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Peripheral effects of three novel non-peptide tachykinin NK_1 receptor antagonists in the anaesthetized rat

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Abstract

Three novel non-peptide tachykinin NK_1 receptor antagonists were assessed on the transient fall in mean arterial blood pressure and the salivation induced by i.v. substance P (0.65 nmol/kg) in the urethane-anaesthetized rat. LY303241 ((*R*)-1-[*N*-(2-methoxybenzyl)acetylamino]-3-(1*H*-indol-3-yl)-2-[*N*-(2-(4-phenylpiperazin-1-yl)acetyl)amino]propane), LY303870 ((*R*)-1-[*N*-(2-methoxybenzyl)acetylamino]-3-(1*H*-indol-3-yl)-2-[*N*-(2-(4-(piperidin-1-yl)piperidin-1-yl)acetyl)amino]propane) and LY306740 ((*R*)-1-[*N*-(2-methoxybenzyl)acetylamino]-3-(1*H*-indol-3-yl)-2-[*N*-(2-(4-cyclohexylpiperazin-1-yl)acetyl)amino]propane) (65 nmol–9 μmol/kg i.v.; 5 min earlier) inhibited both the vasodepressor and salivary responses to substance P in a dose-dependent manner. LY303241 and LY306740 were more potent in inhibiting the vascular response to substance P while LY303870 was more potent in inhibiting the salivary response. LY303870 and LY306740 were devoid of direct effects while LY303241 decreased blood pressure and heart rate for 1 and 10 min, respectively. The antagonists act in a stereoselective and specific manner since the opposite (*S*) enantiomers of LY303870 (LY306155) and LY306740 (LY307679) failed to block the effects of substance P. In addition, LY303241, LY303870 and LY306740 neither affected the hypotension and the salivation induced by carbachol nor the increases in mean arterial pressure and heart rate induced by the tachykinin NK_2 receptor agonist [β-Ala^8]neurokinin A-(4–10). Only LY303241 attenuated the decreases in mean arterial pressure and heart rate evoked by the tachykinin NK_3 receptor agonist senktide. LY303870 and LY306740 appear to be the most interesting antagonists since they act in a specific and selective manner at the tachykinin NK_1 receptor. The difference in the order of potency of the three antagonists to inhibit the hypotension and salivation elicited by substance P could be ascribed to their pharmacodynamic features or to the existence of different signal transduction mechanisms or receptor subtypes.

Keywords: Tachykinin receptor antagonist; Substance P; Tachykinin NK_1 receptor; Salivation; Blood pressure

1. Introduction

Six mammalian tachykinins, namely substance P, neurokinin A, neurokinin A-(3–10), neurokinin B, neuropeptide K and neuropeptide γ, have been identified in both the periphery and the central nervous system (Helke et al., 1990; Otsuka and Yoshioka, 1993). These neuropeptides exert a plethora of biological effects through the activation of three receptors, termed neurokinin$_1$ (NK_1), NK_2, NK_3, for which substance P, neurokinin A and neurokinin B are thought to be the endogenous ligands, respectively (Regoli et al., 1988; Maggi et al., 1993).

In the periphery, substance P is present in the plasma of various species, in perivascular sensory nerves as well as in a subpopulation of endothelial cells (Pernow, 1983; Otsuka and Yoshioka, 1993). The direct activation of the tachykinin NK_1 receptor localized on vascular endothelial cells elicits the release of nitric oxide, leading to vasodilatation (D'Orléans-Juste et al., 1985; Whittle et al., 1989; Persson et al., 1991) and a transient fall in blood pressure in urethane-anaesthetized rats (Maggi et al., 1987; Couture et al., 1989) and in the conscious rat (Pompei et al., 1993). Several observations also support a direct action of substance P on salivary glands to elicit salivar secretion. First, binding studies with labelled substance P or physalaemin suggested the presence of tachykinin NK_1 receptors on rat submaxillary gland membranes (Bahouth et al., 1985; Buck and Burcher, 1985; Lee et al., 1986). Second, neurokinin A and neurokinin B were about 25 times less potent than

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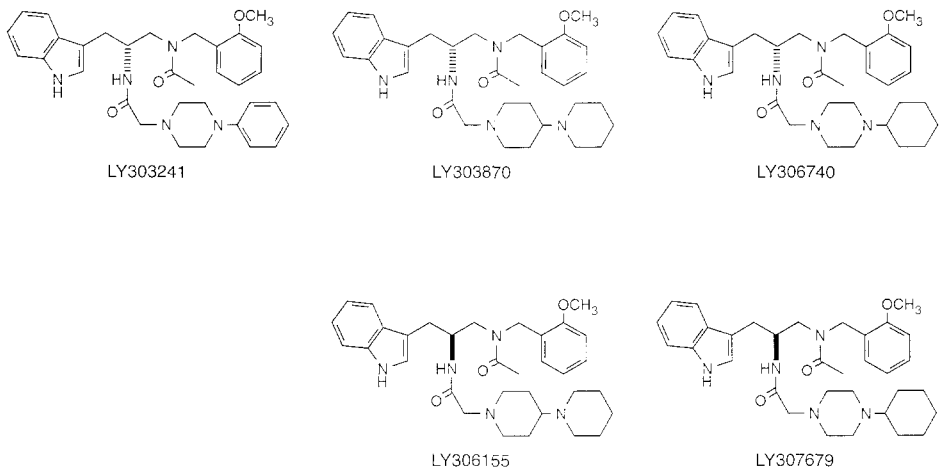
Table 1
Structure of the antagonists



