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Characterization of the antibronchoconstrictor activity of MEN 11420, a tachykinin NK_2 receptor antagonist, in guinea-pigs

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Abstract

We have investigated the antibronchoconstrictor activity of a novel glycosylated bicyclic peptide tachykinin NK_2 receptor antagonist, MEN 11420 c{[(β-D-GlcNAc)Asn-Asp-Trp-Phe-Dpr-Leu]c(2β–5β)}, as compared to MEN 10627 c[(Met-Asp-Trp-Phe-Dpr-Leu)c(2β–5β)] and to the nonpeptide antagonist SR 48968 ((*S*)-*N*-methyl-*N*[4-acetylamino-4-phenylpiperidino-2-(3,4-dichlorophenyl)butyl] benzamide. In the guinea-pig isolated bronchus MEN 11420 (pK_B 8.40 ± 0.07) and MEN 10627 (pK_B 8.67 ± 0.09) competitively antagonized the contraction induced by the tachykinin NK_2 receptor agonist, [βAla^8]neurokinin A-(4–10). SR 48968 showed an apparent pK_B of 9.57 ± 0.2. The atropine-resistant response to electrical stimulation was reduced in a concentration-dependent manner by MEN 11420, MEN 10627 and SR 48968. In urethane-anaesthetized guinea-pigs, MEN 11420 produced a dose-dependent inhibition of bronchoconstriction induced by [βAla^8]neurokinin A-(4–10). Comparable inhibitory effects were observed after i.v. administration of SR 48968 and MEN 10627. Bilateral electrical stimulation of the vagi (20 Hz for 20 s) induced a bronchoconstriction that was dose-dependently inhibited by i.v. MEN 11420, SR 48968 and MEN 10627. MEN 11420 was also effective in inhibiting the capsaicin (20 nmol/kg i.v.)-induced bronchoconstriction. MEN 11420 (1.1 μmol/kg i.v.) showed a longer plasma half-life and a greater area under the plasma concentration-time curve value (AUC) than those of MEN 10627. These findings indicate that MEN 11420 is a potent and selective antagonist of the tachykinin NK_2 receptor in guinea-pig airways with a long duration of action.

Keywords: Tachykinin; Tachykinin NK_2 receptor antagonist; MEN 11420; MEN 10627; Airway; Bronchoconstriction

1. Introduction

Tachykinins are thought to play an important role in the physiology and pathophysiology of guinea-pig and, possibly, human airways (Maggi, 1995 for review): they are involved in mediating biological effects as diverse as mucus secretion (Gashi et al., 1986), increase in microvascular permeability to plasma proteins (Lundberg et al., 1983), vasodilatation and regulation of bronchomotor tone (Hua et al., 1984; Advenier et al., 1987) and recruitment of inflammatory cells (Maggi, 1997 for review).

Two tachykinins, substance P and neurokinin A, are released in the airways from a population of sensory neurons which are sensitive to capsaicin (Maggi, 1995 for review). Substance P has a preferential affinity as a ligand for tachykinin NK_1 receptors whilst neurokinin A possesses high affinity for both tachykinin NK_2 and NK_1 receptors (Mc Kee et al., 1993): neurokinin A can act as a physiological ligand at both receptor types (Maggi, 1995 for review). In guinea-pig airways, the bronchoconstrictor response mediated by capsaicin-sensitive primary afferent neurons roughly follows a proximal-to-distal gradient: it increases from the upper to the lower trachea and is especially prominent in the bronchi (Manzini et al., 1989). In vitro and in vivo studies with selective tachykinin NK_1 and NK_2 receptor antagonists have shown that activation of tachykinin NK_2 receptors is chiefly responsible for the non-cholinergic, atropine-resistant bronchoconstriction produced by chemical stimuli such as capsaicin or by bilateral electrical vagal stimulation. Tachykinin NK_1 receptors, which also mediate bronchoconstriction in guinea-

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pig airways, seems to be activated at a lesser extent during the atropine-resistant bronchoconstriction (Maggi et al., 1990, 1991a; Martin et al., 1992; Satoh et al., 1992; Ballati et al., 1992; Hirayama et al., 1993).

In this study we have characterized the antibronchoconstrictor activity of a novel, water soluble, glycosylated bicyclic peptide tachykinin NK_2 receptor antagonist MEN 11420 (Santicioli et al., 1997; Catalioto et al., 1998), as compared to its parent compound MEN 10627 (Maggi et al., 1994) and to the non peptide tachykinin NK_2 receptor antagonist SR 48968 (Emonds-Alt et al., 1992).

