 to 100 nM) relaxed the noradrenaline-(10 μM) induced tone, and their effects were markedly reduced by (±)-CP-96,345 (100 nM). In noradrenaline-precontracted veins L-nitroarginine (100 μM) reversed the tachykinin-induced vasodilation into a contraction, providing evidence for the involvement of nitric oxide in this response. The tachykinin NK_3 and NK_2 receptor-selective agonists senktide and [βAla^8]neurokinin A-(4–10) were either ineffective, or produced small effects antagonized by (±)-CP-96,345 (100 nM), respectively. In conclusion, tachykinin NK_1 receptors mediate both tachykinin-induced contraction and relaxation in the rabbit jugular vein. This preparation, deprived of the endothelium or pretreated with L-nitroarginine, is suitable for evaluating tachykinin agonists or antagonists.

Keywords: Tachykinin; Tachykinin NK_1 receptor; Endothelium; Nitric oxide (NO); Jugular vein, rabbit

1. Introduction

Tachykinins are a family of peptides which exert a wide variety of biological effects through the stimulation of three distinct receptor types, termed NK_1, NK_2 and NK_3 (Regoli et al., 1989; Guard and Watson, 1991; Maggi et al., 1993). Vasodilatation is one of the most prominent effects elicited by substance P and related tachykinins, which produce transient hypotension in vivo, and relaxation of precontracted blood vessels in vitro (Maggi, 1995, for review). The vasodilator effect of tachykinins is transient in nature, undergoes rapid desensitization and is strictly dependent on the presence of intact endothelium (D'Orléans-Juste et al., 1985, 1986; Duckles, 1986; Franco-Cereceda et al., 1987; McEwan et al., 1988). Early experiments with selective tachykinin receptor-selective agonists suggested that tachykinin NK_1 receptors, located on endothelial cells, mediate the vasodilator effects elicited by tachykinins (see Maggi et al., 1993, for review). Further studies, using some recently developed tachykinin NK_1 receptor-selective antagonists, such as CP-96,345 (Constantine et al., 1991; Lembeck et al., 1992; Rubino et al., 1992), GR 82,334 (Stubbs et al., 1992; Beattie et al., 1993) and SR 140333 (Emonds-Alt et al., 1993; Hall and Brain, 1994), have provided conclusive evidence that the tachykinin-induced vasodilatation and hypotension in vivo require the activation of tachykinin receptors of the NK_1 type. In parallel, the use of inhibitors of nitric oxide generation has allowed the identification of nitric oxide as a mediator involved in the tachykinin-induced endothelium-dependent vasodilation (Whittle et al., 1989; Persson et al., 1991; Pacicca et al., 1992; Hall and Brain, 1994). Most studies on tachykinin-induced vasodilator effects have been either performed in vivo, or on isolated arteries from different species, including humans. Con-

* Corresponding author. Tel. 39-55-5680350, fax 39-55-5680419.

Elsevier Science B.V.
SSDI 0014-2999(95)00361-4

versely, only few studies have described a relaxant effect of substance P on precontracted veins (examples are: Edvinsson et al., 1985; McEwan et al., 1988; McCormack et al., 1989; Luu et al., 1992), and neither the tachykinin receptor(s) involved, nor the nature of such response (direct/indirect) has been investigated.

Besides their vasodilator effect, tachykinins produce vasoconstriction in certain blood vessels, such as the rabbit pulmonary artery deprived of the endothelium (D'Orléans-Juste et al., 1986), the rat isolated portal vein (Mastrangelo et al., 1987) and the rabbit isolated jugular vein (Nantel et al., 1990). In the latter preparation, Nantel et al. (1990), by the use of tachykinin receptor-selective agonists, suggested that tachykinin-induced contractions are mediated only by tachykinin receptors of the NK_1 type, and recommended this organ as a suitable bioassay for evaluating potential tachykinin NK_1 receptor agonists and antagonists. However, we found that, in the rabbit jugular vein, the effects of agonists were small, as compared to the maximal muscle contractility, and hardly reproducible, especially when constructing cumulative concentration-response curves. Since the possibility that tachykinins could exert a muscle relaxant activity in this vessel was not explored by Nantel et al. (1990), and owing to the interest in studying the effects of tachykinins in veins, we decided to re-address the question of the motor responses produced by tachykinins in the rabbit isolated jugular vein, and of the receptor(s) involved.

With this aim we tested septide and [Pro^9]substance P, two agonists reportedly selective for two distinct sites/subtypes of the tachykinin NK_1 receptor (Petitet et al., 1992; Hall et al., 1994), in either unstimulated or precontracted preparations, bearing an intact endothelium or deprived of it. In addition, we evaluated the effect of the tachykinin NK_1 receptor-selective antagonist CP-96,345 (Snider et al., 1991) and of the inhibitor of nitric oxide generation, L-nitroarginine, on both septide and [Pro^9]substance P-induced motor responses.