substance P in producing salivation (Maggi et al., 1987). The tachykinin NK_1 receptor-selective agonist [Pro^9,$Met(O_2)^{11}$]substance P was 10 times more potent than substance P to induce salivation while selective agonists of tachykinin NK_2 ([Nle^{10}]neurokinin A-(4–10)) and NK_3 ([$MePhe^7$]neurokinin B) receptors were ineffective (Giuliani et al., 1988). Third, the sialogogic effect of substance P was unaffected by atropine, which excludes the involvement of the parasympathetic system in this response (Giuliani et al., 1988).

Over the last 5 years, many non-peptide antagonists at the tachykinin NK_1 receptor have been developed, such as CP-96,345 (Snider et al., 1991), RP 67,580 (Garret et al., 1991), SR140,333 (Emonds-Alt et al., 1993), CP122,721 (McLean et al., 1995) and L-733,060 (Rupniak et al., 1995). A recent non-peptide antagonist, namely LY303870 ((*R*)-1-[*N*-(2-methoxybenzyl)acetylamino]-3-(1*H*-indol-3-yl)-2-[*N*-(2-(4-(piperidin-1-yl)piperidin-1-yl)acetyl)amino]-propane), was reported to bind with high affinity and in a selective way to human peripheral ($K_i = 0.15$ nM) and central ($K_i = 0.10$ nM) tachykinin NK_1 receptors. It was found to be active in bioassays such as substance P-induced release of interleukin-6 from U-373 MG human astrocytoma cells, rabbit vena cava contraction and guinea-pig bronchoconstriction (Gitter et al., 1995; Hipskind et al., 1995). It also binds potently to the rat and mouse tachykinin NK_1 receptors, although with lower affinity. In vivo assays demonstrate that LY303870 potently inhibits central tachykinin NK_1-mediated responses such as nociceptive behavioural responses in mice and the hyperalgesia induced by a tachykinin NK_1 receptor agonist in the rat tail-flick test (Iyengar et al., 1995). Binding affinities of two analogues of this series, namely LY303241 ((*R*)-1-[*N*-(2-methoxybenzyl)acetylamino]-3-(1*H*-indol-3-yl)-2-[*N*-(2-(4-phenylpiperazin-1-yl)acetyl)amino]propane) and LY306740 ((*R*)-1-[*N*-(2-methoxybenzyl)acetylamino]-3-(1*H*-indol-3-yl)-2-[*N*-(2-(4-cyclohexylpiperazin-1-yl)acetyl)amino]propane) (Table 1), were lower than that of LY303870 at human tachykinin NK_1 receptors but greater at rodent tachykinin NK_1 receptors (Hipskind et al., 1996; Table 2). However, these three compounds still remain to be fully characterized in vivo and compared for potency, particularly in the periphery. Since the vasodepressor and sialogogic effects of substance P in the urethane-anaesthetized rat are sensitive and well-characterized tachykinin NK_1 receptor-mediated responses that are not subject to desensitization, they can be used as reliable in vivo tachykinin NK_1 receptor assays. Thus, the objectives of this study were: (a) to evaluate LY303241, LY303870 and LY306740 on the cardiovascular response and salivation induced by i.v. substance P; (b) to assess their specificity and selectivity as tachykinin NK_1 receptor antagonists and the duration of receptor blockade; (c) to verify their intrinsic activities.

Table 2
Species differences in affinities: in vitro tachykinin NK_1 binding experiments

Compounds	IC_{50}			
	Human (IM-9) [a]	Guinea pig brain [b]	Rat brain [b]	Mouse brain [b]
LY303241	0.85	0.26	4.5	2.5
LY303870	0.15	0.50	9.3	7.7
LY306740	0.18	ND	1.5	1.3

Binding affinity for the tachykinin NK_1 receptor [a] in human IM-9 cells or [b] in guinea pig, rat or mouse brain homogenates, using ^{125}I-labelled Bolton-Hunter substance P as radioligand, given in nM units. ND = not determined. Adapted from Hipskind et al. (1996).

2. Materials and methods

2.1. Animal preparation

Male Wistar rats (180–200 g; Charles River, St-Constant, Québec, Canada) were anaesthetized with urethane (1.4 g/kg, s.c.) and a polyethylene catheter (PE-240; Intramedics, Clay Adams, NJ, USA) was inserted into the trachea to facilitate spontaneous breathing. One jugular vein and one femoral artery were cannulated with siliconized (Sigmacote, Sigma Company, St. Louis, MO, USA) PE-50 catheters filled with physiological saline containing 50 IU/ml heparin sodium salt (Sigma Company, St. Louis, MO, USA) for intravenous (i.v.) injection (0.5 ml/kg) of peptides and antagonists and direct blood pressure recording, respectively. The rectal temperature was maintained at 37°C during the course of the experiments. The care of animals and research protocols conformed to the guiding principles for animal experimentation as enunciated by the Canadian Council on Animal Care and approved by the committee responsible for animal care at the Université de Montréal.