The effects of MEN 11420, MEN 10627 and SR 48968 were investigated in in vitro models ([βAla^8]neurokinin A-(4–10)) or electrical field stimulation-induced bronchial contractions) in order to compare the in vitro affinity of these drugs with their potency and duration of action in in vivo models of bronchoconstriction.

2. Materials and methods

2.1. In vitro experiments

Male albino guinea-pigs (350–400 g) were stunned and bled. The main bronchi were excised and placed in warmed (37°C) and oxygenated (96% O_2 and 4% CO_2, pH 7.4). Krebs solution of the following composition (mM): NaCl, 119; $NaHCO_3$, 25; KH_2PO_4, 1.2; $MgSO_4$, 1.5; $CaCl_2$, 2.5; KCl, 4.7 and glucose 11. Epithelium-deprived bronchial rings were prepared as described previously (Maggi et al., 1990, 1991a,b).

The preparations were placed in organ baths filled with Krebs solution containing atropine (1 μM) and indomethacin (3 μM). Mechanical activity developed by the preparations was recorded by isotonic transducers (load 0.3 mN).

Cumulative concentration-dependent curves to the tachykinin NK_2 receptor-selective agonist [βAla^8]neurokinin A-(4–10) were constructed, in the absence and presence of MEN 11420 (contact time 15 or 120 min), MEN 10627 (contact time 15 min) or SR 48968 (contact time 15 or 120 min). The concentration-response curves to [βAla^8]neurokinin A-(4–10) were constructed in the presence of the tachykinin NK_1 receptor-selective antagonist SR 140333 (100 nM) (Emonds-Alt et al., 1993a), to rule out any possible involvement of tachykinin NK_1 receptors in the response to [βAla^8]neurokinin A-(4–10). The maximal contraction produced by KCl (80 mM), administered at the end of each concentration-response curve, was used as an internal standard.

In a separate series of experiments, noncholinergic contractile responses were elicited by electrical field stimulation of capsaicin-sensitive afferent nerves (cf. Maggi et al., 1990, 1991a): trains of electrical pulses were delivered at a frequency of 10 Hz for 10 s (60 V, 0.5 ms pulse width) every 30 min, in pairs of bronchi excised from the same animal. After having obtained 2–3 reproducible responses to electrical field stimulation, the effect of MEN 11420 (contact time 15 or 120 min), MEN 10627 (contact time 15 min) and SR 48968 (contact time 15 or 120 min) on the response to electrical field stimulation was studied on one bronchus while the other served as a control for time-dependent changes in responsiveness to electrical field stimulation.

2.2. In vivo experiments

2.2.1. General

Male albino guinea-pigs (Charles River, Italy) weighing 350–400 g were anaesthetized with urethane (1.5 g/kg, s.c.). A polyethylene catheter was inserted into the left jugular vein for drugs administration. The body temperature was kept constant at 36°C. The animals were mechanically ventilated through a tracheal cannula connected to a ventilation pump (Basile mod. 7025) adjusted at a rate of 60 strokes/min and treated with gallamine triethiodide (3.4 μmol/kg i.v. as a bolus, 20 min before starting experiments) to prevent spontaneous respiratory movements. The respiration volume was kept constant by means of a water valve providing a basal insufflation pressure of 7.5 ± 1.7 mmHg ($n = 10$). The insufflation pressure was measured by attaching a pressure transducer (Hewlett Packard 1240) to a side arm of tracheal cannula and was recorded by a MacLab/8s ML 780 via Hewlett Packard carrier amplifier (8805D). Blood pressure was recorded through a polyethylene catheter inserted into the left carotid artery, connected to a pressure transducer and a HP 8805D pressure amplifier. The blood pressure signal was used to trigger a cardiotachometer (HP 15050A) for heart rate recording through a medium gain amplifier HP 8802A. The activity of the tachykinin receptor antagonists was investigated on the responses induced by the selective tachykinin NK_2 receptor agonist [βAla^8]neurokinin A-(4–10), electrical stimulation of the vagi at the cervical level and by capsaicin.

2.2.2. Bronchoconstriction induced by [βAla^8]neurokinin A-(4–10)

A dose–response curve to [βAla^8]neurokinin A-(4–10) was first determined by the administration of increasing doses of the agonist in the same animal at 20–30 min intervals in order to select a dose suitable for evaluating the antibronchoconstrictor activity of tachykinin receptor antagonists. On the basis of these experiments, a dose of 1 nmol/kg, producing about 70% of the maximal response to [βAla^8]neurokinin A-(4–10), was selected to study the effect of the tachykinin NK_2 receptor antagonists. After having obtained two reproducible control responses to [βAla^8]neurokinin A-(4–10) (1 nmol/kg), MEN 11420, SR 48968, MEN 10627 or the vehicle (dmso, 0.1 ml/kg)

were administered intravenously. The challenge with the agonist was performed at 5 min and 30 min and then every 30 min up to 4 h from administration of the antagonists or the vehicle.

