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The dipeptide neurokinin-1 receptor antagonist S19752 is a potent and long-acting inhibitor of bronchoconstriction when administered by aerosol to the guinea pig *in vivo*.

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Abstract. The K+ salt of Tbi-Hyp-Trp -OTfb (S19572, Tbi = 1-[4-(2H-tetrazol-5-yl)-butyl]-1H-indole-3-carbonyl; Tfb = 3,5-di-trifluoromethyl-benzyl) is a chemically modified, water-soluble derivative designed from prolyl-tryptophan using N- and C-terminal substitutions to confer on the analogs receptor selectivity and water solubility. The new NK-1 selective receptor antagonist is both highly potent in binding and bioassays *in vitro*, and long-acting when administered by aerosol to the guinea-pig *in vivo*. Molecular modeling of the antagonist in the seven transmembrane NK-1 receptor model offers a rationale for the observed water solubility and yet retained binding affinity.

Structure-activity relationship studies on the C-terminus of substance P (SP, for a review, see[1]) have led to the discovery of short pseudopeptide receptor antagonists with variable species and receptor specificities[2,3,4] . Some of these compounds lack water solubility and/or prolonged effects in vivo. We have recently described a water-soluble neurokinin-1 (NK-1) dipeptide antagonist (S18523[5]) which displayed high in vitro potency in several binding and bioassays. In this report, we disclose a highly selective dipeptide NK-1 receptor antagonist (S19752) which is water-soluble, potent in *in vitro* assays and which, due to its modified C-terminus, shows higher potency and higher duration of action than the previous analog when adminis-

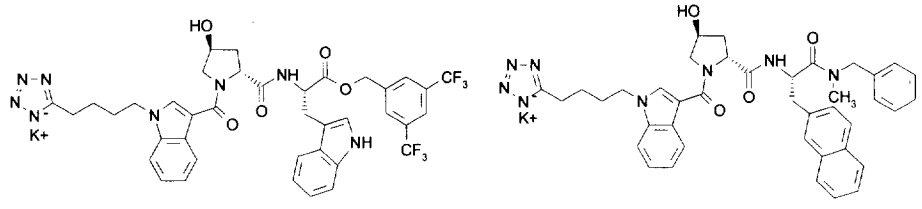

Figure 1. Structure of S19752 (left) and of S18523.

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tered to the guinea-pig *in vivo* against SP-induced bronchoconstriction. Using molecular modeling we further provide an explanation for the observed water solubility and yet retained potency of the two NK-1 antagonists.

Materials and methods

Chemistry. The K^+ salt of the dipeptide 1-[4-(2H-tetrazol-5-yl)-butyl]-1H-indole-3-carbonyl -Hyp-Trp- O-(3,5-di-trifluoromethyl-Bzl) (Hyp=(**R**)-4-hydroxy-**L**-proline) (S19752, Figure 1) was easily obtained by the classical methods of peptide synthesis in solution[6]. Briefly, H-Hyp(tBu)-OBzl was reacted with previously prepared 1-(4-cyanobutyl)-3-indole-carboxylic acid, using the condensation agent bromo-tris-pyrrolidino-phosphonium-PF_6^- (PyBrop). Deprotection by catalytic hydrogen transfer ($Bu_4N^+HSO_4^-$) was followed by coupling with H-**L**-Trp-O-(3-CF_3,5-CF_3-Bzl) in the presence of 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium-BF_4^- and 1H-benzotriazol-1-ol. The nitrile was then converted into the tetrazole by the action of trimethylazidosilane and Bu_2SnO in toluene[7]. Removal of the tBu group was achieved in trifluoroacetic acid in the presence of thiol scavengers and the product was purified by preparative HPLC on reversed phase. Conversion to the K^+ salt was obtained by addition of 1.1 equivalent of 0.1N KOH in acetonitrile. All intermediates were characterized and the final product had a single peak in analytical HPLC, a correct elemental analysis and the expected molecular weight as estimated by FAB mass spectrometry.