2.2. Measure of cardiovascular parameters and salivation

Arterial blood pressure and heart rate were measured with a Statham pressure transducer (P23ID) and a cardiac tachometer (model 7P4) (triggered by the arterial blood pressure pulse) coupled to a Grass polygraph (model 79; Grass Instruments Co., Quincy, MA, USA). Saliva was measured by placing Whatman analytical filter papers in the rat's mouth at 5-min intervals and quantitating the amount of saliva secreted as the difference in the weight of the papers before and after the collection period.

2.3. Experimental protocols

2.3.1. Tachykinin NK_1 receptor antagonists versus substance P

The first series of experiments was designed to evaluate the potency of the three tachykinin NK_1 receptor antagonists on the vasodepressor and salivary responses elicited by substance P. The rats received an i.v. injection of 0.65 nmol/kg substance P and 60 min later, a second injection of substance P was given 5 min after an i.v. pretreatment with either LY303241 (group 1; $n = 16$), LY303870 (group 2; $n = 19$) or LY306740 (group 3; $n = 23$) at 65 nmol/kg, 325 nmol/kg, 1.8 μmol/kg or 9 μmol/kg. The rats received another dose of 0.65 nmol/kg substance P 60 min later, to test the duration of the inhibition. If a complete recovery of the response to substance P occurred, the same protocol was repeated with a higher dose of antagonist. Each antagonist was tested in separate groups of rats.

2.3.2. Stereoselectivity and specificity of the tachykinin NK_1 receptor antagonists

To verify whether the antagonists are enantiomer selective, the opposite (*S*) enantiomers of LY303870 (LY306155) and LY306740 (LY307679) were tested i.v. 5 min prior to 0.65 nmol/kg according to the same protocol as described above. Only one enantiomer was injected into each rat. In addition, to assess the specificity, the highest dose (9 μmol/kg) of each antagonist was tested against carbachol (40 nmol/kg) according to the same protocol.

2.3.3. Selectivity of the tachykinin NK_{-1} receptor antagonists

This series of experiments aims at determining the selectivity of the antagonists for the tachykinin NK_1 receptor. For this purpose, the rats received an i.v. injection of a selective agonist for the tachykinin NK_2 receptor, [β-Ala^8]neurokinin A-(4–10) (65 nmol/kg), and 2 h later a second injection of the agonist was given 5 min after the prior injection of one antagonist (9 μmol/kg). Each rat received randomly only one antagonist.

In a preliminary study, it was observed that a slowly reversible desensitization to the tachykinin NK_3 receptor-selective agonist senktide occurred after i.v. administration. Thus, the cardiovascular effects of senktide in the absence and presence of antagonists were compared in separate groups of rats in which only one injection of senktide (16.3 nmol/kg) was administered per rat. Antagonists were injected (9 μmol/kg) individually 5 min prior to the first injection of senktide and the results were compared to those obtained in the control group injected with senktide alone.

2.4. Agonists, antagonists and other drugs

Substance P was purchased from Hükabel Scientific (Montréal, Québec, Canada). [β-Ala^8]Neurokinin A-(4–10) and senktide were generously provided by Dr. D. Regoli from Université de Sherbrooke (Sherbrooke, Québec, Canada). The three non-peptide antagonists LY303241 (molecular weight (MW) 553.7), LY303870 (MW 559.8) and LY306740 (MW 559.8) and the opposite (*S*) enantiomers LY306155 (MW 559.8) and LY307679 (MW 559.8) were synthesized by Eli Lilly (Indianapolis, IN, USA) (Hipskind et al., 1995, 1996). Urethane (ethyl carbamate), heparin sodium salt (porcine, grade 1-A) and carbachol (carbamyl choline chloride) were purchased from Sigma (St. Louis, MO, USA). Substance P and carbachol were dissolved in physiological saline (0.9% NaCl) whereas [β-Ala^8]neurokinin A-(4–10) and senktide were solubilized in dimethyl sulfoxide (DMSO; Fisher Scientific, Montréal, Québec, Canada). The Eli Lilly compounds were dissolved in a mixture of DMSO and Tween 80 (Fisher Scientific, Montréal, Québec, Canada). The stock solutions (10 mg/ml) were stored in aliquots of 100 μl at −20°C

until used. Substance P and carbachol were injected in pure physiological saline whereas [β-Ala^8]neurokinin A-(4–10), senktide and the antagonists were injected in saline containing DMSO (between 1 and 40% of DMSO).

2.5. Statistical analysis of data

Results are expressed as means ± S.E.M. for n rats. Time-dependent effects were evaluated with a two-way analysis of variance (ANOVA) and a posteriori Dunnett test. Pairwise comparisons were made with Student's t-test for paired samples. For multiple comparisons, statistical differences were evaluated with a one-way analysis of variance (ANOVA) followed by a Dunnett test. The uniformity of the variances among different groups was verified with the BARTLETT test. Only probability values (P) smaller than 0.05 were considered to be statistically significant.