In other series of experiments, the selectivity of MEN 11420 at the highest dose tested in vivo (300 nmol/kg i.v.), was checked against the bronchoconstrictor responses induced by intravenous administration of histamine (10–3000 nmol/kg).

2.2.3. Bronchoconstriction induced by electrical stimulation of the vagi

In these experiments, the animals were pretreated, 30 min before the beginning of stimulation, with SR 140333 (1 μmol/kg i.v.) and atropine (5.5 μmol/kg i.v.) to prevent bronchoconstrictor responses mediated by tachykinin NK_1 and muscarinic receptors, respectively.

The vagi were exposed bilaterally in the cervical region and arranged for electrical stimulation by means of bipolar electrodes connected to a Grass S88 stimulator through a stimulus isolation unit (Hewlett Packard SIU 5). The nerves and the electrodes were immersed in mineral oil to avoid dehydration and spread of current. Train of pulses were delivered for 20 s at a frequency of 20 Hz (10 V, 1 ms pulse width). After having recorded two reproducible bronchoconstrictor responses to vagal stimulation, MEN 11420, MEN 10627, SR 48968 or their vehicle (saline 0.9% NaCl) were administered intravenously. Vagal nerve stimulation was performed at 5, 30, 60 and 90 min from tachykinin NK_2 receptor antagonists or vehicle administration.

2.2.4. Bronchoconstriction induced by capsaicin

In these experiments bronchoconstrictor responses were induced by administration of capsaicin (20 nmol/kg i.v.) in animals pretreated with atropine (5.5 μmol/kg i.v.) and SR 140333 (1 μmol/kg i.v.). After having recorded 2 reproducible control responses to capsaicin, MEN 11420 or the vehicle (saline 0.9% NaCl) were administered intravenously. The challenge with capsaicin was repeated at 5, 30 and 60 min after treatment.

2.2.5. Pharmacokinetic studies

Guinea-pigs were anaesthetized with a combination of i.p. Hypnorm (1 ml/kg; 24 μg/ml fentanyl base, 1.2 mg/ml fluanisone) and i.v. pentobarbital sodium (1 ml/kg; 30 mg/ml) and were implanted with a Silastic catheter in a jugular vein. The free end of the catheter was exteriorized in the back of the neck, the cannula was filled with saline and closed with a stainless steel pin. Eighteen to twenty hours later, MEN 10627 and MEN 11420 were administered through the cannula to the unanaesthetized animals, at a dose of 1 mg/kg corresponding to 1.3 and 1.1 μmol/kg, respectively.

Blood samples (0.5 ml) were collected from the same cannula into heparinized plastic tubes, at suitable intervals up to 360 min from administration. Aliquots of plasma (0.1 ml) obtained after centrifugation were deproteinized with 0.5 ml of acetonitrile and centrifuged. The supernatants (0.5 ml) were evaporated to dryness under an N_2 gas stream, the residue was dissolved in 100 μl of water and analyzed by HPLC using a reversed-phase column (Nucleosil 100 C18, 5 μm, 150 × 4.6 mm, Saulentechnik, Berlin, Germany). The mobile phase was composed of water, acetonitrile and methanol, each containing 0.1% trifluoroacetic acid, in the ratios 50/40/10 and 60/30/10 v/v for MEN 10627 and MEN 11420, respectively. Peaks were detected by spectrofluorometry (λex 280 nm, λem 350 nm). The sensitivity of the assay was 20 ng/ml.

2.3. Evaluation of data

The nature of the interaction of MEN 11420 with tachykinin NK_2 receptors was studied by the 'Schild plot' method: agonist dose ratios, obtained from each individual experiment, were averaged and plotted (as log [dose ratio − 1]) against log [antagonist concentration], according to Arunlakshana and Schild (1959). The antagonism was considered competitive when providing a plot with a linear regression line and slope not significantly different from unity. Antagonist affinity was expressed in terms of pK_B (negative logarithm of the antagonist dissociation constant), and, assuming a slope of −1, was estimated as the mean of the individual values obtained with the equation: pK_B = log [dose ratio − 1] − log [antagonist concentration].

Since the antagonism of SR 48968 was reported to be time-dependent in certain preparations (Emonds-Alt et al., 1992), we have studied the effect of this compound after a 15 or 120 min incubation period: the intensity of antagonism was expressed as A′/A or ratio between two agonist concentration producing 50% of the control maximal response, in the presence or in the absence of the antagonist, respectively.