2. Materials and methods

2.1. General

Male albino New Zealand rabbits (2.5–3.0 kg) were stunned and bled. The jugular veins were removed and placed in oxygenated Krebs solution having the following composition: NaCl, 119 mM; $NaHCO_3$, 25 mM; KH_2PO_4, 1.2 mM; $MgSO_4$, 1.5 mM; $CaCl_2$, 2.5 mM; KCl, 4.7 mM and glucose 11 mM. From each pair of vessels 4–6 preparations were obtained: either rings with intact endothelium, or circular strips from which the vascular endothelium was removed by gently rubbing their intimal surface with a cotton-tip applicator. The effectiveness of this manoeuvre was assessed by checking the absence of a vasorelaxant response to acetylcholine (100 μM). All preparations were placed in 5 ml organ baths, filled with oxygenated (96% O_2 and 4% CO_2) Krebs solution containing indomethacin (3 μM), at 37°C, and were connected to isotonic force transducers (load 3 mN). The experiments commenced after a 120 min equilibration period, during which the preparations received noradrenaline (10 μM; every 30–45 min) and, when the tone raised by noradrenaline was stable, acetylcholine 100 μM.

The contractile responses to the tachykinin NK_1 receptor-selective agonists septide and [Pro^9]substance P were studied either in intact or in endothelium-denuded preparations. Cumulative concentration-dependent curves to these agonists were constructed before and after incubation with L-nitroarginine (100 μM; 60 min before). Both septide and [Pro^9]substance P-induced contractile responses underwent a prolonged desensitization, particulary for those responses elicited by [Pro^9]substance P. For this reason concentration-response curves to septide were repeated after 90 min from the first ones, whereas only one concentration-response curve to [Pro^9]substance P could be constructed in each individual preparation, and matched specimens of jugular vein from the same animal were used for studying the effect of L-nitroarginine and (±)-CP-96,345 (see below) on [Pro^9]substance P-mediated contractions. The contractile response to KCl (80 mM) was used as the internal standard for the above experiments.

In a separate series of experiments relaxant responses to septide, [Pro^9]substance P, senktide and [βAla^8]neurokinin A-(4–10) were studied, in the presence of a stable tone raised by noradrenaline (10 μM). Owing to the marked desensitization produced, only consecutive concentration-response curves for both septide and [Pro^9]substance P could be constructed. Experiments aiming to assess the role of endothelium in the vasomotor responses to septide and [Pro^9]substance P were performed on matched preparations from the same animal.

The tachykinin NK_1 receptor-selective antagonist (±)-CP-96,345 was used to block both vasoconstrictor (in endothelium-deprived preparations) and vasodilator (in preparations with intact endothelium) responses to the agonists used.

All the experiments were performed in the presence of a mixture of peptidase inhibitors: thiorphan (10 μM; 15 min before), captopril and bestatin (1 μM each; 15 min before).

2.2. Evaluation of data

Agonist activity was expressed as EC_{50}, or molar concentration of peptide producing 50% of maximal

effect. Antagonist affinity was expressed as pK_B (negative logarithm of the antagonist dissociation constant) when 'Schild plot' analysis (Arunlakshana and Schild, 1959) showed no significant departure from unity slope. pK_B values were estimated as the means of the individual values obtained with the equation: pK_B = log[dose ratio − 1] − log[antagonist concentration] (Kenakin, 1993; Jenkinson, 1991).

2.3. Statistical analysis

The values in the text, tables or figures are expressed as means ± 95% confidence limits, or ± S.E.M. Statistical analysis was performed by means of Student's *t*-test for paired or unpaired data or by means of two-way analysis of variance (ANOVA), when applicable. Regression analysis of log concentration-effect curves was performed by the least-squares method, considering the curves linear between 20 and 80% of the maximal response.

2.4. Drugs

(±)-CP-96,345 was synthesized in our laboratories as a racemic mixture containing both [(2*R*,3*R*)-*cis*-] and [(2*S*,3*S*)-*cis*-] enantiomers of [2-(diphenylmethyl)-*N*-[(2-methoxyphenyl)-methyl]-1-azabicyclo[2,2,2]octan-3-amine], according to the method described by Lowe (1990). [βAla^8]Neurokinin A-(4–10) was synthesized in our laboratories, by conventional solid-phase synthesis. Other drugs used were: atropine (Serva, Heidelberg, Germany), noradrenaline, indomethacin, thiorphan, bestatin, captopril and L-nitroarginine (Sigma, St. Louis, MO, USA); septide and senktide (Peninsula, St. Helens, UK); acetylcholine (Fluka, Buchs, Switzerland).

[Pro^9]Substance P was a generous gift of Dr. S. Lavielle, CNRS URA 493, Université Paris VI, Paris, France.

