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Pharmacology of the peptidomimetic, MEN 11149, a new potent, selective and orally effective tachykinin NK_1 receptor antagonist

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Abstract

In this study we investigated the pharmacological properties of MEN 11149, 2-(2-naphthyl)-1-*N*-{(1*R*,2*S*)-2-*N*-[1(H)indol-3-ylcarbonyl]aminocyclohexanecarbonyl}-1-[*N*′-methyl-*N*′-(4-methylphenylacetyl)]diaminoethane, a novel partially retro-inverse pseudo peptide antagonist of tachykinin NK_1 receptors. MEN 11149 potently inhibits the binding of [^{3}H]substance P to tachykinin NK_1 sites in IM9 cells ($pK_i = 8.5 \pm 0.1$). The compound is highly specific for the human tachykinin NK_1 receptors, since it has negligible effects ($pK_i < 6$) on the binding of specific ligands to tachykinin NK_2, NK_3 receptors and a battery of central and peripheral receptors or ion channels. The tachykinin NK_1 receptor antagonism of MEN 11149 appears to be insurmountable since, in saturation binding experiments, both K_D and B_{max} are significantly affected by incubation with the compound (1–30 nM). In isolated guinea-pig ileum, MEN 11149 (0.1–100 nM) shifts to the right in a non-parallel way the substance P methyl ester-induced cumulative concentration–response curve with progressive inhibition of the maximal response ($pK_B = 9.6 \pm 0.1$). When tested for reversibility at 5 nM in the same preparation, the compound displays a slow dissociation rate compared to the fast dissociation rate with FK888 (*N*^{2}-[(4*R*)-4-hydroxy-1-(1-methyl-1H-indol-3-yl)carbonyl-L-prolyl]-*N*-methyl-*N*-phenylmethyl-L-3-(2-naphthyl)alaninamide) at 5 nM. In the same preparation, MEN 11149 (10 μM) did not affect the cumulative concentration–response curve to acetylcholine. In vivo, MEN 11149 dose dependently antagonizes [Sar^9,Met(O$_2$)^{11}]substance P-induced bronchoconstriction in anaesthetized guinea-pigs ($ID_{50} = 83 \pm 31$ nmol/kg i.v.). The duration of the effect exceeds 3 h. MEN 11149 does not affect the bronchoconstriction induced by neurokinin A. The compound dose dependently inhibits [Sar^9,Met(O$_2$)^{11}]substance P-induced plasma protein extravasation in guinea-pig bronchi whether administered intravenously ($ID_{50} = 0.22 \pm 0.02$ μmol/kg) or orally ($ID_{50} = 0.97 \pm 0.21$ μmol/kg). These results demonstrate that MEN 11149 is a potent, highly selective and orally effective insurmountable antagonist of tachykinin NK_1 receptors with a long duration of action. © 1998 Elsevier Science B.V.

Keywords: Tachykinin; Tachykinin NK_1 receptor antagonist; Substance P

1. Introduction

The undecapeptide substance P belongs, together with neurokinin A and neurokinin B, to the tachykinin family of neuropeptides. These peptides act through stimulation of three major receptor subtypes, termed tachykinin NK_1, NK_2 and NK_3, having preferential affinity for substance P, neurokinin A and neurokinin B, respectively (Regoli et al., 1994; Maggi, 1995). Substance P is expressed and released from primary sensory nerve fibers and elicits a variety of biological responses centrally and peripherally (Maggi et al., 1993). Substance P is thought to be a key mediator for pain perception as well as for neurogenic inflammation (Jancso et al., 1968; Cuello et al., 1993). This latter term indicates the wide range of inflammatory responses (smooth muscle contraction, vasodilatation, increase in vascular permeability, recruitment of inflammatory cells and secretion) that follows the stimulation of the efferent function of these sensory neurons and the consequent release of tachykinins (Otsuka and Yoshioka, 1993). These biological responses induced by substance P are mediated by activation of tachykinin NK_1 receptors and therefore it has been suggested that specific and selective antagonists of this receptor could represent a new class of analgesic and/or antiinflammatory substances (Beattie et al., 1995a; Lowe and McLean, 1995).

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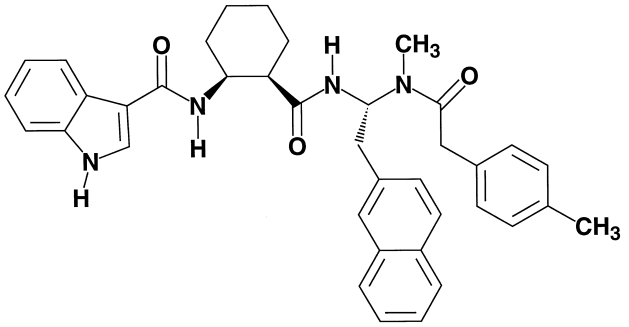

Fig. 1. Chemical structure of MEN 11149.