For in vivo experiments, the bronchoconstriction was calculated as amplitude (mm Hg) of the response over the basal value of insufflation pressure. The effect of the antagonists was expressed as percent variation of the control response at various times after treatment.

Pharmacokinetic parameters were obtained by analysing individual plasma concentration-time curves by a bi-exponential model using the fitting software EasyFit for Macintosh.

2.4. Statistical analysis

The values in the text, tables or figures are expressed as mean ± S.E.M. of mean and 95% confidence limits (c.l.). Statistical analysis was performed by means of Student's *t*-test for paired or unpaired data or by means of One-way analysis of variance (ANOVA) followed by Dunnett's test. The differences were considered statistically significant at

a level of $P < 0.05$. Regression analysis of log concentration-effect curves was performed by the least squares method, considering the curves linear between 20 and 80% of the maximal response.

2.5. Drugs

MEN 11420 or c{[(β-D-GlcNAc)Asn-Asp-Trp-Phe-Dpr-Leu]c(2β–5β)}, MEN 10627 or c[(Met-Asp-Trp-Phe-Dpr-Leu)c(2β–5β)] and [βAla^8]neurokinin A-(4–10) were synthesized by conventional solid phase methods at the Chemistry Department of Menarini Ricerche. SR 48968 ((*S*)*N*-methyl-*N*[4-acetylamino-4-phenylpiperidino-2-(3,4-dichlorophenyl)butyl] benzamide and SR 140333 ((*S*)1-{2-[3-(3,4-dichlorophenyl)-1-(3-iso-propoxyphenylacetyl)-piperidin-3-yl]ethyl}-4-phenyl-1-azoniabicyclo[2.2.2.] octane, chloride) were a gift of Dr. X. Emonds-Alt, Sanofi Recherche, Montpellier, France. [Sar^9]substance P sulphone (Peninsula, St. Helens, UK), gallamine triethiodide, histamine dihydrochloride, capsaicin and pentobarbital were obtained from Sigma (St. Louis, USA). Atropine hydrochloride was obtained from Serva (Heidelberg, Germany). Trifluoroacetic acid was from Aldrich (Milwaukee, USA). Methanol and acetonitrile were from Merck (Darmstadt, Germany).

3. Results

3.1. In vitro experiments

3.1.1. Antagonism of contractions produced by [βAla^8]neurokinin A-(4–10)

In the presence of atropine (1 μM), indomethacin (3 μM) and SR 140333 (0.1 μM), [βAla^8]neurokinin A-(4–10) elicited reproducible contractile responses of the guinea-pig isolated bronchus (EC_{50} = 4.0 nM, 95% c.l. = 2.2, 6.0 nM; $n = 12$) averaging 98 ± 1% of the response to KCl 80 mM (Fig. 1). None of the antagonists displayed any residual agonist activity.

MEN 11420 (30 nM–1000 nM) concentration-dependently antagonized the contraction induced by [βAla^8]neurokinin A-(4–10) without producing depression of the E_{max} to the agonist (Fig. 1a). The slope of Schild plot (−0.96, $n = 12$) was consistent with competitive antagonism and a pK_B value of 8.40 ± 0.07 ($n = 12$) was calculated (Fig. 1d). Extending the contact time from 15 to 120 min did not affect the antagonist potency of MEN 11420 in this preparation (apparent pK_B = 8.46 ± 0.03; $n = 4$).

The parent compound of MEN 11420, MEN 10627 (10–300 nM) likewise produced a concentration-dependent rightward shift of the curve to the agonist compatible with