3. Results

3.1. Effect of septide and [Pro^9]substance P on unstimulated jugular vein preparations

Noradrenaline (10 μM) produced a contractile response of the rabbit isolated jugular vein, which averaged 55 ± 4% (n = 19) and 64 ± 3.5% (n = 19) of the maximal contraction produced by KCl (80 mM) in intact and in endothelium-deprived preparations, re-

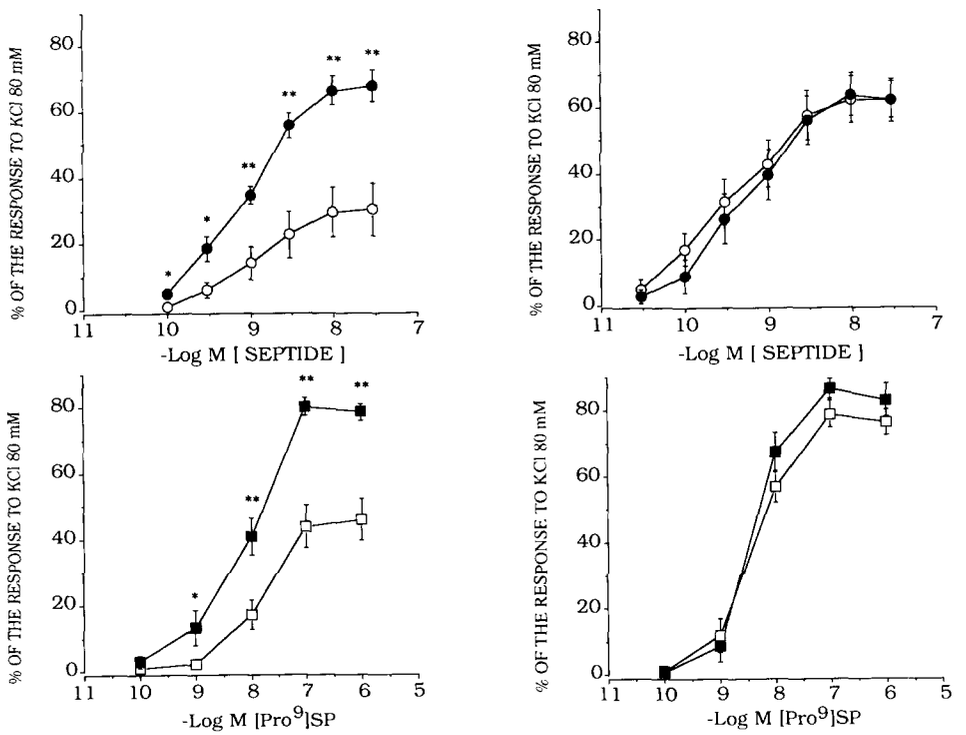

Fig. 1. Effect of endothelium removal and L-nitroarginine on the contractions produced by septide and [Pro^9]substance P in the rabbit isolated jugular vein. Upper panels: Concentration-response curves for septide in the absence (○) and after a 60-min incubation with L-nitroarginine (100 μM) (●), in preparations with intact endothelium (upper left panel) or with endothelium removed (upper right panel). Lower panels: Concentration-response curves for [Pro^9]substance P in the absence (□) and after a 60-min incubation with L-nitroarginine (100 μM) (■), in preparations with intact endothelium (lower left panel) or with endothelium removed (lower right panel). Each value in the figure is the mean ± S.E.M. of 5–7 experiments.

spectively. Acetylcholine (100 μM) completely relaxed ($-94.5 \pm 1\%$; $n = 19$) noradrenaline-contracted preparations with intact endothelium, while it produced a further small contraction of endothelium-denuded strips ($3.7 \pm 1\%$ of maximal response to KCl; $n = 18$).

The tachykinin NK_1 receptor-selective agonists septide and [Pro^9]substance P produced concentration-dependent contractile responses in both intact and endothelium-deprived preparations (Fig. 1, Table 1), which underwent a long-lasting desensitization, particulary for those responses elicited by [Pro^9]substance P (see Materials and methods). Septide was about 10-fold more potent than [Pro^9]substance P, either in intact or in endothelium-deprived preparations (Table 1). Removal of the endothelium from the jugular vein significantly increased the maximal contractions elicited by both septide and [Pro^9]substance P ($E_{max} = +106\%$ and $+72\%$, respectively, Table 1), without modifying their apparent affinities (EC_{50} values) for the tachykinin NK_1 receptors (Table 1; Fig. 1). L-Nitroarginine (100 μM), administered to prevent the generation of endogenous nitric oxide, produced a slowly developing increase of the basal tone of intact preparations ($28.8 \pm 3\%$ of maximal response to KCl (80 mM); $n = 12$) while leaving the tone of endothelium-deprived veins unaffected ($n = 12$). In the presence of L-nitroarginine (100 μM; 60 min before) the responsiveness of intact preparations to both septide and [Pro^9]substance P was increased ($E_{max} = +123\%$ and $+71\%$, respectively, Table 1), without modification of their apparent affinities, while the responsiveness of endothelium-denuded preparations was unaffected (Table 1; Fig. 1). The tachykinin NK_1 receptor-selective antagonist (±)-CP-96,345 (10–300 nM; 15 min before) competitively antagonized septide-($pK_B = 9.0$; 95% confidence limits = 8.7 –9.3) and [Pro^9]substance P-($pK_B = 8.0$; 95% confidence limits = 7.8–8.1) induced contractions in the endothelium-deprived jugular vein (Fig. 2), leaving unaffected the contractile response to noradrenaline (10 μM) ($108 \pm 2.5\%$ of the control response; $n = 5$).