Our understanding of the biological actions of substance P and the involvement of tachykinin NK_1 receptors has progressed significantly following the identification of several non-peptide tachykinin NK_1 receptor antagonists with high affinity and selectivity (Snider et al., 1991; Fujii et al., 1992; Emonds-Alt et al., 1993; Beattie et al., 1995b; Gitter et al., 1995; Gardner et al., 1996; McLean et al., 1996). In addition, peptide antagonists of tachykinin NK_1 receptor have also been described (Hagan et al., 1991; Morimoto et al., 1992; Hagiwara et al., 1994), although their duration of action is short and their oral efficacy is generally low (Hagan et al., 1991; Fujii et al., 1992; Hagiwara et al., 1992; Hashimoto et al., 1992). We now report on the discovery of MEN 11149, 2-(2-naphthyl)-1-*N*-{(1*R*,2*S*)-2-*N*-[1(H) indol-3-yl-carbonyl] aminocyclohexanecarbonyl}-1-[*N*′-methyl-*N*′-(4-methylphenylacetyl)] diaminoethane (Fig. 1), as the prototype of a new class of partially retro-inverse pseudopeptides, characterized by a retro amide bond, a *N*-methyl gem-diamine moiety and a β-aminocycloalkyl carboxylic acid residue. This compound is the result of a progressive site-modification of the structure of the peptidic antagonist, FK 888 (N^2-[(4*R*)-4-hydroxy-1-(1-methyl-1H-indol-3-yl) carbonyl-L-prolyl]-*N*-methyl-*N*-phenylmethyl-L-3-(2-naphthyl)alaninamide), with the aim to protect the analogue from enzymatic processing by alteration of the peptide backbone and preserving the correct three-dimensional arrangement of the aromatic rings of the molecule, a key requisite for its biological activity (Sisto et al., 1994). The pharmacology of MEN 11149 has been examined in a variety of in vitro and in vivo preparations, in comparison to that of FK 888. In principle it is possible to design a long-acting and orally effective peptidomimetic compound showing a potent and insurmountable selective antagonism for the tachykinin NK_1 receptor.

2. Materials and methods

2.1. Tachykinin NK_1 receptor binding in human lymphoblastoma IM9 cells

Tachykinin NK_1 receptor binding was assessed using the human lymphoblastoma IM9 cell line. The IM9 cell line was obtained from the American Type Culture Collection (Rockville, MD) and cultured as previously described (Goso et al., 1994). Binding to IM9 cells was determined by incubating 4×10^6 cells/tube with 0.3 nM [^{3}H]substance P in the presence of increasing concentrations of MEN 11149 or FK888 for 60 min at room temperature. The reaction was terminated by centrifugation in a Beckman 12 microfuge ($12{,}000 \times g$ for 6 min). Non specific binding was determined in the presence of 10 μM substance P.

To evaluate the type of the antagonism of MEN 11149 on [^{3}H]substance P binding to IM9 cells, families of homologous competition curves for substance P were obtained in the absence or presence of increasing concentrations of MEN 11149 (1–30 nM).

2.2. Tachykinin NK_2 and NK_3 receptor binding

In order to assess selectivity, the interactions of MEN 11149 with tachykinin NK_2 and NK_3 receptors were investigated using hamster urinary bladder (Goso et al., 1995) and guinea-pig cerebral cortex membranes (Renzetti et al., 1991), respectively.

Binding of [^{3}H][β-Ala^8]neurokinin A-(4–10) to hamster urinary bladder membranes (tachykinin NK_2 sites) was evaluated by incubating in triplicate aliquots of membrane preparation (100 μg) in 0.5 ml buffer (50 mM Tris–HCl, pH 7.4 containing 2 mM $MnCl_2$, 0.1% bovine serum albumin, 4 μM chymostatin, 40 μM bacitracin, 4 μM leupeptin and 1 μM thiorphan) for 90 min at room temperature in the presence of 0.5 nM [^{3}H][β-Ala^8]neurokinin A-(4–10). Non-specific binding was determined in the presence of 1 μM non-radioactive [β-Ala^8]neurokinin A-(4–10).

The affinity of MEN 11149 for tachykinin NK_3 receptors was investigated by measuring the displacement of [^{3}H]senktide to guinea-pig cerebral cortex membranes. Membranes (ca. 0.8 mg/tube) were incubated at 20°C for 1 h in the presence of 1 nM [^{3}H]senktide in a final volume of 0.5 ml Krebs–HEPES buffer, pH 7.4, composed of (mM): NaCl (120), KCl (4.8), $CaCl_2$ (1), $MgSO_4$ (1) and HEPES (20) plus $MnCl_2$ (2), bovine serum albumin 0.1% and peptidase inhibitors (μg/ml): bacitracin (40), chymostatin (2), trypsin inhibitor (4), phosphoramidon (1.1). Non-specific binding was determined in the presence of 1 μM non radioactive senktide.

2.3. Non-tachykinin receptor binding

The affinity of MEN 11149 for other non-tachykinin receptors was investigated at Cerep (Celle l'Evescault) according to their established proprietary protocols. Radioligands, tissue preparations and incubation conditions are summarized in Table 2. In addition, the affinity of MEN

11149 for the verapamil-sensitive L-type Ca^{2+} channel was determined in rat brain membranes labeled with [^{3}H]desmethoxyverapamil according to Reynolds et al. (1986).