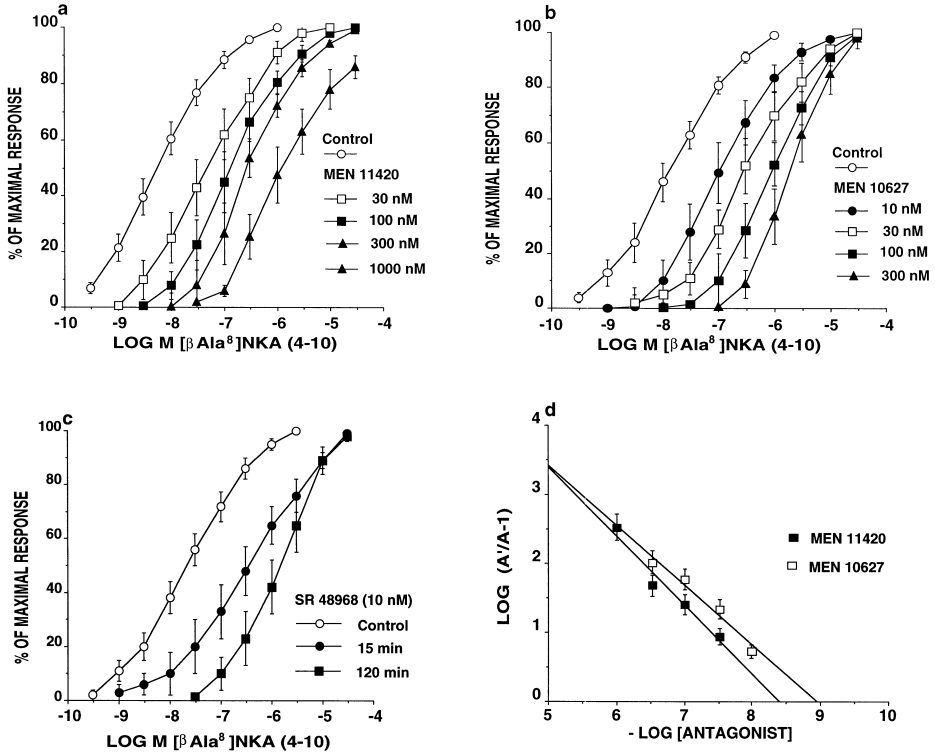

Fig. 1. Antagonism by MEN 11420, MEN 10627 and SR 48968 of [βAla^8]neurokinin A-(4–10)-induced contractions in the guinea-pig isolated bronchus. Concentration–response curves for [βAla^8]neurokinin A-(4–10) in the absence and presence of MEN 11420 (contact time 15 min), MEN 10627 (contact time 15 min) and SR 48968 (contact times 15 and 120 min) are reported in panels (a) to (c). Schild plots of agonist dose ratios vs. MEN 11420 (slope = −0.96; 95% confidence limits = −1.2; −0.7) or MEN 10627 (slope = −0.90; 95% confidence limits = −1.3; −0.5) are reported in panel (d). All the experiments were performed in the presence of 1000 nM SR 140333. Each value is expressed as % of maximal response to KCl (80 mM) and is mean ± S.E.M. of 3–12 experiments.

competitive antagonism (Fig. 1b and d): the estimated affinity ($pK_B = 8.67 \pm 0.09$; Schild plot slope $= -0.89$, $n = 12$) was slightly, but not significantly, higher than that of MEN 11420.

SR 48968 has been shown to produce insurmountable and time-dependent antagonism of certain tachykinin NK_2 receptors (Emonds-Alt et al., 1992; Advenier et al., 1992). In the present study the effect of SR 48968 (10 nM) on the response to [βAla^8]neurokinin A-(4–10) was tested after a 15 or 120 min contact time ($n = 4$). At this concentration SR 48968 did not reduce the maximal response to the agonist (Fig. 1c). However SR 48968 produced a larger rightward shift of the concentration-response curve to the agonist after 120 min than 15 min contact time ($A'/A = 17 \pm 5$ and 57 ± 10 after 15 or 120 min of incubation, $n = 4$, respectively). The apparent pK_B values were 9.0 ± 0.2, $n = 5$, and 9.57 ± 0.2 with 10 nM SR 48968, respectively.

3.1.2. Inhibition of the atropine-resistant contraction produced by electrical field stimulation

In the presence of atropine (1 μM) and indomethacin (3 μM), electrical field stimulation (10 Hz for 10 s) induced a slowly developing contraction which averaged $33 \pm 2\%$ of the maximal response to KCl 80 mM ($n = 6$). MEN 11420 (10–1000 nM, 15 min before) produced a concentration-dependent inhibition of this response (Fig. 2). At the highest concentration tested (1000 nM), MEN 11420 almost abolished the electrical field stimulation-induced bronchoconstriction ($89 \pm 3\%$ inhibition; $n = 4$).

SR 48968 and MEN 10627 (10–1000 nM, 15 min before each) likewise produced a concentration-dependent inhibition of the response to electrical field stimulation. While SR 48968 appeared to be as potent as MEN 11420 in this test, MEN 10627 at 100 nM was slightly more effective (Fig. 2). Both MEN 10627 and SR 48968 completely inhibited ($100 \pm 0\%$ and $99.6 \pm 0.4\%$ inhibition; $n = 5$, respectively) the bronchoconstriction produced by electrical field stimulation at the highest concentration tested (1000 nM) (Fig. 2).

