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A water-soluble, stable dipeptide NK_1 receptor-selective neurokinin receptor antagonist with potent in vivo pharmacological effects: S18523

Jacqueline Bonnet [a], Nathalie Kucharczyk [a], Pascale Robineau [a], Michel Lonchampt [a], Catherine Dacquet [a], Domenico Regoli [b], Jean-Luc Fauchère [a,*], Emmanuel Canet [a]

[a] *Institut de Recherches SERVIER, 11 rue des Moulineaux, 92150 Suresnes, France*
[b] *Département de Pharmacologie, Université Sherbrooke, Sherbrooke, Québec J1H 5N4, Canada*

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Abstract

The potassium salt of a chemically stabilized dipeptide, {1-[4-(1*H*-tetrazol-5-yl)butyl]indol-3-yl}carbonyl-Hyp-Nal-*N*(methyl)-Bzl, (Hyp = (*R*)-4-hydroxy-L-proline; Nal = 3-L-(β-naphthyl)-alanine), S18523, is described as a new water-soluble, potent and selective NK_1 receptor antagonist. The low molecular weight antagonist ($M_r = 736$) displays nanomolar potency ($pA_2 = 9.6$) in the rabbit vena cava (NK_1) bioassay and nanomolar affinity ($pK_i = 9.1$) on the human NK_1 receptor expressed by lymphoblastoma cells. It is devoid of μ-opiate affinity ($K_i > 10^{-4}$ M with respect to tritiated Tyr-DAla-Gly-MePhe-Gly-ol), has negligible calcium-channel affinity (estimated $K_i = 2.6 \times 10^{-5}$ M, with respect to isradipine) and does not cause peritoneal mast-cell degranulation. S18523 has strong antinociceptive effects in three classical pain tests in vivo both by i.v. and p.o. routes. The dipeptide potently antagonizes bronchoconstriction provoked by exogenous substance P in the guinea-pig and acts longer than the non-peptide antagonist CP99994, when administered as aerosol. Finally, S18523 displays antiinflammatory properties, since it dose-dependently inhibits substance P-induced plasma extravasation both in the bladder ($ID_{50} = 0.18$ mg/kg i.v.) and bronchi ($ID_{50} = 0.14$ mg/kg i.v.) of the guinea-pig.

Keywords: Neurokinin; NK_1 receptor antagonist; Bronchoconstriction; Pain; Plasma extravasation; Neuropeptide

1. Introduction

Neurokinins are a family of neuropeptides which share a common pentapeptide sequence Phe-Xaa-Gly-Leu-Met-NH_2, Xaa = variable amino-acid residue. Mammalian representatives are substance P, neurokinin A and neurokinin B, which are synthesized as segments of protein precursors and are released by specific processing endopeptidases under physiological conditions (Carter and Krause, 1990). The neurokinins have been shown to act via three receptor types named NK_1, NK_2 and NK_3 (Henry, 1987). These receptors identified by molecular cloning (review: Otsuka and Yoshioka, 1993) belong to the seven-transmembrane rhodopsin-like proteins. They are activated to various degrees by the natural ligands substance P, neurokinin A and neurokinin B, which show low selectivity. However, selective agonist ligands have been optimized using chemical peptide synthesis, structure-activity studies and selective bioassay tissue preparations (Regoli et al., 1988). These ligands have been instrumental for the development of neurokinin receptor antagonists. A comprehensive review on receptors and antagonists for substance P and related peptides covers these topics (Regoli et al., 1994a).

In the periphery, tachykinins are found in the neuronal plexus of the gastrointestinal tract, in thin sensory afferent nerves and in sympathetic and parasympathetic neuronal cell types. Peptides synthesized in the cell bodies of sensory neurones are transported both centrally and peripherally, and can be released from spinal or peripheral nerve terminals. Peripheral release of neurokinins causes vasodilation, plasma extravasation, salivary gland secretion, activation of the immune system and mast-cell degranulation (Holzer, 1988), whereas central release of neurokinins is involved in the transmission of nociceptive signals in the spinal cord (Duggan et al., 1988). It has been suggested that development of selective antagonists for the NK_1 receptor may lead to compounds that could be useful in the treatment of pain, inflammation, gastrointestinal diseases,

* Corresponding author. Tel.: +33 1 41 18 23 65; fax: +33 1 41 18 24 30.

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allergy, lesions of the urinary system and migraine. More recently, it has also been suggested that NK_1 receptor antagonists may be useful in the treatment of emesis (Tattershall et al., 1994) and even in psychotic disorders, such as schizophrenia (Otsuka and Yoshioka, 1993). Furthermore, the release of neurokinins from lung sensory nerve endings (C-fibers) has been implicated in non-adrenergic non-cholinergic (NANC) bronchoconstriction, neurogenic mucosal plasma extravasation and mucus hypersecretion in the airways (Lundberg et al., 1983; Barnes et al., 1991), thus suggesting a possible therapeutic role for both NK_1 (Rogers et al., 1988) and NK_2 antagonists (Advenier et al., 1987) in inflammatory diseases of the airways and in asthma.