3. Results

3.1. Cardiovascular and salivary responses to tachykinin agonists and carbachol

As depicted in Fig. 1, the i.v. injection of 0.65 nmol/kg substance P induced a transient fall in mean arterial pressure (−27.5 ± 1.0 mmHg) and an increase in salivation (47.5 ± 4.3 mg per rat); these effects were significant when compared to saline ($P < 0.001$; $n = 44$) and occurred within the first 30 s and 5 min post-injection, respectively. However, at this dose, substance P had no significant effect on heart rate. The i.v. injection of the muscarinic receptor agonist carbachol (40 nmol/kg) elicited a transient fall in mean arterial pressure (−45.1 ± 2.9 mmHg) and heart rate (−110 ± 15 beats/min) which occurred during the first 30 s post-injection ($P < 0.01$ compared to saline; $n = 13$). These cardiovascular effects were accompanied by intense salivation (37.1 ± 4.3 mg per rat) within the first 5 min post-injection ($P < 0.01$; $n = 13$). The tachykinin NK_2 receptor-selective agonist [β-Ala^8]neurokinin A-(4–10) (65 nmol/kg) evoked transient and slight increases in mean arterial pressure (7.1 ± 0.8 mmHg) and heart rate (25 ± 4 beats/min); the maximal effects ($P < 0.001$; $n = 20$) were at 30 s and 3 min post-injection, respectively. The tachykinin NK_3 receptor-selective agonist senktide (16.3 nmol/kg) induced prolonged ($t = 30$ s–4 min; $P < 0.001$; $n = 7$) decreases in mean arterial pressure (−28.9 ± 3.9 mmHg at $t = 1$ min) and heart rate (−28 ± 4 beats/min at $t = 3$ min). Both [β-Ala^8]neurokinin A-(4–10) and senktide failed to cause salivation. As illustrated in Table 3, the basal values for mean arterial pressure and heart rate were similar among the different groups of rats (84.0 ± 1.4 mmHg and 418 ± 4 beats/min, respectively; $n = 107$), except the group which received [β-Ala^8]neurokinin A-(4–10) had a slightly lower mean arterial pressure (74.8 ± 3.2 mmHg; $P < 0.01$). The

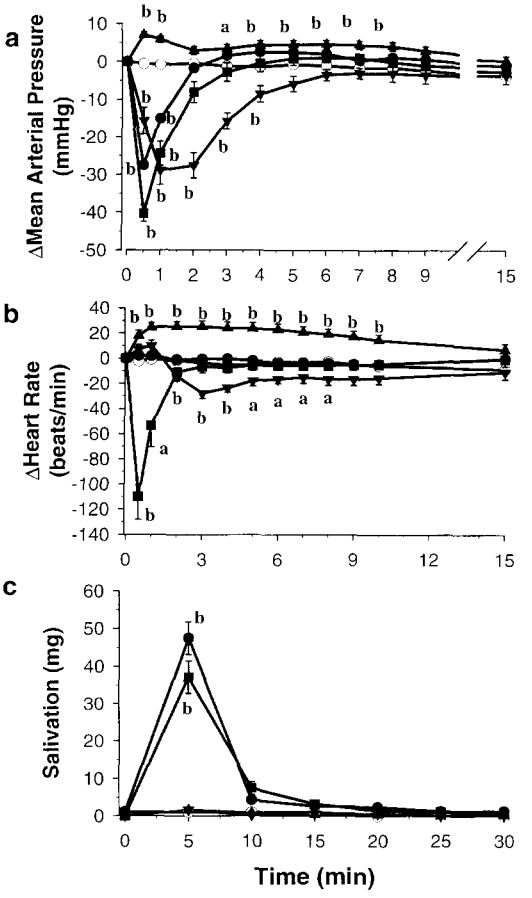


Fig. 1. Time-dependent effects of 0.65 nmol/kg substance P (●), 40 nmol/kg carbachol (■), 65 nmol/kg [β-Ala^8]neurokinin A-(4–10) (▲) and 16.3 nmol/kg senktide (▼) on changes in mean arterial blood pressure, heart rate and salivation ($n = 7$–44). Significance to saline (○): $^a P < 0.05$; $^b P < 0.01$.

Table 3
Basal values of mean arterial pressure and heart rate in the different groups of rats

Treatments	n	Basal mean arterial pressure (mmHg)	Basal heart rate (beats/min)
Substance P	45	89.7 ± 1.8	435 ± 6
Carbachol	13	84.0 ± 3.9	398 ± 15 ^{a}
[β-Ala^8]Neurokinin A-(4–10)	20	74.8 ± 3.2 ^{b}	424 ± 9
Senktide	29	81.7 ± 2.9	394 ± 6 ^{b}

Data are means ± S.E.M. for n rats. Significance to the substance P-treated group: $^a P < 0.05$; $^b P < 0.01$.

groups treated with carbachol and senktide had a lower heart rate (398 ± 15 and 394 ± 6 beats/min, respectively; $P < 0.05$ and $P < 0.01$).

3.2. Effects of three tachykinin NK_1 receptor antagonists on the peripheral effects of substance P

The antagonists LY303241, LY303870 and LY306740 inhibited the vasodepressor and salivary responses to substance P in a dose-dependent manner (Figs. 2 and 3). The rank order of potency of antagonists to inhibit the substance P-induced vasodepressor effect was LY303241 (IC_{50} = 450 nmol/kg) ≥ LY306740 (IC_{50} = 500 nmol/kg) > LY303870 (IC_{50} = 600 nmol/kg) while that for inhibition of the salivary response to substance P was LY303870 (IC_{50} = 225 nmol/kg) > LY306740 (IC_{50} = 700 nmol/kg) ≥ LY303241 (IC_{50} = 750 nmol/kg). Whereas LY306740 (9 μmol/kg) inhibited the substance P-induced decreases in mean arterial pressure for more than 60 min, the blockade by LY303241 and LY303870 at the same dose was almost over after 1 h (Fig. 4). In contrast, the inhibition elicited by the three compounds of the salivary response to substance P was still prominent after 1 h. The injection of the vehicle had no significant effects on either the vascular effect or the salivation induced by substance P (Fig. 4).