Since the inhibitory effect of SR 48968 toward the contraction induced by [βAla^8]neurokinin A-(4–10) was larger after 120 than 15 min contact time, we also studied the effect of SR 48968 (10–100 nM, $n = 4$) after 120 min contact time. Indeed prolonging the contact time from 15 to 120 min, consistently increased the efficacy of SR 48968 in inhibiting the atropine-resistant contraction to electrical field stimulation: the mean values of percent inhibition averaged 16 ± 5 and $75 \pm 14\%$ at 10 nM ($n = 5$ and 4, $P < 0.05$) and 74 ± 8 and $96 \pm 2\%$ at 100 nM after 15 and 120 min contact time, respectively.

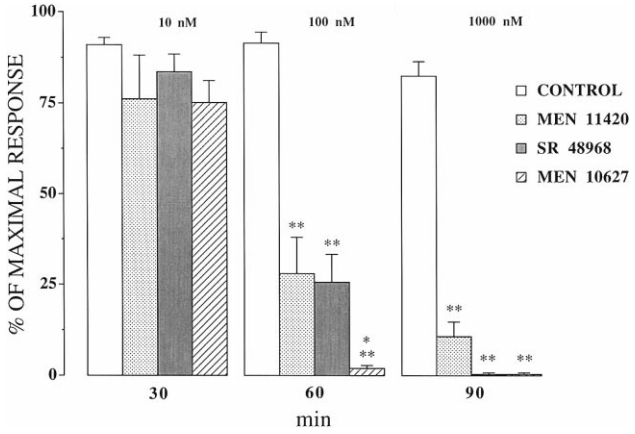

Fig. 2. Antagonism by MEN 11420, MEN 10627 and SR 48968 of electrically-induced NANC contractions of the isolated guinea-pig bronchus. The panel shows the responses obtained by application of electrical field stimulation (EFS: trains of 60 V, 0.5 ms duration, 10 Hz, for 10 s) in the guinea-pig isolated bronchus. After having elicited control response, at time = 0, the preparations were incubated with the antagonists or the vehicle (control), and electrical field stimulation applied again after 30, 60 and 90 min from the first application. MEN 11420, SR 48968 and MEN 10627 were added at increasing concentrations (10 nM, 100 nM and 1 μM) 15 min before the application of the next cycle EFS. All the responses to electrical field stimulation are expressed as % of the response obtained at time = 0. Each value is mean ± S.E.M. of 4–5 experiments. $**P < 0.01$, significantly different from control response obtained in time-matched preparations,. $*P < 0.05$, significantly different from the responses produced by electrical field stimulation in preparations pretreated with MEN 11420 or SR 48968 100 nM each.

3.2. In vivo experiments

3.2.1. Inhibition of bronchoconstrictor response induced by [βAla^8]neurokinin A-(4–10)

[βAla^8]neurokinin A-(4–10) (0.1–10 nmol/kg i.v., $n = 6$) produced a dose-dependent (Fig. 3a) increase of respiratory insufflation pressure in anaesthetized guinea-pigs ($ED_{50} = 0.71$ nmol/kg, 0.58–0.77 nmol/kg are 95% c.l.). The dose of 1 nmol/kg, inducing about 70% of the maximal response, was selected for further studies. Administration of [βAla^8]neurokinin A-(4–10) (1 nmol/kg i.v.) at 30 min intervals produced a reproducible bronchoconstriction averaging 32 ± 2 mmHg ($n = 45$). In vehicle-treated animals the amplitude of this response did not show significant time-dependent changes over 4 h observation period (Fig. 3).

MEN 11420 (30–300 nmol/kg) produced a dose-dependent inhibition of the bronchoconstrictor response to [βAla^8]neurokinin A-(4–10) (Fig. 3b). At 30 nmol/kg MEN 11420 inhibited the response to the agonist by $62 \pm 7\%$ within 5 min from administration and at 300 nmol/kg i.v. produced a total blockade of the response to [βAla^8]neurokinin A-(4–10) up to 90 min: the effect of the agonist slowly recovered thereafter, but it was still depressed by about 60% after 4 h. MEN 11420 (300 nmol/kg