A considerable effort has been put into the discovery of peptide and non-peptide antagonists of the neurokinins, mainly due to the therapeutic potential of this class of compounds. While both size and structure of the first peptide antagonists spantides I and II (Folkers et al., 1990) were clearly related to the natural hormones substance P, neurokinin A and neurokinin B, a number of much smaller pseudopeptide and non-peptide antagonists were discovered later on.

Representative peptide-derived NK_1 antagonists include the modified tetrapeptide S16474 (Kucharczyk et al., 1993), tripeptide FR113 680 (Morimoto et al., 1992) and dipeptide FK888 (Fujii et al., 1992). Even simpler Trp derivatives (the *N*-acyl-L-Trp-benzyl esters) with trifluoromethyl substituents on the benzyl rings (L732138; MacLeod et al., 1993) are potent and selective NK_1 (human) receptor antagonists. Non-peptide antagonists were also described (Watling and Krause, 1993) originating both from peptide drug design or from screening of chemical files or combinatorial libraries. The precursor of the NK_1-selective CP96345 (Snider et al., 1991) was followed by RP67580 (Garret et al., 1991), SR140333 (Emonds-Alt et al., 1993), CP99994 (McLean et al., 1993) and RPR100893 (Tabart and Peyronel, 1994). Although some of the most potent compounds are currently evaluated in clinical studies, little is known about the therapeutic potential of these antagonists (Longmore et al., 1995).

We report here on the rational design, synthesis and pharmacological evaluation of the potent dipeptide-derived NK_1 antagonist {1-[4-(1*H*-tetrazol-5-yl)butyl]indol-3-yl}carbonyl-Hyp-Nal-*N*(methyl)-Bzl, (Hyp = (*R*)-4-hydroxy-L-proline; Nal = 3-L-(β-naphthyl)-alanine), S18523 (for structure formula, see Fig. 1).

2. Materials and methods

2.1. Materials

The tritiated ligands ($Sar^9MetO_2^{11}$)substance P and DAGO were purchased from NEN-Dupont, [^{3}H]neurokinin A from Euromedex (Strasbourg, France), while the unlabelled reference peptides substance P and neurokinin A were obtained from Bachem. Phenylbenzoquinone, formalin and Evans blue were products from Sigma, Carlo Erba and Fluka, respectively. Mepyramine, propanolol, capsaicin and *o*-phthalaldehyde were Sigma compounds.

2.2. Chemistry

Optimization started from the structure of the NK_1 antagonist S16474 (Kucharczyk et al., 1993) which was water-soluble, stable to proteolysis and devoid of adverse effects, such as mast-cell degranulation or calcium-channel affinity and of μ-opioid receptor binding. Extensive structure-activity relationships studies towards a reduction of size and a minimization of the peptide character, while maintaining high potency and water solubility, finally led to S18523. One key step was the introduction of a solubilizing group on the indole nitrogen with an optimized

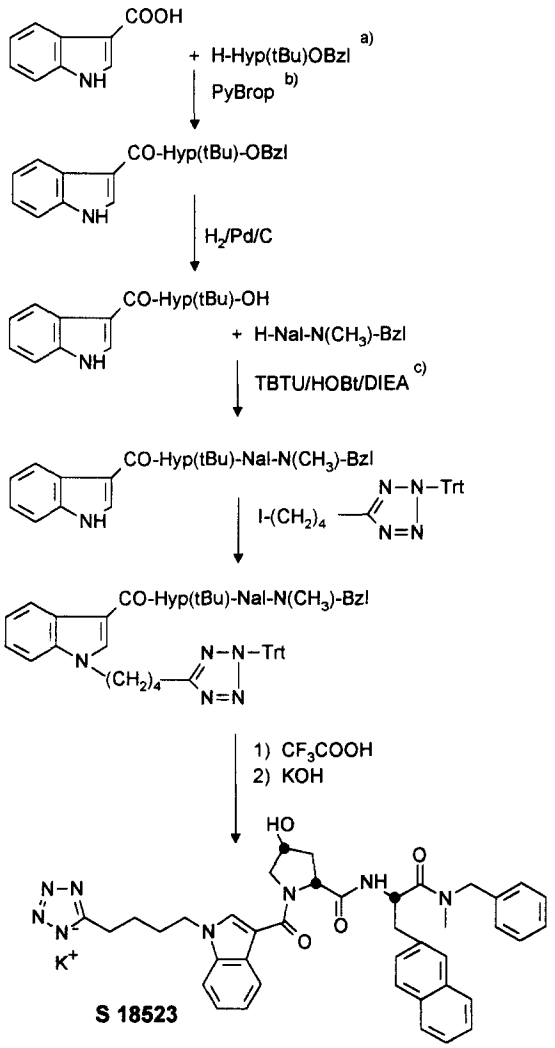

Fig. 1. Synthesis scheme of S18523. [a] Abbreviations for amino acids and protecting groups according to IUPAC/IUB (1984, Biochem. J. 219, 345); [b] Coste et al. (1991); [c] Knorr et al. (1989).