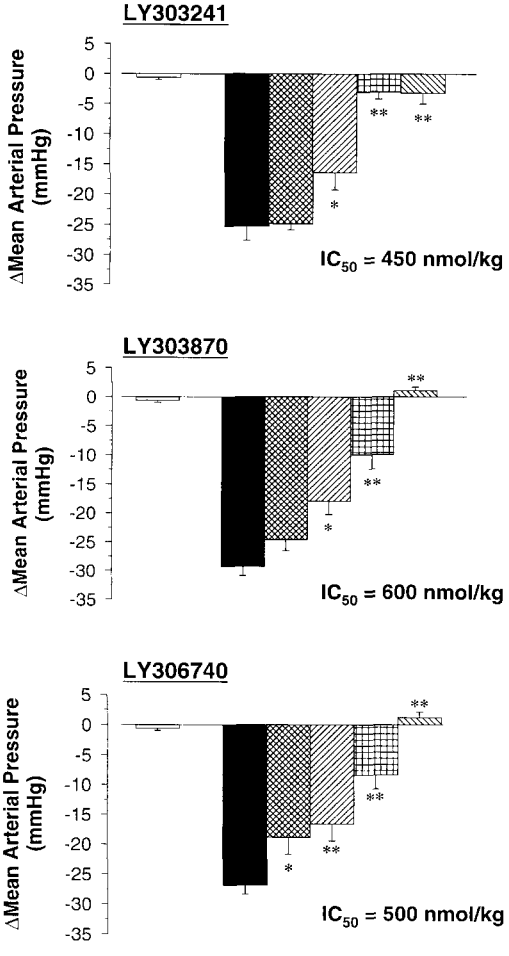


Fig. 2. Dose-dependent inhibition of the maximal changes in mean arterial blood pressure elicited by 0.65 nmol/kg substance P (2nd column) after treatment with LY303241, LY303870 or LY306740 at 65 nmol/kg (3rd column), 325 nmol/kg (4th column), 1.8 μmol/kg (5th column) and 9 μmol/kg (6th column) (n = 4–8 rats). Vehicle is also shown (1st column). Significance to substance P alone: * $P < 0.05$; ** $P < 0.01$.

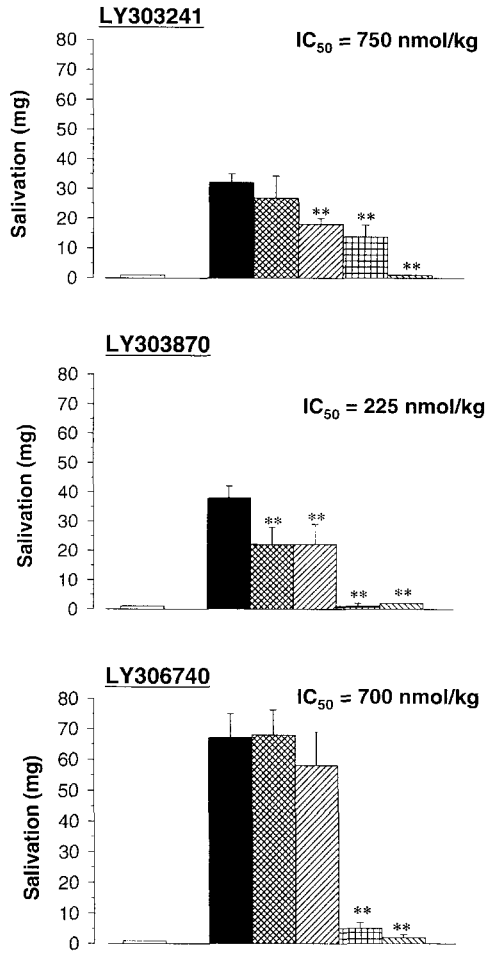


Fig. 3. Dose-dependent inhibition of the maximal salivary response induced by 0.65 nmol/kg substance P (2nd column) after treatment with LY303241, LY303870 or LY306740 at 65 nmol/kg (3rd column), 325 nmol/kg (4th column), 1.8 μmol/kg (5th column) and 9 μmol/kg (6th column) (n = 4–8 rats). Vehicle is also shown (1st column). Significance to substance P alone: * $P < 0.05$; ** $P < 0.01$.