2.4. Contractions of guinea-pig ileum induced by substance P methyl ester

Male Dunkin–Hartley guinea-pigs (Charles River, Calco; 400–450 g) were killed by cervical dislocation. A segment of ileum (about 10 cm) was removed. A 1 ml glass pipette was placed in the ileal lumen and the longitudinal muscle layer was carefully removed and divided in 4 equal segments (1.5–2 cm long). Each segment was mounted under an initial tension of 0.5 g in a 5 ml organ bath containing Krebs solution (in the presence of atropine 1 μM, (±)-chlorpheniramine 1 μM and indomethacin 3 μM) gassed with 5% CO_2 and 95% O_2 at 37°C. Responses were recorded with an isometric transducer connected to a 7050 Unirecord polygraph (Basile, Comerio). The preparation was left to stabilize for 60 min, and three responses to 100 nM substance P methyl ester at 15 min intervals were then obtained to achieve stabilization in the preparation. After 15 min, a first cumulative concentration–response curve to substance P methyl ester (1–300 nM) was obtained. After 15 min, the tachykinin NK_1 receptor antagonist was incubated for 60 min and then, a second curve for the agonist was made. The data are expressed as percentages of the maximum response to the first agonist cumulative concentration–response curve.

A kinetic study was also performed in order to investigate the rate of drug dissociation from receptors. Tissue were stimulated every 20 min by a submaximally effective concentration of substance P methyl ester (30 nM). After obtaining two or three reproducible responses in the absence of the antagonist, MEN 11149 or FK888 at 5 nM or their vehicle was incubated for 60 min and responses to substance P methyl ester were produced every 20 min. After withdrawal of the antagonist, the responses to substance P methyl ester were challenged every 20 min for another 4 h. The data are expressed as percentages relative to time 0.

Drug specificity towards muscarinic receptors was determined from cumulative concentration–response curve to acetylcholine (10 nM–30 μM) in the absence and presence of 0.1 μM MEN 11149.

2.5. Bronchoconstriction in guinea-pigs

Bronchoconstriction was induced with [Sar^9,$Met(O_2)^{11}$]substance P in male Dunkin Hartley guinea-pigs (300–400 g). The animals were anaesthetized with intraperitoneal urethane (1.2 g/kg) and artificially ventilated (tidal volume = 10 ml/kg; 60 strokes/min; Basile 7025 rodent ventilator). D-Tubocurarine (3.9 μmol/kg i.v.) was administered to prevent spontaneous respiratory movements. The jugular vein was cannulated and body temperature was maintained at 37°C with a heating pad. A side-arm from the tracheal cannula was attached to a Statham pressure transducer connected to a 8805D (Hewlett Packard, Andover, MA) preamplifier to measure pulmonary insufflation pressure. [Sar^9,$Met(O_2)^{11}$]substance P was intravenously administered at a dose of 1 nmol/kg 15, 30 and 45 min before and 5, 30, 60, 90, 120, 150 and 180 min after the intravenous administration of vehicle, MEN 11149 or FK 888 (0.03–1 μmol/kg). Bronchoconstriction was quantified as an increase in pulmonary insufflation pressure and recorded on a Hewlett Packard 7754A polygraph.

In order to assess the selectivity of the tachykinin NK_1 antagonism of MEN 11149 (at 1 μmol/kg i.v.), its inhibitory effect on the bronchoconstriction induced by a submaximal dose of neurokinin A (3 nmol/kg i.v.) was evaluated in separate experiments.

2.6. Plasma protein extravasation in guinea-pig bronchi

Male albino Dunkin–Hartley guinea-pigs weighing 300–400 g were anaesthetized with urethane (1.2 g/kg i.p.) and the jugular vein was cannulated for drug administration. The animals were allowed to recover for at least 30 min after surgery. Evans blue was administered intravenously (20 mg/kg in saline containing 1,000 I.U./ml heparin) 5 min before intravenous [Sar^9,$Met(O_2)^{11}$]substance P (1 nmol/kg). Five min later the animals were perfused via the thoracic aorta with saline (about 100 ml in 10 min) to wash the dye out of the vascular system. MEN 11149 or FK 888 (0.03–3 μmol/kg) was administered intravenously 10 min before the tachykinin NK_1 receptor agonist. The oral effect of MEN 11149 (0.5–5 μmol/kg) was investigated with a 60 min pretreatment before the challenge with the agonist. The caudal segment of the trachea and the main stem bronchi were excised, weighed and the dye was extracted in 2 ml formamide (50°C for 24 h). The tissue content of Evans blue was determined by spectrophotometry (Beckman DU-7; $\lambda = 620$ nm) and expressed as ng/mg of wet tissue. The characteristic amount of non-specific dye leakage in guinea-pig bronchi was 20.8 ± 2 ng Evans blue/mg tissue ($n = 9$). This value was subtracted from all the data relative to plasma protein extravasation presented in Section 3 and in Figs. 5 and 6.

2.7. Data analysis

All the data in the text, tables and figures are means ± S.E.M. Binding data were analyzed by means of LIGAND, a non-linear curve-fitting program (Munson and Rodbard, 1980). Families of homologous competition curves for [^{3}H]substance P in the absence and in the presence of increasing concentrations of the antagonist MEN 11149

were simultaneously analyzed with the LIGAND program. An apparent pK_B was derived from a double-reciprocal regression plot from at least 3 or 4 couples of curves (control and in the presence of the antagonist) for each concentration of antagonist (Kenakin, 1987). IC_{50} and ID_{50} values were calculated by means of the least squares method, considering the curves linear between 20 and 80% of the maximal effect. ID_{50} values for the antibronchospastic effect were determined considering the inhibition at peak effect (30 min after the administration of the antagonist). Significance was assessed by one-way analysis of variance followed by Bonferroni's test.