Pharmacology. Radioligand binding assays with the IM9 human lymphoblastoma cell line which expressed NK-1 receptors and with transfected chinese hamster ovary cells expressing human NK-2 receptors were performed as described[5]. Bioassays in vitro on isolated organs (rabbit vena cava (NK-1), rabbit pulmonary artery (NK-2), rat portal vein (NK-3)) were carried out as described by Regoli et al.[8] Capsaicin-induced plasma extravasation was also estimated according to a described procedure[5]. Finally, the effect of the new antagonist on SP-induced bronchoconstriction in the guinea-pig was measured both after i.v. and aerosol administration. Briefly, male Hartley guinea-pigs (360-460g) were anaesthetized with urethane (1.5 g/kg i.p.) and 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 10ml/kg), and curarized (gallamine triethiodide 2 mg/kg i.v.) to prevent interference from spontaneous respiration. 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. All animals were pretreated with mepyramine (1 mg/kg i.v.) to block the effects of possible histamine release, and with propanolol (1 mg/kg i.v.). SP (2 nmol/kg i.v.) was injected 15 min before intravenous treatment with either S19752 (50, 200, 500 nmol/kg), or the reference non-peptide antagonist CP 99994[9] (50, 200, 500 nmol/kg), or saline. Furthermore, the effects of CP 99994 ($5x10^{-4}$, 10^{-3}M) and S19752 ($5x\ 10^{-4}$, 10^{-3}M) by aerosol (40 sec) were also tested. Bronchoconstrictive responses to SP were then examined 3 min, 10 min, 20 min and 40 min following drug treatment and expressed as increases in pulmonary inflation pressure.

Molecular modeling was carried out with the SYBYL program (v. 6.1a) and the Tripos force field[10] running on a Silicon Graphics Indigo R4400 workstation. The structure of the human NK-1 receptor was based on that of bacteriorhodopsin using the alignment of Röper et al.[11]

Results

The solubility in water of the chemically characterized final compound (K^+ salt) was higher than 100mg/ml at room temperature (pH 5-8). Table 1 compares the binding affinity and the antagonistic potency of S19752 with those of other known antagonists for which these parameters were obtained under the same experimental conditions[1,12]. The new antagonist appeared to be a potent ligand of the NK-1 receptor in the IM9 cell and in the RVC assays, with K_I and pA_2 values in the low nanomolar range. It compared well with the peptide (FK888 and L732138) and non peptide (CP99994) antagonists. The compound was also selective with respect to the NK-2 and NK-1 receptor subtypes by at least 3 orders of magnitude, as seen from the low binding affinity on the CHO cells and the low potency in the RPA and RPV assays. The inhibition by S19752 of the bronchoconstriction induced by substance P was investigated in the guinea-pig both by the i.v. and aerosol routes (Figure 2). The new antagonist, after i.v. administration, was found to significantly inhibit responses to SP with an ID50 of approximately 50 nmol/kg at 3 min. Most importantly, this effect did not fade at 40 min after treatment. When the compound was administered as an aerosol, it reduced the bronchoconstrictive response to SP at the dose of $5x10^{-4}$M to 70% of the control

Table 1. Binding affinity (Ki, nM)[a] and antagonistic potency (pA_2)[a]

Preparation →	IM9 [b]	CHO [c]	RVC [d]	RPA [e]	RPV [f]
Receptor subtype →	hNK-1	hNK-2	NK-1	NK-2	NK-3
S 19752	2.4 ± 0.4	4200 ± 300	9.0 ± 0.3	5.2 ± 0.6	5.5 ± 0.4
S 18523 [5]	1.5 ± 0.4	2400 ± 450	9.6 ± 0.6	5.6 ± 0.5	5.0 ± 0.5
FK888 [1,2]	1.3	1200	9.1	4.5	4.8
L732138 [1,4]	1.9	10^4	8.4	5.2	5.2
CP99994 [1,7]	0.3	10^4	8.9	< 4	< 4

a) number of independent experiments n ≥ 5 ; b) IM9 human lymphoblastoma ; c) transfected chinese hamster ovary cells ; d) rabbit vena cava; e) rabbit pulmonary artery ; f) rat portal vein

at 3 min, an effect which was observed for CP99994 only at higher doses. The antagonistic action of S19752 slightly increased at 10 and 20 min and was still lasting after 40 min at the two doses of $5x10^{-4}$ and 10^{-3} M. This was in contrast to the effect of CP99994 which decreased in function of time at the same doses, under the same conditions. S19752, intravenously, also strongly (IC50 26 μg/kg) and significantly ($p< 0.03$) reduced the capsaicin-induced plasma extravasation in the bronchi of guinea-pigs, which is comparable to the value for CP99994 (IC50 14μg/kg) under the same conditions.

Molecular fitting of the antagonists S19752 and S18523 into the modelled G-protein coupled NK-1 receptor provides convincing evidence that the alkyl tetrazole moiety protrudes outside the membrane-spanning domain and does not significantly interfere with the binding site (Jacoby et al., Poster P14C, 11. Eur. Symposium on QSAR, Lausanne, Sept.1-6, 1996).