3.3. Effects of inactive enantiomers on the peripheral effects of substance P

The opposite (S) enantiomers of LY303870 (LY306155) and LY306740 (LY307679) were tested against the effects of substance P. As shown in Fig. 5, neither LY306155 nor LY307679 (65 nmol/kg–9 μmol/kg) affected the decreases in mean arterial pressure induced by substance P. However, both compounds potentiated the salivation induced by substance P; this enhancing effect was not

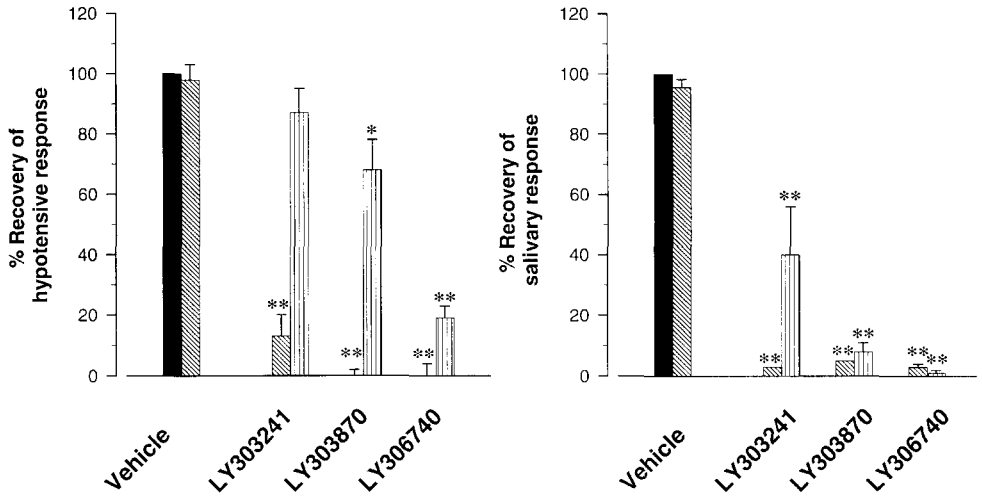


Fig. 4. Recovery of the vasodepressor and salivary responses to i.v. 0.65 nmol/kg substance P in the presence of 9 μmol/kg antagonists (n = 4–8 rats). The effects induced by the first injection of substance P (1st column) were considered as 100% and were not significantly altered 60 min later after the second injection of substance P if the rats were pretreated with the vehicle (2nd column). The antagonists were injected i.v. 5 min (3rd, 5th and 7th columns) before the second i.v. injection of substance P. A third injection of substance P was given alone 60 min later (4th, 6th and 8th columns). Significance to substance P alone: * $P < 0.05$; ** $P < 0.01$.

dose-dependent. The two (S) enantiomers had no direct effect on salivation.

3.4. Effects of the tachykinin NK_1 receptor antagonists on muscarinic, tachykinin NK_2 and NK_3 receptors

Pretreatment with 9 μmol/kg of LY303241, LY303870 or LY306740 did not significantly alter the maximal responses evoked by 40 μmol/kg carbachol ($n = 6$) on mean arterial pressure (LY303241: -39 ± 4 vs. -33 ± 3 mmHg; LY303870: -42 ± 3 vs. -38 ± 4 mmHg; LY306740: -37 ± 5 vs. -38 ± 7 mmHg) and salivation (LY303241: 18 ± 2 vs. 24 ± 5 mg; LY303870: 35 ± 6 vs. 39 ± 9 mg; LY306740: 35 ± 5 vs. 35 ± 3 mg). Moreover, the three compounds failed to affect significantly the pressor and chronotropic responses elicited by the tachykinin NK_2 receptor-selective agonist [β-Ala^8]neurokinin A-(4–10) (65 nmol/kg) (Table 4). Whereas 9 μmol/kg

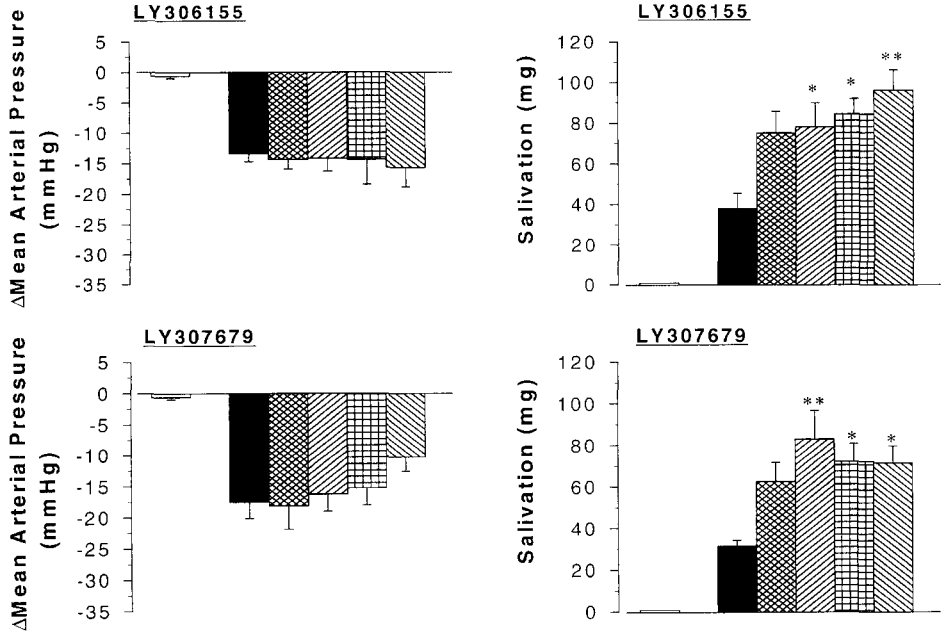


Fig. 5. Effects of the (S) enantiomers of LY303870 (LY306155) and of LY306740 (LY307679) at 65 nmol/kg (3rd column), 325 nmol/kg (4th column), 1.8 μmol/kg (5th column) and 9 μmol/kg (6th column) on the maximal changes in mean arterial blood pressure and salivation produced by 0.65 nmol/kg substance P (2nd column) (n = 4–8 rats). The direct effect of vehicle is also shown (1st column). Significance to substance P alone: * $P < 0.05$; ** $P < 0.01$.