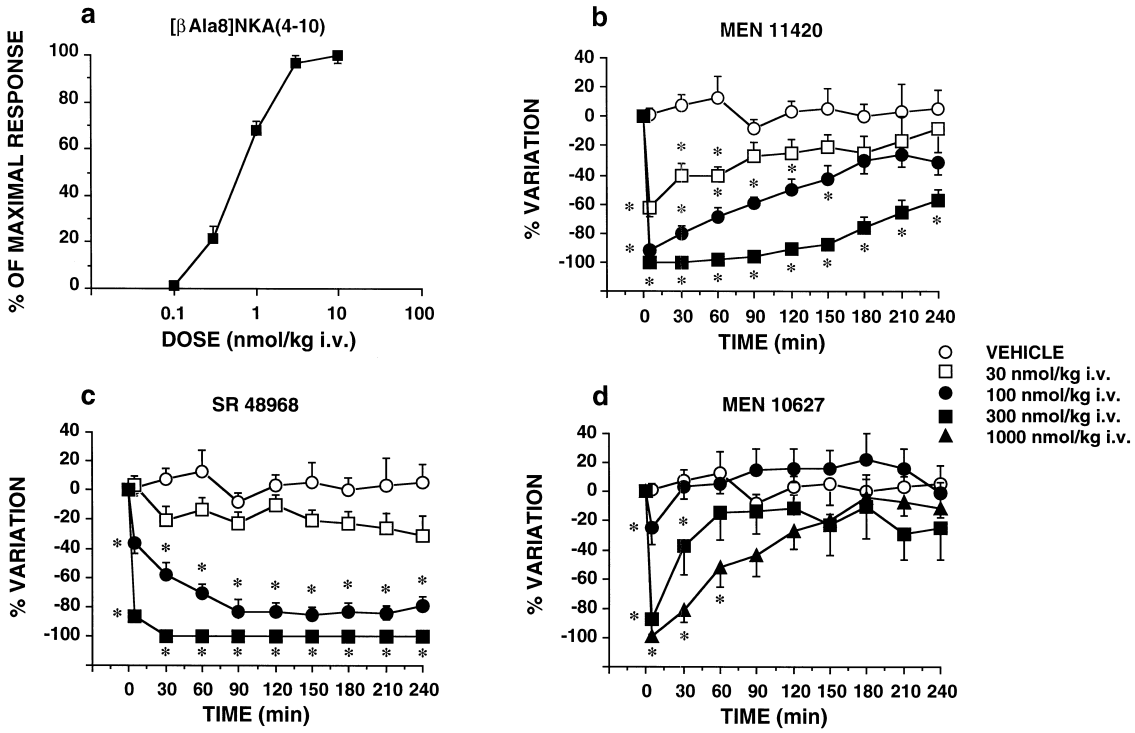

Fig. 3. Effect of MEN 11420, MEN 10627 and SR 48968 on bronchoconstriction induced by i.v. administration of [βAla^8]neurokinin A-(4–10) in guinea-pigs. Panel a: dose-dependent bronchoconstriction produced by intravenous administration of the selective NK_2 receptor agonist [βAla^8]neurokinin A-(4–10) in anaesthetized animals. Panels b–d: dose-dependent inhibition of the bronchoconstriction, induced by 1 nmol/kg [βAla^8]neurokinin A-(4–10), by i.v. administered MEN 11420, SR 48968 and MEN 10627. In panel a each value is expressed as % of maximal response to [βAla^8]neurokinin A-(4–10). In panels b–d the results are expressed as percent variation of the control response to [βAla^8]neurokinin A-(4–10) recorded before treatment. Each value is mean ± S.E.M. of 4–6 experiments. $* P < 0.05$, significantly different from the respective value in the vehicle group.

i.v., 5 min before) did not significantly affect the dose-dependent bronchoconstriction induced by the intravenous administration of histamine (10–3000 nmol/kg, $n = 6$) (Fig. 4). At the highest dose tested (300 nmol/kg i.v.) MEN 11420 did not significantly affect resting cardiovascular parameters: systolic (67 ± 3 mmHg) and diastolic (39 ± 3 mmHg) blood pressure, heart rate (197 ± 29 beats/min) over a 30 min observation period ($n = 4$, data not shown)

SR 48968 (30–300 nmol/kg) produced a dose-dependent inhibition of the bronchoconstrictor response to [βAla^8]neurokinin A-(4–10) (Fig. 3c): a significant inhibition was observed at doses of 100 and 300 nmol/kg which inhibited the response to the agonist by $83 \pm 8\%$ (peak effect at 90 min) and 100% (peak effect at 30 min), respectively (Fig. 3c). At 300 nmol/kg the blockade of contractions was maintained up to 240 min.

MEN 10627 (100–1000 nmol/kg) also produced a dose-dependent inhibition of the bronchoconstrictor response to [βAla^8]neurokinin A-(4–10): the inhibitory effect was rapid in onset but was shorter in duration than that of MEN 11420 (Fig. 3d). At all doses tested the peak inhibitory effect (25 ± 11, 88 ± 4 and $99 \pm 1\%$ inhibition at 100, 300 and 1000 nmol/kg i.v., respectively) was attained at 5 min from antagonist administration.