Table 1
pA_2 (±S.E.M.) [a] of S18523 against selective standard agonists in isolated tissue bioassays, compared to representative peptide and non-peptide antagonists and binding affinity pK_i (nM) to human NK_1 and NK_2 receptors

Antagonist	Preparation	RVC (NK_1) [b]	RPA (NK_2) [c]	RPV (NK_3) [d]	IM9 (NK_1) [e]	CHO (NK_2) [f]
	Reference agonist	SP	NKA	[MePhe^7]NKB	[^{3}H][Sar^{9}Met(O_2)^{11}]SP	[^{3}H]NKA
S18523		9.6 ± 0.6	5.6 ± 0.5	5.0 ± 0.5	9.10 ± 0.30	5.48 ± 0.13
Spantide I [g]		6.8	5.8	6.2	n.d.	n.d.
S16474 [h]		7.0	5.5	5.2	7.52 ± 0.15	6.38 ± 0.02
FK888 [i]		9.1	5.2	5.2	8.88	5.92
L732138 [j]		8.4	5.5	5.2	8.72	~ 4
CP99994		8.9	Inactive	Inactive	9.60	< 4
SR140333		9.8	5.8	5.8	9.69	n.d.

[a] Number of independent experiments ≥ 6; [b] RVC, rabbit vena cava; [c] RPA, rabbit pulmonary artery; [d] RPV, rat portal vein; [e] IM9 human lymphoblastoma; [f] transfected Chinese hamster ovary cells; [g] spantide I = *H*-D-Arg-Pro-Lys-Pro-Gln-Gln-D-Trp-Phe-D-Trp-Leu-Leu-NH_2; [h] S16474, cyclo[-Abo-Asp(D-Trp(OC(CH_2)$_2$ COOH)-Phe-*N*(Me)-Bzl)-] and Abo, (*S,S,S*)-azabicyclooctane-carboxylic acid; [i] FK888, N^2-[(*R*)-4-hydroxy-1-(1-methyl-1 *H*-indol-3-yl) carbonyl-L-prolyl]-*N*-methyl-*N*-benzyl-3-(2-naphthyl)-L-alaninamide; [j] Ac-Trp-*O*-(3,5-bis-trifluoromethyl)-Bzl; n.d., not determined.

spacer length of four methylene groups. The final compound (Fig. 1) is formally a dipeptide, although the only residual amide bond links two non-natural amino acids, Hyp and Nal, and is hardly recognized by proteolytic enzymes, as confirmed by degradation studies (see Results). The C-terminal substituent, *N*-methyl-benzyl, is also a stabilizing unit against carboxypeptidases, while the tetrazole group on the indole-carbonyl moiety (a substitute for tryptophan) provides water solubility. The new antagonist was easily obtained by the classical methods of peptide synthesis in solution using differential protection and selective deprotection (Fauchère and Schwyzer, 1981) following the synthesis scheme given in Fig. 1. The intermediates were characterized and the final product purified by preparative high-pressure liquid chromatography (HPLC) on reverse phase before being converted into its potassium salt. It had a single peak in HPLC, a correct elemental analysis and the expected molecular weight as estimated by fast atom bombardment mass spectrometry.

2.3. Binding assays on human NK_1 and NK_2 receptors

Radioligand binding assays were performed with the IM9 human lymphoblastoma cell line which expressed NK_1 receptors (Payan et al., 1986) or with transfected Chinese hamster ovary (CHO) cells, a cell line expressing a single class of high-affinity human NK_2 receptor sites (about 600 000 binding sites/cell) with an apparent K_D of 2.1 nM for neurokinin A (Takeda et al., 1992). Cells were incubated for 45 min at 23°C with [^{3}H][Sar^9-Met(O_2)^{11}]substance P or [^{3}H]neurokinin A (approximately 0.1 nM) to label NK_1 and NK_2 sites, respectively. Binding in the presence of defined concentrations of the test compound was then estimated with a conventional filtration assay. Receptor selectivity with respect to opiate μ-receptors was assessed in a classical binding assay on rat brain membranes using tritiated DAGO as the μ-selective ligand (Goldstein and Naidu, 1989).

2.4. Bioassays

In vitro tests were performed on three isolated organs, the rabbit vena cava, the rabbit pulmonary artery and the rat portal vein, the contractile response of which are mediated exclusively by NK_1, NK_2 and NK_3 receptors, respectively. Experiments were carried out as described by Regoli et al. (1994b) and the results are expressed as pA_2 values (Arunlakshana and Schild, 1959) obtained against the same standard agonists indicated in Table 1.

Table 2
Blood levels (μg/ml) of pseudopeptide and non-peptide neurokinin receptor antagonists after oral administration (25 mg/kg, rat)

Compound	Rat portal vein			Rat jugular vein			log *P* [a]
	30′	60′	90′	30′	60′	90′	
± CP96345 [b]	1.79 ± 0.01	0.77 ± 0.01	0.50 ± 0.02	1.24 ± 0.02	0.55 ± 0.02	0.36 ± 0.02	1.44
S18523	0.24 ± 0.01	0.14 ± 0.01	0.10 ± 0.01	0.19 ± 0.01	0.10 ± 0.01	0.08 ± 0.01	2.61
S15890 [c]	0.18 ± 0.01	0.26 ± 0.01	0.11 ± 0.01	0.11 ± 0.01	0.15 ± 0.01	0.04 ± 0.01	3.17
FK888 [d]	0.15	0.16	0.13	0.09	0.11	0.06	3.90
S17773 [e]	0.12 ± 0.01	0.13 ± 0.02	0.14 ± 0.02	0.08 ± 0.01	0.10 ± 0.02	0.08 ± 0.02	4.50

[a] Log of partition coefficient in octanol/water at pH 7.1; [b] non-peptide reference antagonist (Snider et al., 1991); [c] (des-methyl)-FK888 (Kucharczyk et al., 1993); [d] estimated from correlation with log *P* values; [e] 5,6,7,8-tetrahydro-FK888.