Discussion

Results of in vitro studies on isolated organs and on human lymphoblastoma cells indicate that S19752 is a very potent and selective NK-1 receptor antagonist, with potency and selectivity similar to those of the most active non-peptide antagonists described so far. Due to the introduction of an anionic alkyltetrazole substituent, and in contrast to the peptide antagonists FK888 [1,2] and L732138 [1,4], the new NK-1 antagonist is water-soluble, a desirable

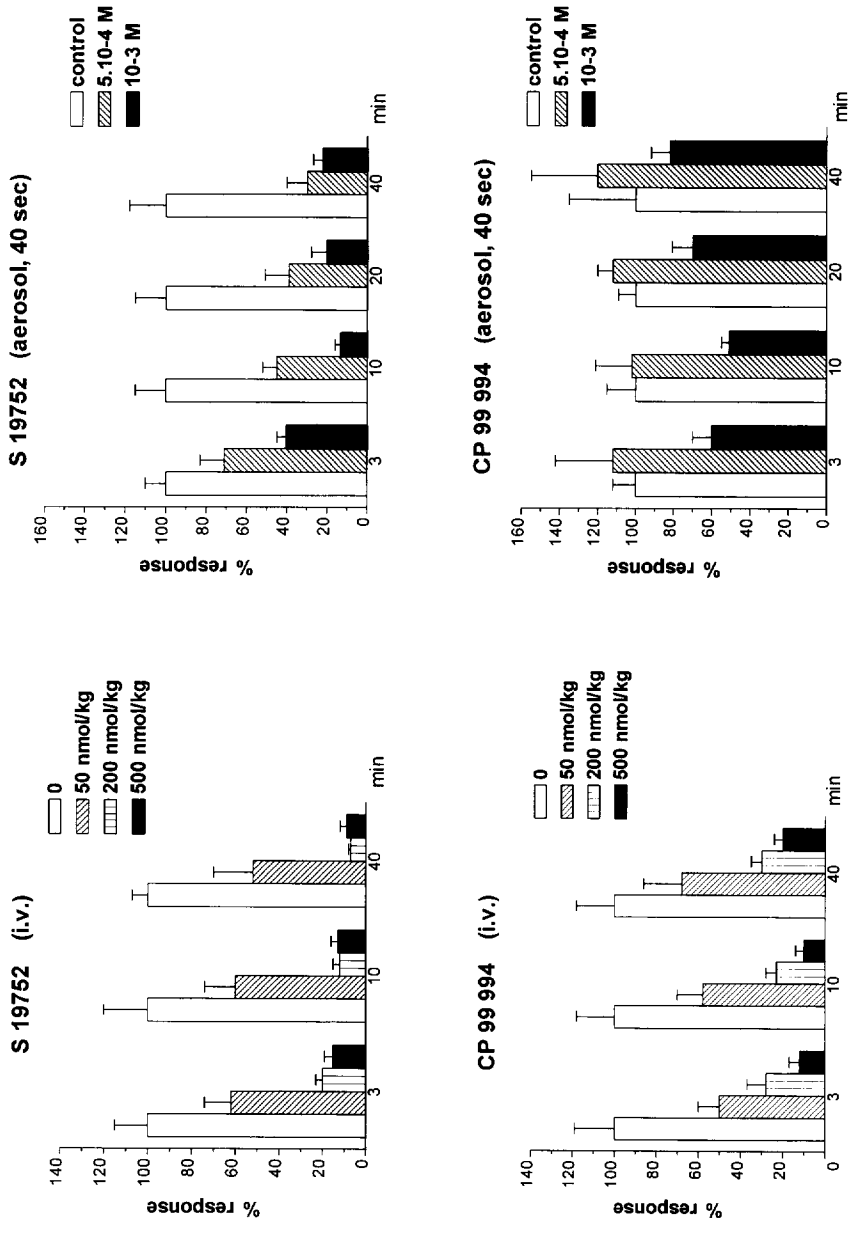

Figure 2. Effects of S19752 and of CP99994 (50, 200, 500 nmol/kg, i.v.) and of the same compounds ($5x10^{-4}$ and 10^{-3} mol/L, aerosol) on substance P-induced bronchoconstriction in the guinea-pig.

property for its pharmacological evaluation, especially in vivo. Molecular modeling suggests that this should be a general way to modify the peptide transport properties without altering the receptor-binding interaction. Binding assays performed on a number of receptors for other endogenous ligands (not shown) confirmed that S19752 interacted only with the NK-1 site. In the guinea-pig, the inhibition by S19752 (i.v.) of both airway bronchoconstriction and mucosal plasma extravasation produced by exogenous and endogenous SP, respectively, provided further evidence that the compound was as effective as the representative non-peptide antagonist CP99994. In addition, its potency and duration of action after aerosol administration are of particular interest in view of the possible clinical use of the new antagonist for the treatment of chronic inflammatory airway diseases.

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