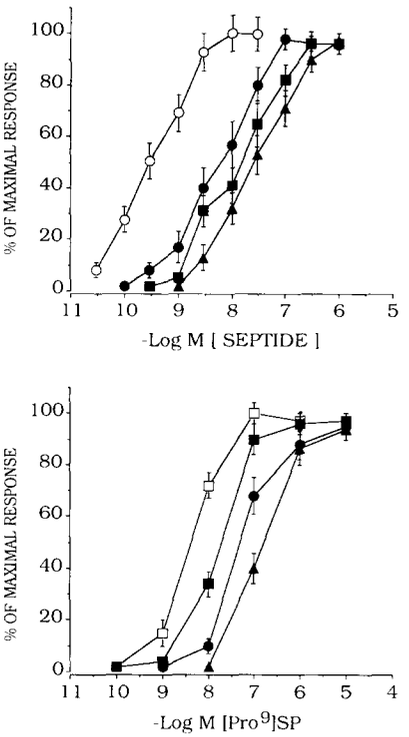

Fig. 2. Antagonism by (±)-CP-96,345 of septide-and [Pro^9]substance P-induced contractions in the endothelium-deprived rabbit jugular vein. Upper panel: Concentration-response curves for septide in the absence (○) and in the presence of (±)-CP-96,345 10 nM (●), 30 nM (■) and 100 nM (▲). Each value is the mean ± S.E.M. of 3–4 experiments. Lower panel: Concentration-response curves for [Pro^9]substance P in the absence (□) and in the presence of (±)-CP-96,345 30 nM (■), 100 nM (●) and 300 nM (▲). Each value is the mean ± S.E.M. of 3–4 experiments.

3.2. Effect of septide, [Pro^9]substance P, senktide and [βAla^8]neurokinin A-(4–10) on precontracted jugular vein preparations

Both septide and [Pro^9]substance P were tested for their ability to relax preparations precontracted with noradrenaline (10 μM). In the presence of intact en-

Table 1
Effect of L-nitroarginine on tachykinin NK_1 receptor-mediated contractile responses elicited by septide or [Pro^9]substance P in intact or endothelium-deprived rabbit jugular vein

Peptide	With endothelium				Without endothelium			
	Control		L-NOArg		Control		L-NOArg	
	EC_{50}	E_{max}	EC_{50}	E_{max}	EC_{50}	E_{max}	EC_{50}	E_{max}
Septide	2.0 ± 0.6	30.2 ± 8	1.0 ± 0.2	67.5 ± 4^a	0.4 ± 0.1	62.2 ± 7^b	0.7 ± 0.2	63.7 ± 7
[Pro^9]Substance P	18.0 ± 5	45.9 ± 6	10.1 ± 3	78.5 ± 2^a	5.1 ± 2	79.0 ± 4^c	4.5 ± 1	86.8 ± 5

Each value in the table is mean ± S.E.M. of 5–7 determinations. EC_{50} = nmolar concentration of peptide producing 50% of maximal effect. E_{max} = maximal response expressed as percentage of that to KCl 80 mM. L-NOArg = in the presence of L-nitroarginine (100 μM; 60 min before). [a]Significantly different from control response: $P < 0.01$. [b]Significantly different from the corresponding response obtained in preparations with intact endothelium: $P < 0.05$ and [c]$P < 0.01$.

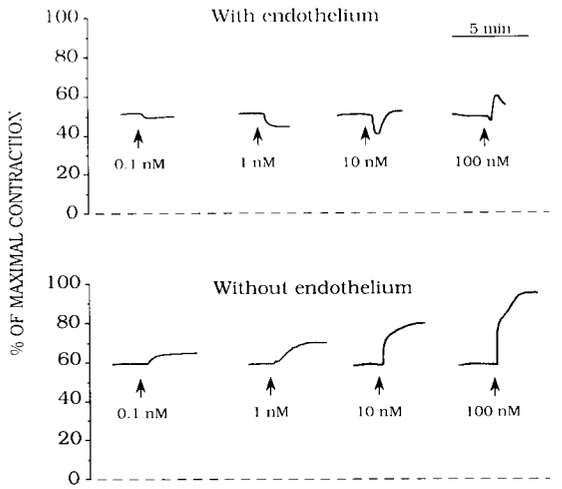

Fig. 3. Typical tracings showing the effect of septide in the rabbit isolated jugular vein precontracted with noradrenaline (10 μM), in one preparation with intact endothelium (upper panel) and in a matched one with endothelium removed (lower panel). Maximal contraction is that produced by KCl 80 mM.

dothelium, both peptides (0.1–100 nM) produced either no change of the muscular tone or a relaxation which, at higher concentrations (10–100 nM), was followed by a delayed contraction (Fig. 3 and Fig. 4). Concentrations of septide or [Pro^9]substance P higher than 100 nM produced a contraction as primary response ($n = 6$ for each peptide). The relaxant responses to both septide and [Pro^9]substance P underwent a marked desensitization, which did not allow construction of cumulative curves for these peptides. In