2.5. Estimation of blood levels

Estimation of blood levels in the portal and jugular veins, after oral administration to the rat of S18523 and other peptide and non-peptide antagonists (Table 2), were performed according to Paladino et al. (1994), as a preliminary test for evaluating intestinal absorption and hepatic metabolism of the compound. Briefly, the antagonist suspended in arabic gum, was administered to the rat by oesophagal intubation at the dose of 25 mg/kg. Blood samples taken from the same rat by puncture at the portal and jugular vein at 30, 60 and 90 min after gavage (3 rats at each time point), were analyzed photometrically after extraction and separation by HPLC.

2.6. Hot-plate test, phenylbenzoquinone-induced writhing and formalin test

The hot-plate test was carried out in mice (male CD1 26–30 g) randomly assigned to groups of 12 animals using the method described by Eddy and Leimbach (1953). S18523 was administered i.v. 10 min and the reference CP99994 5 min before the test. Results were expressed as an ED_{50} value, corresponding to the dose required to increase the reaction time by 50%.

Writhing responses were provoked in mice (male CD1 26–30 g) by i.p. injection of a solution of phenylbenzoquinone, as originally described by Siegmund et al. (1957). 5 min after the injection of phenylbenzoquinone, the number of abdominal constrictions observed over a 5-min period were recorded on mice randomly assigned to groups of 12 animals. S18523 was administered either i.v. or p.o. 5 or 20 min, respectively, prior to the injection of phenylbenzoquinone. The reference CP99994 was administered 5 min before the phenylbenzoquinone. Results expressed as ID_{50} value, correspond to the dose required to produce 50% inhibition of the number of phenylbenzoquinone-evoked constrictions.

Behavioural nociceptive responses to the intraplantar injection of 0.1 ml of a 5% v/v aqueous formalin solution were measured in rats (CD males 130–160 g) using a modification of the method described by Dubuisson and Dennis (1977). Rats randomly assigned to groups of 8 animals, were placed in a perspex observation chamber and the response to formalin was measured as the time spent licking the injected hindpaw over a 10-min period during the delayed phase response (25–35 min after formalin injection). The effect of S18523 (or of CP99994) on the delayed, tonic phase response was examined by administering the compound i.v., 20 min after the formalin injection.

2.7. Substance P-induced bronchoconstriction

Male Hartley guinea-pigs (360–460g, Charles River) were anaesthetized with urethane (1.5 g/kg i.p.) and anaesthesia was monitored in a classical way using clinical criteria. The trachea, left jugular vein and right carotid artery were cannulated. Body temperature was maintained at 37° ± 1°C using a blanket control unit. The animal was attached to a respiratory pump, artificially ventilated (60 breaths/min, tidal volume 10 ml/kg) and curarized (gallamine triethiodide 2 mg/kg i.v.) to prevent interference from spontaneous respiration. Artificial ventilation was defined so as to keep both blodd gases and pH in the normal range, as determined in preliminary experiments. Bronchoconstriction experiments were terminated by an overdose of pentobarbital. Pulmonary inflation pressure was recorded on a breath-by-breath basis using a Statham pressure transducer connected to a side arm of the tracheal cannula. Carotid blood pressure was measured with a similar transducer (Spectramed P23xL, Bilthoven, The Netherlands). Both transducers were connected to amplifiers and a recorder (Gould RS3400, Valley View, OH, USA). All animals were pretreated with mepyramine (1 mg/kg i.v.) to block the effects of histamine release that may be induced by tachykinins, and with propanolol (1 mg/kg i.v.). Substance P (2 nmol/kg i.v.) was injected 15 min before i.v. treatment with either S18523 (200 and 500 nmol/kg), CP99994 (50, 200 and 500 nmol/kg), or saline. Furthermore, the effects of CP99994 (10–3 M) and S18523 (10–3 M) by aerosol (40 s) were also tested (aerosol device: Portasonic 9, Devilbiss, Somerset, PA, USA). Bronchoconstrictive responses to substance P were then examined 3, 20 and 40 min following drug treatment and expressed as increases in pulmonary inflation pressure (Bertrand et al., 1993). For each group, the results were given as percentages of the mean response in the control group at the same time (mean ± S.E.M., $n = 5$).