2.8. Chemicals

MEN 11149 (2-(2-naphthyl)-1-*N*-{(1*R*,2*S*)-2-N-[1(H)indol-3-yl-carbonyl]aminocyclohexanecarbonyl}-1-[*N*′-methyl-*N*′-(4-methylphenylacetyl)]diaminoethane) was synthesized at the Chemistry Department, Menarini Ricerche, Pomezia. FK 888 (N^2-[(4*R*)-4-hydroxy-1-(1-methyl-1H-indol-3-yl)carbonyl-L-prolyl]-*N*-methyl-*N*-phenylmethyl-L-3-(2-naphthyl)alaninamide) was synthesized at Tocris Cookson, Langford, Bristol. SR 48968 ((*S*)-*N*-methyl-*N*[4-(4-acetylamino-4-phenyl piperidino)-2-(3,4-dichlorophenyl)-butyl}benzamide) was a kind gift of Dr. X. Edmonds-Alt, Sanofi Recherche, Montpellier. MEN 10627 (cyclo(Met–Asp–Trp–Phe–Dap–Leu)cyclo(2β–5β)] and [β-Ala^8]neurokinin A-(4–10) were synthesized at the Chemistry Department, Menarini Ricerche, Florence. Senktide was from Peninsula Laboratories, Belmont, CA. [Sar^9,Met(O$_2$)^{11}]substance P was from Neosystem, Strasbourg. Substance P-methyl ester was from Bachem, Bubendorf. Neurokinin A and substance P were from Novabiochem, Postfach. [^{3}H]SP (specific activity = 46 Ci/mmol) and [^{3}H]desmethoxyverapamil (specific activity = 84 Ci/mmol) were purchased from Amersham International, Buckinghamshire. [^{3}H]Senktide (specific activity = 46.8 Ci/mmol) and [^{3}H][β-Ala^8]neurokinin A-(4–10) (specific activity = 87.5 Ci/mmol) were obtained from New England Nuclear, Boston. If not otherwise stated all other materials were from Sigma, St. Louis, MA.

MEN 11149 was dissolved in dimethylsulfoxide (maximal final concentration = 0.3%) in all the in vitro experiments. For the in vivo studies, MEN 11149 was solubilized in DMSO (in bronchoconstriction and plasma protein extravasation, effect per i.v.) or in saline containing 6% Tween 80 (plasma protein extravasation, effect per os).

3. Results

3.1. Effect on tachykinin receptors

The affinity of MEN 11149 for the tachykinin NK_1 receptor was assessed by measuring the displacement of [^{3}H]substance P binding to human IM9 cells. MEN 11149 inhibited [^{3}H]substance P binding to IM9 cells in a concentration-dependent manner giving a pK_i value of 8.5 ± 0.1 ($n = 6$). The pK_i value for FK 888 was 8.9 ± 0.2 ($n = 5$).

Table 1
Effects of MEN 11149 on the binding parameters of [^{3}H]substance P to tachykinin NK_1 receptors expressed on IM9 cells

	K_D (nM)	B_{max} (sites/cell)	
Control	0.10 ± 0.02	4200 ± 252	—
1 nM	0.20 ± 0.02	3720 ± 186	N.S.
3 nM	0.28 ± 0.04	3000 ± 270	$P < 0.001$
10 nM	0.56 ± 0.09	2460 ± 246	$P < 0.001$
30 nM	0.98 ± 0.12	2100 ± 294	$P < 0.001$

Data are the means ± S.E.M. of at least 4 separate experiments performed in triplicate. Simultaneous statistical analysis was performed with the LIGAND program.

The nature of MEN 11149 inhibition was studied by Scatchard analysis of specific [^{3}H]substance P binding to IM9 cells in the absence and presence of increasing concentrations (1–30 nM) of the antagonist. MEN 11149 ($n = 4$ for each concentration of antagonist) concentration dependently increased K_D values with a concentration-dependent significant decrease of B_{max} values (Table 1).

In classical binding assays for tachykinin NK_2 (hamster urinary bladder) and NK_3 (guinea-pig cerebral cortex) receptors, MEN 11149 did not significantly inhibit the binding of [^{3}H][β-Ala^8]neurokinin A-(4–10) to hamster urinary bladder membranes (tachykinin NK_2 sites) and that of [^{3}H]senktide to guinea-pig cerebral cortex membranes (tachykinin NK_3 sites), showing at least three orders of magnitude selectivity for the tachykinin NK_1 receptors in comparison with tachykinin NK_2 ($pK_i = 5.8 \pm 0.6$, $n = 3$) and NK_3 ($pK_i < 5$, $n = 3$) receptors.

3.2. Non-tachykinin receptor and ion channel binding affinity

MEN 11149 lacked appreciable affinity for L-type voltage-sensitive calcium channel, displacing [^{3}H]desmethoxyverapamil binding with a pK_i value of 5.5 ± 0.5 ($n = 3$).

MEN 11149 was essentially inactive in an extensive receptogram for a number of receptors and ion channels. In all assays, MEN 11149 had K_i values greater than 1 μM (Table 2).