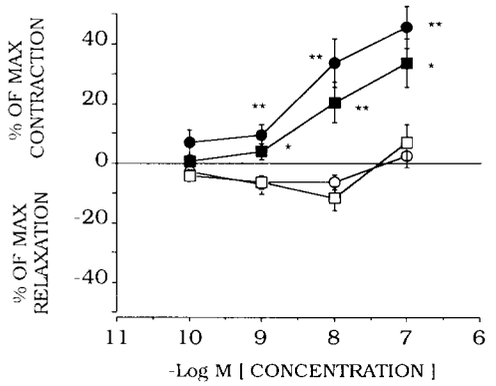

Fig. 4. Effect of endothelium removal on the relaxant effect produced by septide and [Pro^9]substance P in the precontracted rabbit isolated jugular vein. Consecutive concentration-response curves for septide (○) and for [Pro^9]substance P (□) are shown in preparations with intact endothelium, or in preparations with endothelium removed (●) and (■), respectively. All preparations were precontracted with noradrenaline (10 μM). Each value is the mean ± S.E.M. of 5–6 experiments. Maximal contraction is that produced by KCl 80 mM. Maximal relaxation is that obtained by return to basal tone, preceding the administration of noradrenaline. * Significantly different from the corresponding response produced in preparations with intact endothelium, $P < 0.05$ and ** $P < 0.01$.

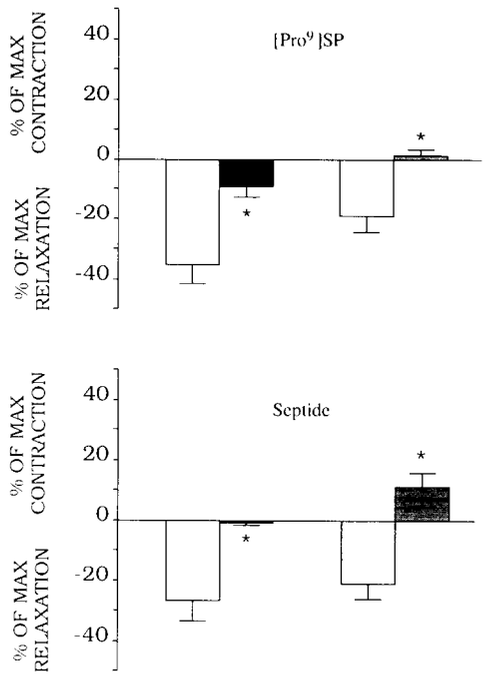

Fig. 5. Inhibition by (±)-CP-96,345 and L-nitroarginine of the relaxation produced by septide and [Pro^9]substance P in the precontracted rabbit isolated jugular vein with intact endothelium. Upper panel: Relaxation produced by [Pro^9]substance P (1 nM) in preparations precontracted with noradrenaline (10 μM), in control experiments (white columns) and in the presence of (±)-CP-96,345 (100 nM; 15 min before) (black column) or L-nitroarginine (100 μM; 60 min before) (gray column). Lower panel: Relaxation produced by septide (1 nM) in preparations precontracted with noradrenaline (10 μM), in control experiments (white columns) and in the presence of (±)-CP-96,345 (100 nM; 15 min before) (black column) or L-nitroarginine (100 μM; 60 min before) (gray column). Each column is the mean ± S.E.M. of 5–6 experiments. Maximal contraction is that produced by KCl 80 mM. Maximal relaxation is that obtained by return to basal tone, preceding the administration of noradrenaline. * Significantly different from the control response $P < 0.05$.

sharp contrast, neither septide nor [Pro^9]substance P were able to relax strips of jugular vein deprived of the endothelium: both peptides invariably produced a contraction as primary response (Fig. 3 and Fig. 4). L-Nitroarginine (100 μM; 60 min before) and (±)-CP-96,345 (100 nM; 15 min before) were studied against a single relaxing concentration (1 nM) of both septide and [Pro^9]substance P in preparations with intact endothelium precontracted with noradrenaline (10 μM) (Fig. 5). (±)-CP-96,345 markedly reduced (−78 ± 7%; $n = 6$) the relaxant effect of [Pro^9]substance P and almost abolished (−99 ± 1%; $n = 6$) that produced by septide. L-Nitroarginine reversed the relaxation produced by [Pro^9]substance P (18 ± 5% of maximal relaxation; $n = 5$) or septide (20 ± 5% of maximal relaxation; $n = 6$) into a contraction (1.3 ± 1% and 10.5 ± 5% of maximal contraction, respectively) (Fig. 5).

The tachykinin NK_2 receptor-selective agonist [βAla^8]neurokinin A-(4–10) and the tachykinin NK_3

receptor-selective agonist senktide were tested in jugular vein preparations with intact endothelium, to ascertain whether other tachykinin receptors may be involved in producing endothelium-dependent relaxation. Senktide, up to 1 μM ($n = 4$), failed to relax and/or produce additional contractions in preparations precontracted with noradrenaline (10 μM). [βAla^8]Neurokinin A-(4–10) did not affect the tone of precontracted veins up to 10 nM ($n = 10$). [βAla^8]Neurokinin A-(4–10) (100 nM) produced, as primary response, a slight relaxation (6.6 ± 2% of maximal relaxation) in 3 out of 10 preparations tested, while in the remaining ones it produced an additional contraction (19.3 ± 7% of maximal contraction; $n = 7$). (±)-CP-96,345 (100 nM; 15 min before) completely suppressed [βAla^8]neurokinin A-(4–10)-induced relaxations (100% inhibition; $n = 3$) and almost completely (89 ± 5% inhibition; $n = 4$) reduced [βAla^8]neurokinin A-(4–10)-induced additional contractions.