2.8. Capsaicin-induced neurogenic plasma extravasation

Male Hartley guinea-pigs (360–460 g, Charles River) were anaesthetized and mechanically ventilated as described above. Left jugular vein and right carotid artery were cannulated for administration of drugs and for monitoring of systemic arterial pressure, respectively. After a 15-min rest period, the animals were injected i.v. with S18523 (0.1, 0.3 and 1 mg/kg) or saline ($t = 0$) and then, 3 min later, with Evans blue dye (30 mg/kg). 1 min later, capsaicin (200 μg/kg in 1 min) was infused. In separate experiments, S18523 was tested by the oral route (10 mg/kg p.o., 1 h before capsaicin injection). At $t = 9$ min, the animals were disconnected from the ventilator. The chest was opened and a cannula was inserted into the aorta through the left ventricle. Intravascular dye was eliminated using a perfusion through this cannula with 100 ml saline at a pressure of 80 mm Hg. Main bronchi were then excised, cleaned, blotted, weighed, placed in 2 ml formamide for dye extraction and incubated for 16 h at 37°C. Absorption of the extracted dye as measured at 620 nm and the results, expressed as ng Evans blue/mg wet tissue ($n = 6$; Rogers et al., 1988).

2.9. Substance P-induced plasma extravasation in the bladder

Plasma extravasation in the guinea-pig was examined by measuring the leakage of i.v. injected Evans blue into the tissues of the bladder, evoked by substance P (Saria et al., 1983). Male Hartley guinea-pigs (300–350 g) were anaesthetised with isoflurane and a catheter was inserted into the jugular vein for i.v. injection of Evans blue (30 mg/kg), substance P (1 μg/kg), S18523 or CP99994. After 5 min, the animals were exsanguinated under isoflurane anaesthesia and the bladder was removed, minced, placed into 50-ml centrifuge tubes containing 5 ml of an aqueous solution of acetone and sodium sulphate (0.15% w/v) and left overnight at a temperature of 4°C. After centrifugation, 0.2 ml of the supernatant was removed from each tube and the absorbance read at 620 nm. Results were expressed as ng Evans blue/mg tissue as calculated from a calibration curve.

2.10. Mast-cell degranulation

Rat peritoneal mast cells: Male Sprague-Dawley rats (350–400g, Charles River) were sacrificed by CO_2 asphyxiation and mast cells were obtained by rinsing the peritoneal cavity as described by Johnson and Moran (1966). Pooled washed cells were centrifuged at 400g for 10 min at 4°C and resuspended in a glucose and albumin-containing phosphate buffer (pH 7) to a density of 2×10^4 cells/ml. After a 10-min incubation at 37°C, S18523 or spantide I was added to the cell suspension and incubation was carried on for 10 min in order to allow histamine release. The reaction was then quenched by placing the tubes in ice. The cell and supernatant fractions were separated by centrifugation ($700 \times g$ for 10 min at 4°C) and assayed fluorometrically for histamine after condensation with *o*-phthalaldehyde. The amount of histamine released from mast cells into the supernatant was expressed as a percentage of the total histamine content.

2.11. Data analysis

The pA_2 values in the tissue bioassays were the means ± S.E.M. of 6 independent experiments. The pK_i (means ± S.E.M. of 3 independent experiments) were obtained from binding after conversion of the IC_{50} values using the Cheng and Prusoff (1973) equation. The blood levels measured after oral administration were the means ± S.E.M. of 3 measurements of the samples from 3 rats at each vein and each time.

All in vivo data were expressed as means ± S.E.M. ED_{50} and ID_{50} values were determined by simple linear regression with replications and expressed as ±95% confidence limits. Statistical significance of the inhibition of bronchoconstriction was determined with one-way analysis of variance followed by the Newman-Keuls test. Data from the capsaicin-induced extravasation assay were processed using a two-way analysis of variance (Rogers et al., 1988). The significance level was set at $P \leq 0.05$.

2.12. Animal care

The animals were maintained in rooms with controlled temperature (22 ± 2°C) and hygrometry (45–65%) and with a 12 h–12 h light dark cycle. They had free access to tap water and to commercial standard diet. Ethical guidelines for experimental investigations in animals were followed (Zimmermann, 1983) and experimental protocols set up after consultation of the Institute internal ethic committee.

3. Results

3.1. Partition coefficient and water solubility

The log *P* value in octanol/water was estimated from the value of the capacity factor *k'* in reverse-phase HPLC, after calibration with directly measured partition coefficients (shake-flask method, pH 7.1) for compounds of the same series (Kucharczyk et al., 1993). The value for S18523 was: log $P = 2.61 \pm 0.2$. The water solubility of S18523 (potassium salt) was higher than 100 mg/ml at room temperature, in contrast to that of FK888 which was less than 0.01 mg/ml.

3.2. Degradation studies

Incubation at 37°C of S18523 was carried out both with heparinized blood plasma or with rat renal cortex homogenates (protein concentration 500 μg/ml) and followed by addition of trifluoroacetic acid, centrifugation and HPLC analysis of the supernatants. No significant degradation was detectable, even after 24 h incubation in the two media, in contrast to the extensive proteolysis of control peptides, such as substance P or bradykinin (data not shown).