Table 4
Effects of tachykinin NK_1 receptor antagonists on maximal cardiovascular changes in response to tachykinin NK_2- and NK_3-selective agonists

	ΔMean arterial pressure (mmHg)	ΔHeart rate (beats/min)
Vehicle	−1±0	−2±1
[β-Ala^8]Neurokinin A-(4–10)	8±1 [b]	16±4 [a]
LY303241 + [β-Ala^8]neurokinin A-(4–10)	6±1 [b]	16±1 [a]
[β-Ala^8]Neurokinin A-(4–10)	8±2 [a]	34±8 [a]
LY303870 + [β-Ala^8]neurokinin A-(4–10)	9±2 [b]	23±5 [a]
[β-Ala^8]Neurokinin A-(4–10)	6±1 [a]	17±3 [a]
LY306740 + [β-Ala^8]neurokinin A-(4–10)	6±1 [a]	19±5 [a]
Senktide	−30±3 [b]	−28±3 [b]
LY303241 + senktide	−15±3 [b,c]	−18±2 [b,c]
LY303870 + senktide	−30±6 [b]	−30±2 [b]
LY306740 + senktide	−23±3 [b]	−28±3 [b]

Data are means ± S.E.M. for 6 rats; significance is indicated by [a] $P < 0.05$; [b] $P < 0.01$ (to vehicle) or [c] $P < 0.05$ (to senktide alone). Antagonists were given at the dose of 9 μmol/kg i.v. while [β-Ala^8]neurokinin A-(4–10) and senktide were given at 65 nmol/kg and 16.3 nmol/kg, respectively.

LY303241 reduced partly the hypotension and bradycardia induced by senktide (16.3 nmol/kg), LY303870 and LY306740 did not affect these responses (Table 4).

3.5. Intrinsic effects of the tachykinin NK_1 receptor antagonists

Between 65 nmol/kg to 9 μmol/kg, no cardiovascular effects were seen with LY303870 and LY306740. However, at 9 μmol/kg, LY303241 decreased mean arterial pressure (≈ −25 mmHg) and heart rate (≈ −50 beats/min) for 1 min and 10 min, respectively. The three tachykinin NK_1 receptor antagonists had no direct effects on salivation at doses up to 9 μmol/kg.

4. Discussion

4.1. Peripheral effects of tachykinin receptor agonists

The i.v. injection of 0.65 nmol/kg substance P elicited a transient fall in mean arterial pressure, which is in agreement with previous results obtained in the urethane-anaesthetized rat (Maggi et al., 1987; Couture et al., 1989) and the conscious rat (Pompei et al., 1993). Substance P activates tachykinin NK_1 receptors situated on the vascular endothelium to cause the release of endothelial nitric oxide, which leads to the relaxation of vascular smooth muscles (D'Orléans-Juste et al., 1985; Whittle et al., 1989). At this dose, substance P failed to induce significant chronotropic changes. Chronotropic responses were previously seen with higher doses of substance P (Couture et al., 1989). Hancock and Lindsay (1995) found that i.v. injection of substance P (0.6 and 1.0 nmol/kg) caused a lowering of blood pressure which lasted less than 70 s while higher doses (3.3–33 nmol/kg) elicited an initial fall in blood pressure and a secondary hypertension which was accompanied by a rise in heart rate. The pressor and chronotropic effects of substance P were ascribed to the release of catecholamines from sympathetic nerves and the adrenal medulla. Even at high doses (up to 32.5 nmol/kg) no rises in mean arterial pressure were seen with substance P in the urethane-anaesthetized rat (Couture et al., 1989). This discrepancy may be due to the use of different anaesthetics. In the study of Hancock and Lindsay (1995), the rats were anaesthetized with ketamine and sodium pentobarbital. The present results confirm previous studies showing that substance P increases salivation (Maggi et al., 1987). This effect appears to be due to the direct activation of tachykinin NK_1 receptors located on the parotid glands (Bahouth et al., 1985; Buck and Burcher, 1985; Lee et al., 1986; Giuliani et al., 1988). Since the salivation elicited by the tachykinin NK_1 receptor selective agonist, [Sar^9,Met(O$_2$)^{11}]substance P, in contrast to substance P was subjected to desensitization (Cellier and Couture, unpublished observations), substance P was used as a tachykinin NK_1 receptor agonist in this study.

The i.v. injection of the tachykinin NK_2 receptor-selective agonist [β-Ala^8]neurokinin A-(4–10) induced transient rises in mean arterial pressure and heart rate. In our previous study, the chronotropic effect induced by a similar tachykinin NK_2 receptor agonist, namely neurokinin A-(4–10), was ascribed to the release of catecholamines from sympathetic nerve terminals and the adrenal medulla (Couture et al., 1989). In the latter study, however, neurokinin A-(4–10) had a weak hypotensive effect similar to that produced by [β-Ala^8]neurokinin A-(4–10) in the conscious rat (Pompei et al., 1993).