4. Discussion

In keeping with the results obtained by Nantel et al. (1990), the present results, obtained with the tachykinin NK_1 receptor-selective antagonist (±)-CP-96,345 (Snider et al., 1991), show that, in the rabbit jugular vein, tachykinins induce a contractile effect through activation of tachykinin receptors of the NK_1 type.

Petitet and coworkers proposed the existence of a 'septide-sensitive' subtype of the tachykinin NK_1 receptor in the guinea-pig ileum, for which septide and other tachykinin NK_1 receptor-selective agonists would have higher affinity/efficacy than for the 'classical' tachykinin NK_1 receptor (Petitet et al., 1992). The 'septide-sensitive' receptor site/subtype has been subsequently recognized in other tissues from the guinea-pig and also from other species by the use of various tachykinin NK_1 receptor-selective antagonists which have been found to possess higher affinity at this latter site/subtype than at the 'classical' tachykinin NK_1 receptor (see Maggi, 1994, for review).

In our experiments, the greater potency (about 10-fold) shown by (±)-CP-96,345 against septide as compared to [Pro^9]substance P –two agonists reportedly selective for two distinct sites/subtypes of the tachykinin NK_1 receptor (Petitet et al., 1992; Hall et al., 1994) –provide evidence for the presence of different sites/subtypes of the tachykinin NK_1 receptor in the rabbit jugular vein, as shown previously in the rabbit iris sphincter muscle (Hall et al., 1994). The present results are unable to answer the question whether, in the rabbit, the 'septide-sensitive' is a distinct receptor protein or a different agonist recognition site(s) present on one and the same NK_1 receptor protein.

Our experiments on precontracted jugular vein preparations show that tachykinins, besides producing contraction, are also able to produce a relaxation through the activation of tachykinin NK_1 receptors. The antagonism exerted by (±)-CP-96,345 against septide and [Pro^9]substance P-induced relaxations reinforces this conclusion. The tachykinin-mediated relaxation is strictly dependent on the presence of intact endothelium, suggesting that those tachykinin NK_1 receptors involved in the vasodilator response to tachykinins could be located on endothelial cells, as has been shown to occur in other vessels, like the pig aorta (Saito et al., 1990) or the dog carotid artery (Stephenson et al., 1986). The present results provide also pharmacological evidence that activation of tachykinin NK_1 receptors leads to generation of endogenous nitric oxide, which in turn relaxes the tone of precontracted veins, as demonstrated by the inhibition of tachykinin-mediated relaxation exerted by L-nitroarginine. It is noteworthy that, in the jugular vein, the tachykinin-induced relaxation seems *completely* mediated by endogenous nitric oxide, whereas the same is not true for in vivo vasodilation (e.g. Santicioli et al., 1993) and arterial relaxation (e.g. Pacicca et al., 1992; Hoover and Hossler, 1993) whereby tachykinins appear to induce generation of other endothelium-derived relaxing factor(s), in addition to nitric oxide, in the arterial vascular bed (see Maggi, 1995, for further discussion of this point).

The ineffectiveness of the tachykinin NK_3 receptor-selective agonist senktide to relax and/or to contract the rabbit jugular vein rules out the hypothesis that tachykinin NK_3 receptors may participate in the motor effects produced by tachykinins in this vessel. The tachykinin NK_2 receptor-selective agonist [βAla^8]neurokinin A-(4–10) produced small relaxations and/or contractions at a concentration (100 nM) which may stimulate also tachykinin receptors of the NK_1 type (e.g. see Patacchini et al., 1994). This was further demonstrated here by the sensitivity of these responses to the blocking action of (±)-CP-96,345. Thus, tachykinin NK_1 receptors are the only mediators of tachykinin-induced vasodilation, as well as vasoconstriction, in this vessel.

The existence of different populations of tachykinin NK_1 receptors mediating opposite effects (vasodilation and contraction) in the rabbit jugular vein is also supported by the observation that either removal of the endothelium, or addition of L-nitroarginine, results in a greater contractile response to tachykinin NK_1 receptor-selective agonists. Moreover, the effects of L-nitroarginine and endothelium removal were non-additive, indicating that nitric oxide generation, following tachykinin NK_1 receptor stimulation, occurs exclusively in endothelial cells.

L-Nitroarginine, per se, produced a slowly develop-

ing, endothelium-dependent contraction of jugular vein preparations; a similar effect, elicited by L-nitroarginine itself, or by other inhibitors of nitric oxide formation, has been noted also in certain mammalian arteries (Rees et al., 1989; Pacicca et al., 1992) and arterioles (Hall and Brain, 1994), and may be accounted for by a tonic or resting release of nitric oxide, which could contribute to the basal tone of this vessel in situ.

From a methodological point of view, we found that following removal of the endothelium, or pretreatment with L-nitroarginine, reproducible concentration-response curves to the agonists can be easily constructed in this preparation, making it a useful bioassay for evaluating compounds with agonist/antagonist activity at the tachykinin NK_1 receptor.