3.3. Binding affinity and inhibitory potency in in vitro assays

Table 1 compares the binding affinity and antagonist potency of S18523 with those of other known antagonists from which the values were obtained under the same experimental conditions (Regoli et al., 1994a; Dacquet et al., 1995). The new antagonist S18523 appears to be a potent ligand of the NK_1 receptor in the rabbit vena cava bioassay and the IM9 cells with pA_2 and pK_i values in the nanomolar range. The compound is selective for the NK_1 receptor since its affinities in the NK_1 systems are 3.5-4 orders of magnitude higher than in the NK_2 and NK_3 receptor assays. On the rabbit vena cava bioassay,

S18523 is more active than CP99994, one of the best non-peptide antagonists (McLean et al., 1993) and shows comparable activity to SR140333 (Emonds-Alt et al., 1993). Moreover, S18523 is more potent than the peptide antagonists spantide I and S16474 and is as active as the water-insoluble parent dipeptide FK888. Similar findings were obtained for the binding affinities measured on cell lines expressing human NK_1 and NK_2 receptors, respectively (last two columns of Table 1). Species selectivity was observed, since in competitive binding studies on rat brain membranes with the NK_1 radioligand ($Sar^9Met\text{-}O_2^{11}$)substance P, S18523 had a K_i value of 10 ± 2 μM, comparable to that of FK888 (2.4 μM), but clearly lower than that of RP67580 (60 nM).

3.4. Selectivity with respect to opiate μ-receptor binding and other assays

S18523 was unable to displace the μ-selective ligand [^{3}H]DAGO from its binding sites on rat brain membranes (estimated $K_i > 10^{-4}$ M). The absence of morphinomimetic activity was confirmed by the fact that in the hot-plate test, the effect of S18523 was not reversed by naloxone. S18523 also showed NK_1 receptor specificity with respect to a number of other receptors, since it was at least 3 orders of magnitude less potent than the reference ligand (in parentheses) in the following binding assays: dopamine D1 (SCH23390: Hess et al., 1986); D2 (raclopride: Köhler et al., 1985); α_1-adreno- (prazosin: Glossmann et al., 1980); α_2-adreno- (RX821002: Hudson et al., 1992); 5-HT_{1A} (8-hydroxy-2-(di-*n*-propylamino)tetralin: Hall et al., 1985); 5-HT_2 (ketanserin: Leysen et al., 1982); 5-HT_3 (BRL43694 (granisetron): Nelson and Thomas, 1989); histamine H_1 (pyrilamine: Hill and Young, 1980); muscarinic (quinuclidinylbenzylate: Roskoski et al., 1985) receptors.

3.5. Blood levels

Blood levels in the portal and jugular vein after oral administration of the antagonists to the rat are compared in Table 2. S18523 was found to be less well absorbed than the non-peptide CP96345, while it was better absorbed than the two derivatives of FK888 which were available (S15890 and S17773). The apparent correlation of the absorption with log P in this series (see Paladino et al., 1994, for discussion) would indicate for FK888 (not tested) an intermediate absorption between that of S15890 and S17773, clearly lower than the absorption of S18523. No extensive metabolism of S18523 or of any of the antagonists is apparent from comparison of the values at the portal and jugular vein.

3.6. Analgesic effects in hot-plate test, phenylbenzoquinone-induced writhing response and the formalin test

In the hot-plate test, S18523 had an ED_{50} of 0.018 mg/kg i.v. (confidence interval: 0.001–0.32 mg/kg). Results are illustrated in Fig. 2. In this test, when administered at 0.05 mg/kg i.v., S18523 was maximally effective

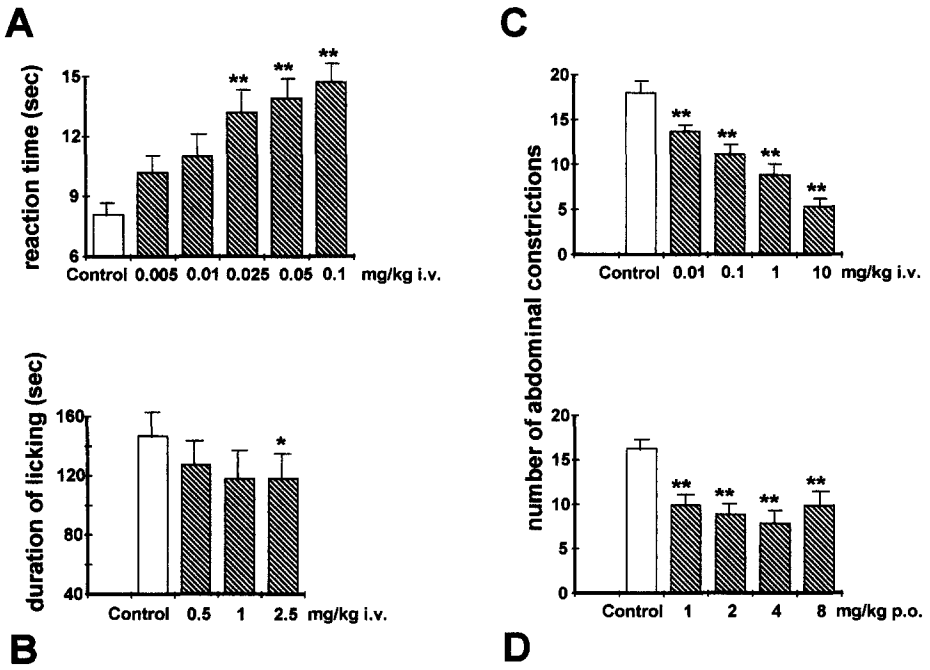

Fig. 2. Antinociceptive effects of S18523 in vivo. (A) Mouse hot-plate test; (B) formalin-delayed response; (C,D) phenylbenzoquinone-induced writhing. Data are means ± S.E.M. of 12 (S18523) or 24 animals (control) per group. * $P < 0.05$; ** $P < 0.01$.