The i.v. injection of 16.3 nmol/kg of the tachykinin NK_3 receptor-selective agonist senktide induced decreases in mean arterial pressure and heart rate; this is fully in agreement with the effects observed by Couture et al. (1989). The hypotensive effect of senktide was associated with a vagal reflex induced by the activation of sensory nerves. Pompei et al. (1993) have reported that senktide has no peripheral effects on the cardiovascular system of the conscious rat. The differences between the two studies may be attributable to the presence or not of anaesthesia. Indeed, we have reported that 4 nmol/kg senktide increases mean arterial pressure and heart rate in the conscious rat (Cellier et al., 1995).

4.2. Effects of the antagonists on the peripheral response to substance P

The decreases in mean arterial pressure and salivation induced by substance P were inhibited by the three non-peptide tachykinin NK_1 receptor antagonists in a dose-de-

pendent manner. The inhibition was complete with the highest dose of antagonist, thus confirming the involvement of tachykinin NK_1 receptors in both the vasodepressor and sialogogic responses as revealed with other non-peptide tachykinin NK_1 receptor antagonists (Snider et al., 1991; Lembeck et al., 1992; Rouissi et al., 1993; Jung et al., 1994). The order of potencies of the three Eli Lilly compounds was dissimilar for the two parameters. Whereas LY303241 and LY306740 were more potent than LY303870 to inhibit the substance P-induced hypotension, LY303870 was the most potent to inhibit the salivary response to substance P. These differences could be ascribed to the existence of different receptor subtype or transduction mechanisms linked to the tachykinin NK_1 receptors found on the vascular endothelium and salivary glands. For instance, Clerc et al. (1992) found that the tachykinin NK_1 receptor-selective agonist septide was 7 times more potent in displacing binding of [^{3}H]substance P in the rat submaxillary gland (K_i = 161 nM) than in the rat brain (K_i = 1157 nM). Septide is resistant to metabolic degradation by peptidases in both tissues and therefore the stability of the agonist cannot explain this difference in the order of potency. In addition, Sagan et al. (1995) have reported that, on human tachykinin NK_1 receptor transfected CHO cells, the tachykinin NK_1 receptor-selective agonists [Pro^9]substance P and septide display different affinity (100-fold lower for septide) and activate different second messengers (septide does not activate cyclic AMP production in contrast to [Pro^9]substance P). Alternatively, these differences could be related to the pharmacodynamic features of the compounds which cause a more persisting inhibition of the salivary response.

The direct cardiovascular effects elicited by the highest doses of LY303241 remain to be explained but are unlikely to be due to agonist activity since no salivation was induced by this compound.

4.3. Stereoselectivity and specificity of the three tachykinin NK_1 receptor antagonists

The opposite (*S*) enantiomers of LY303870 (LY306155) and LY306740 (LY307679) did not affect the vasodepressor response to substance P. These compounds potentiated the salivation induced by substance P, although not in a dose-dependent manner. The mechanism of such hypersalivation remains obscure and does not appear to be due to a residual intrinsic activity. Interestingly, this potentiation of the substance P sialogogic effect has been seen in the presence of neuropeptide K and neuropeptide γ (Takeda and Krause, 1989a,b). Also, the non-peptide tachykinin NK_1 receptor antagonist RP67,580 stereoselectively potentiates at high doses the hypertension induced by i.c.v. injection of substance P (Culman et al., 1995). Further studies are necessary to see if there is a link between these synergistic effects. However, the lack of inhibition of the vasodepressor and salivary responses to substance P by these enantiomers underscores the specificity of action of LY303870 and LY306740 at the tachykinin NK_1 receptor.

The decreases in mean arterial pressure and salivation elicited by the muscarinic receptor agonist carbachol were not affected by LY303241, LY303870 and LY306740. This also confirms the specificity of action of the three compounds at tachykinin NK_1 receptors.

4.4. Selectivity of the three tachykinin NK_1 receptor antagonists

LY303241, LY303870 and LY306740 had no effect on the increases in mean arterial pressure and heart rate induced by [β-Ala^8]neurokinin A-(4–10). This indicates that they do not act at tachykinin NK_2 receptors in this in vivo model. Similarly, LY303870 and LY306740 did not interfere with tachykinin NK_3 receptor-mediated responses. In contrast, LY303241 slightly reduced the cardiovascular effects of senktide by a mechanism which remains to be studied.

In conclusion, the three antagonists potently inhibit the vasodepressor and salivary effects of substance P in a dose-dependent, stereoselective and specific manner in the urethane-anaesthetized rat. LY303870 and LY306740 appear to be the most interesting compounds since they act selectively at the tachykinin NK_1 receptor and have no direct effects on the cardiovascular system and salivation even at high doses. Finally, the difference in the order of potency of the three antagonists to prevent the effects of substance P on the two parameters could be ascribed to pharmacodynamic features of the antagonists, different second messenger mechanisms or to the existence of tachykinin NK_1 receptor heterogeneity in the same species. Hence, LY303870 and LY306740 represent a new class of tachykinin NK_1 receptor antagonists to further our understanding of the roles played by tachykinins in various physiological and pathological processes.

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