3.3. Receptor antagonism in guinea-pig ileum

MEN 11149 produced a concentration-dependent, non-parallel rightward shift of the concentration–response curve of substance P methyl ester. Moreover, MEN 11149 also induced a progressive reduction of the maximal response to the agonist, suggesting that MEN 11149 exerted a not purely competitive antagonism. The resulting value of pK_B was 9.6 ± 0.1 ($n = 14$; Fig. 2). On the other hand,

Table 2
Affinity profile of MEN 11149 in different receptor assays

Binding site	Radioligand, tissue, incubation conditions	K_i (μM)
α_1 (non sel.)	[^{3}H]prazosin (0.25 nM), rat cerebral cortex, 60 min/25°C	> 1
α_2 (non sel.)	[^{3}H]RX821002 (0.5 nM), rat cerebral cortex, 30 min/22°C	> 1
β_1	[^{3}H](-)CGP 12177 (0.5 nM), rat heart, 20 min/25°C	> 1
β_2	[^{3}H](-)CGP 12177 (0.4 nM), guinea-pig lung, 20 min/25°C	> 1
BDZ_c	[^{3}H]flunitrazepam (0.4 nM), rat cerebral cortex, 60 min/4°C	> 1
BDZ_p	[^{3}H]PK 11195 (0.2 nM), rat heart, 15 min/25°C	> 1
D_1	[^{3}H]SCH 23390 (0.3 nM), rat striatum, 45 min/25°C	> 1
D_2	[^{3}H]YM-09151-2 (0.1 nM), rat striatum, 60 min/25°C	> 1
ET_B	[^{125}I]endothelin-1 (10 pM), rat cerebellum, 60 min/37°C	> 1
$GABA_A$	[^{3}H]muscimol (2.5 nM), rat cerebral cortex, 10 min/4°C	> 1
$GABA_B$	[^{3}H]GABA (10 nM), rat cerebral cortex, 10 min/22°C	> 1
H_1	[^{3}H]pyrilamine (1 nM), guinea-pig lung, 15 min/25°C	> 1
H_2	[^{125}I]APT (0.1 nM), guinea-pig striatum, 150 min/22°C	> 1
Muscarinic	[^{3}H]QNB (50 pM), rat cerebral cortex, 120 min/25°C	> 1
Nicotinic	[^{3}H]cytisine (1.5 nM), rat cerebral cortex, 75 min/4°C	> 1
NMDA	[^{3}H]CGP 39653 (2 nM), rat cerebral cortex, 60 min/4°C	> 1
σ	[^{3}H]pCl-Phe-DPDPE (0.75 nM), rat cerebral cortex, 15 h/25°C	> 1
k	[^{3}H]U 69593 (0.7 nM), guinea-pig cerebellum, 80 min/25°C	> 1
μ	[^{3}H]DAMGO (1 nM), rat cerebral cortex, 60 min/25°C	> 1
$5\text{-}HT_{1A}$	[^{3}H]8-OH-DPAT (0.5 nM), rat cerebral cortex, 30 min/25°C	> 1
$5\text{-}HT_{2A}$	[^{3}H]ketanserin (0.5 nM), rat cerebral cortex, 15 min/37°C	> 1
$5\text{-}HT_3$	[^{3}H]BRL 43694 (1 nM), N1E-115 cells, 180 min/4°C	> 1
Somatostatin	[^{125}I]Tyr^{111}-somatostatin (50 pM), AtT-20 cells, 60 min/37°C	> 1
Ca^{2+} channel (L, DHP site)	[^{3}H]PN-200-110 (40 pM), rat cerebral cortex, 90 min/25°C	~ 1
Ca^{2+} channel (N)	[^{125}I]w-conotoxin GVIA (1 pM), rat cerebral cortex, 30 min/25°C	> 1
Na^+ chann. (site 1)	[^{3}H]saxitoxin (2 nM), rat cerebral cortex, 30 min/22°C	> 1
Na^+ chann. (site 2)	[^{3}H]batrachotoxinin (10 nM), rat cerebral cortex, 60 min/25°C	> 1

Abbreviations: α_1, α_2 = adrenoceptor; β_1, β_2 = adrenoceptor; BDZ_c and BDZ_p = central and peripheral benzodiazepine; D_1 and D_2 = dopamine; ET_B = endothelin; $GABA_A$ and $GABA_B$ = γ-amino-*n*-butyric acid; H_1 and H_2 = histamine; σ, k and μ = opioid; $5\text{-}HT_{1A}$, $5\text{-}HT_{2A}$ and $5\text{-}HT_3$ = serotonin.