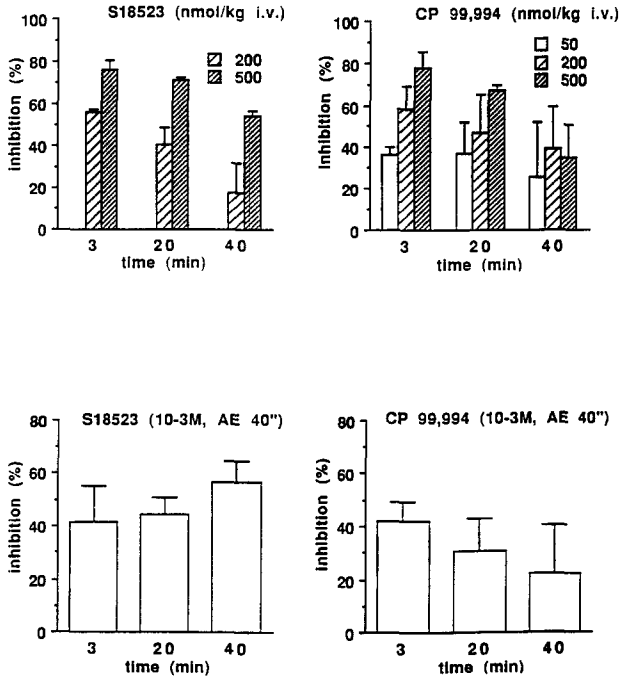

Fig. 3. (Top panel) Effects of S18523 (200 and 500 nmol/kg i.v.) and CP99994 (50, 200 and 500 nmol/kg i.v.) on substance P-induced bronchoconstriction. (Bottom panel) Effects of S18523 (10^{-3} M) and CP99994 (10^{-3} M) by aerosol (AE) on substance P-induced bronchoconstriction. Data (±S.E.M., $n = 5$) are expressed in percentage of the control values (mean of 5 animals treated with vehicle).

at 5–10 min after injection (80–88% activity) and retained 53% activity at 40 min. CP99994 had an ED_{50} of 0.006 mg/kg i.v. and morphine of 0.30 mg/kg i.v. (not shown). In phenylbrenzoquinone-induced writhing, S18523 had an ID_{50} of 0.56 mg/kg i.v. (confidence interval: 0.035–9.6 mg/kg). An effect of 71% reduction in the number of evoked constrictions was obtained with a dose of 10 mg/kg i.v. (Fig. 2). CP99994 produced a 53% inhibition in the writhing response at a dose of 1 mg/kg i.v. When S18523 was administered p.o., a maximum inhibitory effect of 52% was obtained at 4 mg/kg. An inhibition of the delayed phase formalin response in the rat was produced following i.v. administration of S18523. Significant reduction (48%) in pain-related behaviour was obtained at the dose of 2.5 mg/kg i.v. (Fig. 2); the same activity was observed with CP99994 at 2 mg/kg i.v. (not shown).

3.7. Substance P-induced bronchoconstriction in guinea-pigs

The effects of S18523 and CP99994 on substance P-induced bronchoconstriction were first investigated by the i.v. route ($n = 5$ in each group). Results obtained at 3, 20 and 40 min after treatment are reported in Fig. 3 (top panel). While by itself S18523 had no direct effect on baseline airway tone (and on arterial blood pressure), it was found to significantly inhibit responses to substance P with an ID_{50} of approximately 200 nmol/kg at 3 min ($P < 0.05$). A similar effect was obtained with CP99994 at the same dose (Fig. 3), while the dose had to be elevated to 500 nmol/kg to reach the same level of inhibition with FK888 (not shown). By aerosol, results obtained at 3, 20 and 40 min after drug inhalation are shown in Fig. 3 (bottom panel). S18523 was found to significantly inhibit response to substance P ($P < 0.05$). No direct comparison with FK888 was possible by the inhalation route, due to the insolubility of the compound.

3.8. Capsaisin-induced neurogenic plasma extravasation in guinea-pigs

In order to determine the efficiency of S18523 to antagonize endogenously released tachykinins, we studied its activity in capsaicin-induced neurogenic plasma extravasation. S18523 (1 mg/kg i.v.) strongly and significantly reduced capsaicin-induced plasma extravasation in the bronchi, with an ID_{50} of 0.14 mg/kg (confidence interval for $P < 0.05$: 0.003–2.8 mg/kg). By the oral route, S18523 induced a 63% inhibition as compared to non-treated animals, at the dose of 10 mg/kg.