In conclusion, this study provides evidence that tachykinins produce endothelium-dependent relaxations also in veins, through activation of tachykinin NK_1 receptors. In the rabbit jugular vein this effect is sensitive to L-nitroarginine pretreatment, implying a role for nitric oxide, released from endothelial cells, as the endogenous mediator of tachykinin-induced vasodilation. Furthermore, the present results show that, in the rabbit isolated jugular vein, tachykinins produce a dual effect – relaxation and contraction – through activation of the same receptor type – the tachykinin NK_1 – likely located on different target cells: smooth muscle vs. endothelium. The resulting vasomotor effect of tachykinins at this level is dependent on the tone of the vessel. On the basis of these results, it may be speculated that endogenous tachykinins, locally released, might contribute to control of blood flow in the venous vascular bed.

Acknowledgements

We wish to thank Dr. S. Lavielle, CNRS URA 493, Université Paris VI, Paris, France, for the generous gift of [Pro^9]substance P.

References

Arunlakshana, O., and H.O. Schild, 1959, Some quantitative uses of drug antagonists, Br. J. Pharmacol. Chemother. 14, 48.

Beattie, D.T., C.M. Stubs, H.E. Connor and W. Feniuk, 1993, Neurokinin-induced changes in pial artery diameter in the anaesthetized guinea-pig, Br. J. Pharmacol. 108, 146.

Constantine, J.W., W.S. Lebel and H.A. Woody, 1991, Inhibition of tachykinin-induced hypotension in dogs by CP-96,345, a selective blocker of NK-1 receptors, Naunyn-Schmied. Arch. Pharmacol. 344, 471.

D'Orléans-Juste, P., S. Dion, J. Mizrahi and D. Regoli, 1985, Effects of peptides and non-peptides on isolated arterial smooth muscles: role of endothelium, Eur. J. Pharmacol. 114, 9.

D'Orléans-Juste, P., S. Dion, G. Drapeau and D. Regoli, 1986, Different receptors are involved in the endothelium-mediated relaxation and the smooth muscle contraction of the rabbit pulmonary artery in response to substance P and related neurokinins, Eur. J. Pharmacol. 125, 37.

Duckles, S.P., 1986, Effects of capsaicin on vascular smooth muscle, Naunyn-Schmied. Arch. Pharmacol. 333, 59.

Edvinsson, L., R. Hakanson, S. Steen, F. Sundler, R. Uddman and C. Wahlested, 1985, Innervation of human omental arteries and veins and vasomotor responses to noradrenaline, neuropeptide Y, substance P and vasoactive intestinal peptide, Regul. Pept. 12, 67.

Emonds-Alt, X., J.D. Doutremepuich, M. Heaulme, G. Neliat, V. Santucci, R. Steinberg, P. Vilain, D. Bichon, J.P. Ducoux, E. Proietto, D. Van Broeck, P. Soubrie, G. Le Fur and J.C. Breliere, 1993, In vitro and in vivo biological activities of SR 140,333, a novel potent nonpeptide tachykinin NK_1 receptor antagonist, Eur. J. Pharmacol. 250, 403.

Franco-Cereceda, A., A. Rudehill and J.M. Lundberg, 1987, Calcitonin gene-related peptide but not substance P mimics capsaicin-induced coronary vasodilation in the pig, Eur. J. Pharmacol. 142, 235.

Guard, S. and S.P. Watson, 1991, Tachykinin receptor types: classification and membrane signalling mechanisms, Neurochem. Int. 18, 149.

Hall, J.M. and S.D. Brain, 1994, Inhibition by SR 140333 of NK_1 tachykinin receptor-evoked, nitric oxide-dependent vasodilatation in the hamster cheek pouch microvascolature in vivo, Br. J. Pharmacol. 113, 522.

Hall, J.M., D. Mitchell and I.K.M. Morton, 1994, Typical and atypical NK_1 tachykinin receptor characteristics in the rabbit isolated iris sphincter, Br. J. Pharmacol. 112, 985.

Hoover, D.B. and F.E. Hossler, 1993, Vasoconstrictor and dilator responses to neurokinin A in isolated guinea-pig heart, Peptides 14, 29.

Jenkinson, D.H., 1991, How we describe competitive antagonists: three questions of usage, Trends Pharmacol. Sci. 12, 53.

Kenakin, T.P., 1993, Pharmacologic Analysis of Drug-Receptor Interaction, 2nd edn. (Raven Press, New York, NY).

Lembeck, F., J. Donnerer, M. Tsuchiya and A. Nagahisa, 1992, The non-peptide antagonist CP 96,345, is a potent inhibitor of neurogenic inflammation, Br. J. Pharmacol. 105, 527.

Lowe III, J.A., 1990, Quinuclidine Therapeutic Agents, Patent No. WO 90/05729, 1–69.

Luu, T.N., A.H. Chester, G.S. O'Neil, S. Tadjkarimi and M.H. Yacoub, 1992, Effects of vasoactive neuropeptides on human saphenous vein, Br. Heart J. 67, 474.