FK 888 (10, 30 and 100 nM) produced a rightward parallel shift of the concentration–response curve without depression of the maximal response, giving a pA_2 value of 8.8 ± 0.2 ($n = 6$; data not shown). MEN 11149 was then examined in a kinetic study aimed to evaluate the rate of dissociation of tachykinin NK_1 receptor antagonism in comparison to that by FK888. Tachyphylaxis to substance

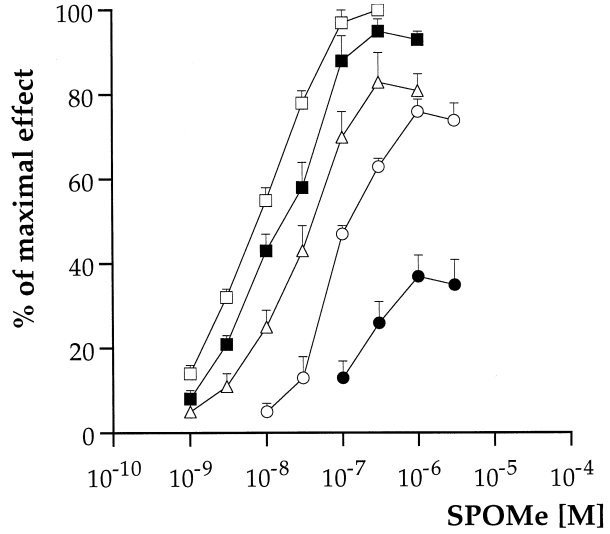

Fig. 2. Concentration–response curves for substance P methyl ester-induced contractions of guinea-pig ileum in the presence of vehicle (□, $n = 23$) or 0.1 (■, $n = 6$), 1 (△, $n = 5$), 10 (○, $n = 9$) and 100 nM (●, $n = 3$) MEN 11149. Values are expressed as percentages relative to the maximum response obtained in the first cumulative agonist-induced contraction curve.

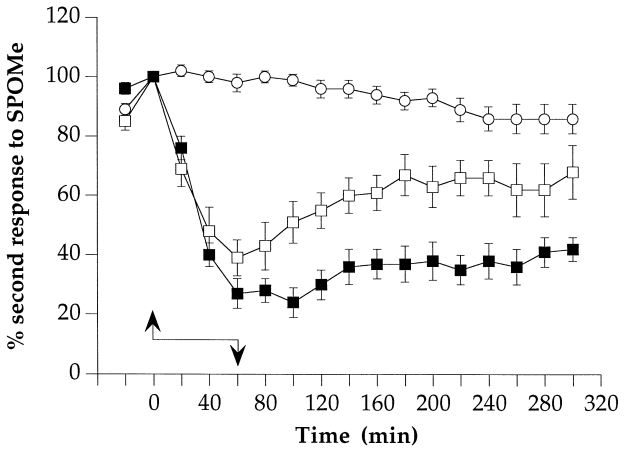

Fig. 3. Time-dependent variations of contractile responses of guinea-pig ileum stimulated every 20 min by substance P methyl ester (30 nM) in the presence of vehicle (○, $n = 10$) and after addition of 5 nM MEN 11149 (■, $n = 6$) or 5 nM FK888 (□, $n = 9$). The antagonists were left to incubate for 60 min before removal. Results are expressed as percentages of the response to substance P methyl ester obtained at time 0.

P methyl ester was moderate, since the response to this agonist at 300 min was 82 ± 5% ($n = 10$) of the basal response. MEN 11149 (5 nM) time-dependently inhibited the response to tachykinin NK_1 receptor agonist, similarly to FK 888, since response to the agonist at 60 min was 27 ± 5%, $n = 3$, and 39 ± 6% of the basal response, $n = 5$; respectively. Duration of the inhibitory effect of MEN 11149 following drug washout was longer (44 ± 4% of basal response at 300 min, $P < 0.05$; $n = 6$) than that by FK 888 (68 ± 9% of basal response at 300 min, $n = 9$; Fig. 3).

In guinea pig ileum, MEN 11149 at 0.1 μM did not affect the cumulative concentration–response curve to acetylcholine (agonist affinity measured as pD_2 was 6.8 ± 0.1, $n = 4$, in controls and, 6.8 ± 0.1, $n = 4$, in the presence of MEN 11149) thus indicating specificity for tachykinin NK_1 receptors.

3.4. Effect on bronchoconstriction induced by [Sar^9,Met(O$_2$)^{11}]substance P

Bronchoconstrictive responses to [Sar^9,Met(O$_2$)^{11}]substance P did not show tachyphylaxis: after 180 min and 10 challenges with [Sar^9,Met(O$_2$)^{11}]substance P the response was decreased only by 6 ± 10% ($n = 10$; Fig. 4).

Administration of MEN 11149 resulted in dose-dependent inhibition of the bronchoconstriction induced by [Sar^9,Met(O$_2$)^{11}]substance P. ID_{50} calculated at the peak effect (30 min) was 83 ± 31 nmol/kg i.v. ($n = 20$). The duration of the MEN 11149 effect exceeded 3 h (Fig. 4). The effect of FK 888 was similar to that shown by MEN 11149, since its ID_{50} (peak effect at 30 min) was 59 ± 12 nmol/kg i.v. ($n = 20$; data not shown). The FK 888 effect lasted at least 3 h (data not shown).