3.9. Antagonism of substance P-induced plasma extravasation in the guinea-pig bladder

S18523 produced dose-dependent inhibition of substance P-induced extravasation of Evans blue dye in the guinea-pig bladder with an ID_{50} of 0.18 mg/kg i.v. (confidence interval: 0.03–2.6 mg/kg). Results are illustrated in Fig. 4. In a separate experiment, 84% inhibition was observed at the dose of 0.5 mg/kg i.v. For comparison, CP99994 produced a 72% inhibition of the response at the lowest dose used of 0.05 mg/kg i.v.

3.10. Mast-cell degranulation

S18523 was tested for its ability to promote degranulation of rat peritoneal mast cells. No degranulation could be

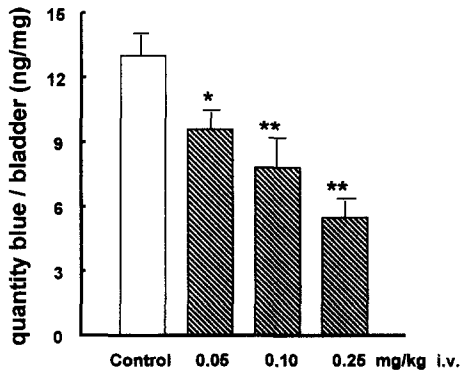

Fig. 4. Effect of S18523 on substance P-induced Evans blue extravasation in the guinea-pig bladder. Data are means ± S.E.M. of 8 animals per group. * $P < 0.05$; ** $P < 0.01$.

detected with S18523 when applied in concentrations up to 100 μM, in contrast to the significant effects of e.g. spantide I (ED_{50} = 1 μM).

3.11. Effects on Ca^{2+} channels

S18523 only weakly affected the binding of the tritiated ligand PN 200–110 (isradipine) on rat cerebral cortex membranes (Guard and Watling, 1992) compared to nifedipine as the reference ligand (K_i 1.5×10^{-8} M). The estimated K_i for S8523 was 2.6×10^{-5} M.

4. Discussion

S18523 is a chemically modified dipeptide, structurally related to FK888. It has the advantage of being readily soluble in water, while FK888 is only sparingly soluble. Water solubility was conferred on the compound by the introduction of an anionic alkyltetrazole substituent on the indole moiety, without impairing its biological activity. This property makes the pharmacological evaluation of S18523 easier, especially in vivo. Despite high water solubility, S18523 is relatively well absorbed when given per os, as estimated by the measurement of the blood levels in the rat portal and jugular vein, and compared to parent derivatives of FK888, although it is less well absorbed than racemic CP96345. Indeed, S18523 has been found to be active in the mouse writhing syndrome after oral administration at doses about 10 times higher than those given i.v.

Results of in vitro studies on isolated organs or on human lymphoblastoma cells (IM9) indicate that S18523 is among the most potent and selective NK_1 receptor antagonists discovered (Table 1). Its pharmacological potency and binding affinity (human receptors) are similar to those of the most active non-peptide antagonists described so far. Its specificity for the NK_1 receptor has been demonstrated in binding assays performed on a large number of receptors for other endogenous agents and indeed S18523 was found to interact exclusively with the NK_1 binding site.

The potent antinociceptive effects in three classical in vivo tests for analgesia provides a first indication that S18523 could be useful for the treatment of pain syndromes. Indeed, its potency was about the same as that of CP99994 in the three tests. When compared to morphine in the hot-plate test, S18523 was about 10 times more potent.

In the guinea-pig, the inhibition by S18523 (i.v.) of both airway bronchoconstriction and mucosal plasma extravasation, produced either by endogenous neurokinins (released after neurogenic stimulation) or by exogenous substance P as well as the inhibition of substance P-induced plasma extravasation in the bladder provide further evidence that this compound is a potent neurokinin antagonist. The compound was as effective as representative non-peptide antagonists, such as CP99994 in the airway models and about 10 times less potent in the bladder test. Since NK_1 receptors in the respiratory tract regulate airway vascular leakage and mucus secretion, potent and selective NK_1 antagonists, such as S18523, could be clinically useful for the treatment of chronic inflammatory airway diseases, such as asthma and chronic bronchitis. In addition, its potency and duration of action after aerosol administration are of particular interest.

The development of several neurokinin receptor antagonists has been impaired by side effects due to hypotension mediated by L-type calcium channels (Schmidt et al., 1992) or histamine release due to peritoneal mast-cell degranulation (Lowman et al., 1988; Folkers et al., 1990). Both the estimated K_i (10 micromolar range) of S18523 on calcium channel microsomal preparations and the absence of detectable mast-cell degranulation at 100 micromolar concentration, leave an ample therapeutic margin for application.

In conclusion, the potency and selectivity of the new antagonist S18523 in pain, bronchoconstriction and inflammation models both in vitro and in vivo, the lack of adverse effects, such as calcium-channel affinity or mast-cell degranulation, and of μ-opiate character and the favourable physico-chemical properties, such as water solubility and stability towards proteolysis in vitro, make it an interesting pharmacological tool and drug candidate among the existing neurokinin NK_1 receptor antagonists. The high yields obtained in the described synthesis in solution also indicate a good feasibility of a scale-up procedure.

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