Maggi, C.A., 1994, Evidence for receptor subtypes/species variants of receptors, in: The Tachykinin Receptors, ed. S.H. Buck (Humana Press, Totowa, NJ) p. 395.

Maggi, C.A., 1995, Tachykinins and calcitonin gene-related peptide (CGRP) as co-transmitters released from peripheral endings of sensory nerves, Prog. Neurobiol. 45, 1.

Maggi, C.A., R. Patacchini, P. Rovero and A. Giachetti, 1993, Tachykinin receptors and tachykinin receptor antagonists, J. Auton. Pharmacol. 13, 23.

Mastrangelo, D., R. Mathison, H.J. Huggel, S. Dion, P. D'Orléans-Juste, N.E. Rhaleb, G. Drapeau, P. Rovero and D. Regoli, 1987, The rat isolated portal vein: a preparation sensitive to neurokinins, particularly neurokinin B, Eur. J. Pharmacol. 134, 321.

McCormack, D.G., R.O. Salonen and P.J. Barnes, 1989, Effect of sensory neuropeptides on canine bronchial and pulmonary vessels in vitro, Life Sci. 45, 2405.

McEwan, J.R., N. Benjamin, S. Larkin, R.W. Fuller, C.T. Dollery and I. MacIntyre, 1988, Vasodilatation by calcitonin gene-related peptide and by substance P: a comparison of their effects on resistance and capacitance vessels of human forearms, Circulation 77, 1072.

Nantel, F., N. Rouissi, N.-E. Rhaleb, S. Dion, G. Drapeau and D. Regoli, 1990, The rabbit jugular vein is a contractile NK-1 receptor system, Eur. J. Pharmacol. 179, 457.

Pacicca, C., P.Y. Von Der Weid and J.L. Bény, 1992, Effect of nitro-L-arginine on endothelium-dependent hyperpolarizations and relaxations of pig coronary arteries, J. Physiol. 457, 247.

Patacchini, R., R. De Giorgio, A. Giachetti and C.A. Maggi, 1994, Different mechanism of tachykinin NK_2 receptor blockade by SR 48968 and MEN 10,627 in the guinea-pig isolated gallbladder and colon, Eur. J. Pharmacol. 271, 111.

Persson, M.G., P. Hedqvist and L.E. Gustafsson, 1991, Nerve-induced tachykinin-mediated vasodilatation in skeletal muscle is dependent on nitric oxide formation, Eur. J. Pharmacol. 205, 295.

Petitet, F., M. Saffroy, Y. Torrens, S. Lavielle, G. Chassaing, D. Loeuillet, J. Glowinski and J.C. Beaujouan, 1992, Possible existence of a new tachykinin receptor subtype in the guinea-pig ileum, Peptides, 13, 383.

Rees, D.D., R.M.J. Palmer, H.F. Hodson and S. Moncada, 1989, A specific inhibitor of nitric oxide formation from L-arginine attenuates endothelium-dependent relaxation, Br. J. Pharmacol. 96, 418.

Regoli, D., G. Drapeau, S. Dion and P. D'Orléans-Juste, 1989, Receptors for substance P and related neurokinins, Pharmacology 38, 1.

Rubino, A., H. Thomann, J.M. Henlin, W. Schilling and L. Criscione, 1992, Endothelium-dependent relaxant effect of neurokinins on rabbit aorta is mediated by the NK_1 receptor, Eur. J. Pharmacol. 212, 237.

Saito, R., H. Konishi, Y. Takano, S. Nonaka, K. Sakaguchi, Y. Shimohigashi and H.-O. Kamiya, 1990, Characterization of tachykinin receptors in endothelial cells of porcine artery, Neurosci Lett. 110, 337.

Santicioli, P., S. Giuliani and C.A. Maggi, 1993, Failure of L-nitroarginine, a nitric oxide synthase inhibitor, to affect hypotension and plasma protein extravasation produced by tachykinin NK-1 receptor activation in rats, J. Auton. Pharmacol. 13, 193.

Snider, M.R., J.W. Constantine, J.A. Lowe III, K.P. Longo, W.S. Lebel, H.A. Woody, S.E. Drozda, M.C. Desai, F.J. Vinick, R.W. Spencer and H.J. Hess, 1991, A potent nonpeptide antagonist of the substance P (NK_1) receptor, Science 251, 435.

Stephenson, J.A., E. Burcher and R.J. Summers, 1986, Autoradiographic demonstration of endothelium-dependent ^{125}I-Bolton-Hunter substance P binding to dog carotid artery, Eur. J. Pharmacol. 124, 377.

Stubbs, C.M., G.J. Waldron, H.E. Connor and W. Feniuk, 1992, Characterization of the receptor mediating relaxation to substance P in canine middle cerebral artery: no evidence for involvement of substance P in neurogenically mediated relaxation, Br. J. Pharmacol. 105, 875.

Whittle, B.J.R., J. Lopez-Belmonte and D.D. Rees, 1989, Modulation of the vasodepressor actions of acetylcholine, bradykinin substance P and endothelin in the rat by a specific inhibitor of nitric oxide formation, Br. J. Pharmacol. 98, 646.