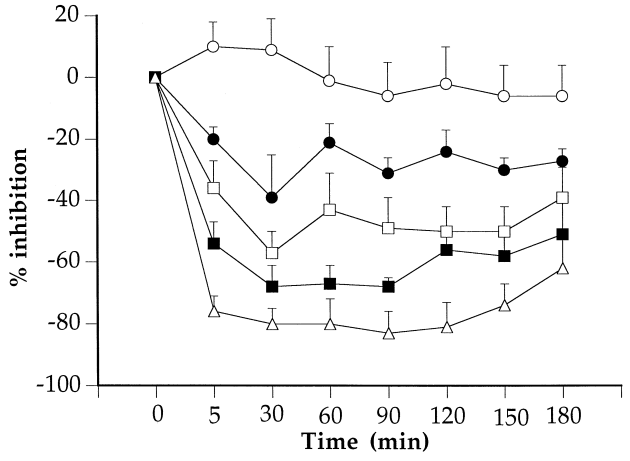

Fig. 4. Bronchoconstriction induced by [Sar^9,Met(O$_2$)^{11}]substance P (1 nmol/kg i.v.) in anaesthetized guinea-pigs. Effect of vehicle ($n = 10$; ○) and MEN 11149 at 0.03 ($n = 5$; ●), 0.1 ($n = 5$; □), 0.3 ($n = 5$, ■) and 1 ($n = 5$; △) μmol/kg i.v.

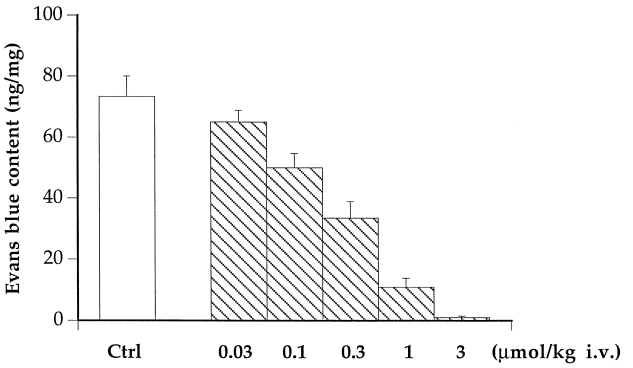

Fig. 5. Plasma protein extravasation induced by [Sar^9,Met(O$_2$)^{11}]substance P (1 nmol/kg i.v.) in guinea-pig bronchi. Effect of the treatment with vehicle ($n = 13$) or MEN 11149 at 0.03 ($n = 6$), 0.1 ($n = 6$), 0.3 ($n = 4$), 1 ($n = 5$) and 3 ($n = 3$) μmol/kg i.v. The antagonist or its vehicle was administered 10 min before the agonist challenge.

3.5. In vivo selectivity of tachykinin NK_1 antagonism in anaesthetized guinea-pigs

The injection of neurokinin A at 3 nmol/kg resulted in reproducible bronchoconstrictive responses in the control group (−4.4 ± 4.3% versus basal value 5 min after injection of vehicle; $n = 3$). The responses were not affected by MEN 11149 at 1 μmol/kg i.v. (−6.9 ± 3.9%; $n = 4$), but were completely abolished by a selective tachykinin NK_2 receptor antagonist, MEN 10627 (−94.4 ± 8.2; 1 μmol/kg i.v.; $n = 3$).

3.6. Effect on plasma protein extravasation induced by [Sar^9,Met(O$_2$)^{11}]substance P

The dose-related effect of MEN 11149 on plasma protein extravasation produced by [Sar^9,Met(O$_2$)^{11}]substance P in guinea-pig bronchi is shown in Fig. 5. In the control group, the plasma protein extravasation was 73.3 ± 6.7 ng of Evans blue/mg tissue ($n = 13$). Pretreatment with 0.03 ($n = 6$), 0.1 ($n = 6$), 0.3 ($n = 4$), 1 ($n = 5$) and 3 ($n = 3$)

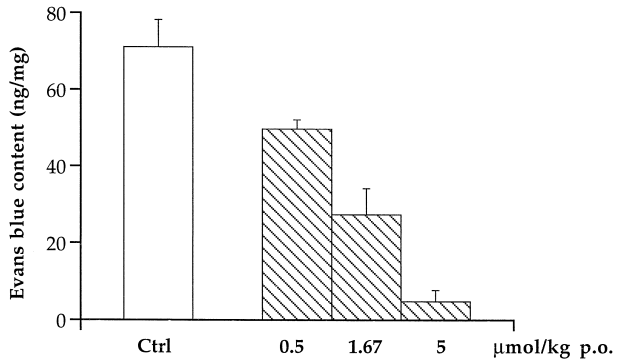

Fig. 6. Effect of oral administration of vehicle ($n = 5$) or MEN 11149 at 0.5 ($n = 4$), 1.67 ($n = 6$) and 5 μmol/kg ($n = 4$) on plasma protein extravasation induced by [Sar^9,Met(O$_2$)^{11}]substance P (1 nmol/kg i.v.) in guinea-pig bronchi. The antagonist or its vehicle was administered orally 60 min before the agonist challenge.

μmol/kg i.v. of MEN 11149 progressively decreased the amount of plasma protein extravasation resulting in an ID_{50} of 0.22 ± 0.02 μmol/kg i.v. The ID_{50} seen with FK 888 was 0.36 ± 0.07 μmol/kg i.v. ($n = 27$; data not shown).

When tested orally, MEN 11149 dose dependently antagonized the [Sar^9,$Met(O_2)^{11}$]substance P-induced plasma protein extravasation in guinea-pig bronchi with an $ID_{50} = 0.97 \pm 0.21$ μmol/kg (Fig. 6).

4. Discussion

MEN 11149 is the prototype of a new class of partially retro-inverse pseudopeptides, structurally related to FK 888. The main modification is the introduction of a β-amino cycloalkyl carboxylic acid residue, aimed at protection of the analogue from enzymatic processing by alteration of the peptide backbone, preserving the correct three-dimensional arrangement of the aromatic rings of the FK 888 molecule, a key requisite for its biological activity (Sisto et al., 1994). The present work demonstrates that MEN 11149 is a highly potent and selective antagonist at human tachykinin NK_1 receptors. The affinity for human tachykinin NK_1 receptors, as evaluated in binding studies, is in the nanomolar range ($pK_i = 8.5 \pm 0.1$) and close to that shown by the parent compound, FK888. The affinity of MEN 11149, measured in functional experiments in longitudinal smooth muscle preparations obtained from guinea-pig ileum, appears to be slightly greater ($pK_B = 9.6 \pm 0.1$).

Increasing concentrations of MEN 11149 induced a concomitant increase of K_D and a decrease of the B_{max} of [^{3}H]substance P in IM9 cells, suggesting that the compound interacts with the tachykinin NK_1 receptor apparently not competitively. In isolated guinea-pig ileum, cumulative concentration–response curves to substance P methyl ester were concentration-dependently shifted in a non-parallel way to the right by MEN 11149. MEN 11149 inhibits the maximal response to the agonist, suggesting that the compound likely behaves as an insurmountable antagonist, whereas FK 888, in the same preparation, behaves as a pure competitive antagonist. In addition, MEN 11149 and FK888 display different levels of reversibility of their inhibitory effect on the contractile response to the tachykinin NK_1 receptor agonist in guinea pig ileum. This finding suggests that, in this system, the different rates of dissociation of the antagonist from tachykinin NK_1 receptors may be due to the different types of antagonism exerted by FK 888 and MEN 11149.

MEN 11149 shows high selectivity for tachykinin NK_1 receptors versus tachykinin NK_2 and NK_3 receptors. Affinity for other receptor types of MEN 11149 is negligible ($K_i > 1$ μM). In addition, similarly to many non peptide tachykinin NK_1 receptor antagonists, MEN 11149 binds to L-type Ca^{2+} channels with very low affinity ($pK_i = 5.5 \pm 0.5$): one order of magnitude less than CP 96,345 (Snider et al., 1991), a compound that interacts with Ca^{2+} channels at micromolar concentrations in binding and functional assays (Delay-Goyet et al., 1992; Schmidt et al., 1992).

In anaesthetized guinea-pigs, the inhibition by MEN 11149 (i.v.) of both airway bronchoconstriction and plasma protein extravasation induced by [Sar^9,$Met(O_2)^{11}$]substance P provides further evidence that this compound is a potent and selective tachykinin NK_1 receptor antagonist under in vivo conditions also. The potency of MEN 11149 as an antibronchospastic agent is twice that reported previously for CP 99,994 (ID_{50} about 200 nmol/kg i.v. at 3 min; Bonnet et al., 1996). In particular, the antibronchospastic effect elicited by MEN 11149 persists over time since the inhibition of [Sar^9,$Met(O_2)^{11}$]substance P-induced bronchoconstriction was still evident 3 h after intravenous administration. MEN 11149 is poorly soluble in water. However, it is relatively well absorbed when given per os, showing a bioavailability higher than that of FK 888. This difference results in a lower effective dose of MEN 11149 to inhibit [Sar^9,$Met(O_2)^{11}$]substance P-induced plasma protein extravasation in guinea-pig bronchi (1.67 μmol/kg = 1 mg/kg p.o.) than that of FK888 (10 mg/kg p.o.; Fujii et al., 1992; Hagiwara et al., 1994).

The main difference seen in the comparison of the pharmacological profile of MEN 11149 and of FK888 is the type of receptor antagonism. The change in the type of antagonism by MEN 11149 may be ascribed to the replacement of the prolyl group present in the FK 888 structure by the β-amino cycloalkyl carboxylic acid residue. The passage from a competitive to an insurmountable/non-competitive antagonism in the same chemical structure may lead to improvement of the pharmacological profile, similarly to what has been described for the antagonists of the angiotensin AT_1 receptor (Wong et al., 1990a,b; Olins et al., 1995). Non-competitive antagonism may contribute to the long duration of action of these compounds in vivo (Wienen et al., 1993; Dickinson et al., 1994). Non-competitive antagonism associated to better oral bioavailability seems to be the reason for the better pharmacological profile of the non-competitive tachykinin NK_1 receptor antagonist CP 122,721 as compared to that of the previous competitive antagonist, CP 99,994 (McLean et al., 1996).

In conclusion MEN 11149 is a peptidomimetic substance P antagonist showing a potent, selective and specific antagonism of tachykinin NK_1 receptors. This antagonism is insurmountable and its in vitro and in vivo inhibitory effects are long-lasting and with a very slow rate of disappearance. Further, MEN 11149 is a potent antagonist of tachykinin NK_1-mediated motor and inflammatory responses in guinea-pig airways and this action is evident and prolonged even after oral administration. These data support the feasibility of designing and synthesizing pep-

tidomimetic tachykinin NK_1 receptor antagonists with pharmacodynamic and pharmacokinetic characteristics that make them suitable drugs for testing in human disease.

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