3.2.2. Inhibition of bronchoconstrictor response induced by MEN 11420, SR 48968 and MEN 10627 on electrical stimulation of the vagi

Bilateral electrical stimulation of the vagi provoked a bronchoconstrictor response averaging 42 ± 6 mmHg which was reduced to 24 ± 2 mmHg ($n = 89$) after administration of atropine (5.5 μmol/kg i.v.) and SR 140333 (1 μmol/kg, i.v.). During repeated cycles of stimulation at 30 min intervals, the amplitude of the control response to vagal stimulation declined progressively to about 60% of the original response at 90 min from vehicle administration (Fig. 4).

MEN 11420 (10–100 nmol/kg i.v.) produced a fast onset dose-dependent inhibition of the atropine-resistant response to vagal stimulation (Fig. 4a). At 100 nmol/kg the response to vagal stimulation was totally blocked during the whole observation period. SR 48968 produced a significant inhibition of the response to vagal stimulation at doses of 100–300 nmol/kg and its inhibitory effect was slow in onset (Fig. 4b). Also MEN 10627 produced a statistically significant inhibition, at 100–300 nmol/kg its inhibitory effect was fast in onset and not sustained (Fig. 4c). When comparing the dose-response curve for the inhibition produced by the three antagonists at 5 and 30 min from their i.v. administration, the following rank order

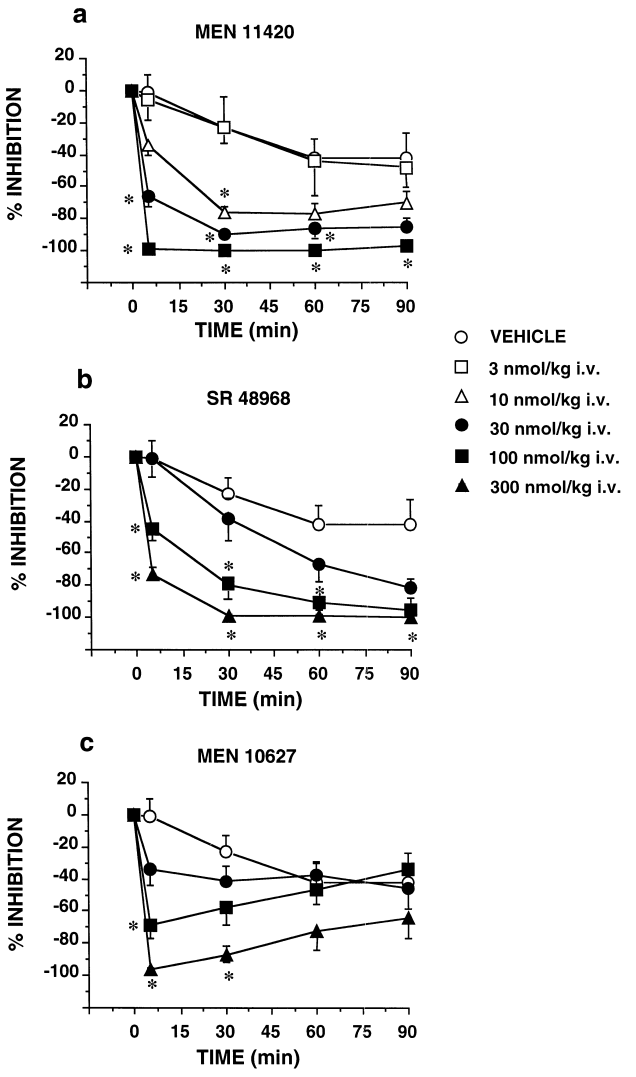

Fig. 4. Effect of MEN 11420, SR 48968 and MEN 10627 on bronchoconstriction induced by electrical stimulation of the vagi in guinea-pigs. Time- and dose-dependent inhibition of bronchoconstriction induced by electrical stimulation (20 Hz, 1 ms pulse width, 10 V for 20 s) of the vagi in anaesthetized animals following intravenous administration of the NK_2 antagonists. In all experiments the animals were pretreated with atropine (5.5 μmol/kg i.v.) and SR 140333 (1 μmol/kg i.v.). Each value is mean ± S.E.M. of 5–6 experiments. $* P < 0.05$, significantly different from the corresponding value in the vehicle-treated group.

of potency was observed MEN 11420 > MEN 10627 > SR 48968 at 5 min which became MEN 11420 > SR 48968 = MEN 10627 at 30 min from antagonists administration.

3.2.3. Effect of MEN 11420 on capsaicin-induced bronchoconstriction

The administration of capsaicin (20 nmol/kg i.v.) at 25–30 min intervals produced reproducible bronchoconstrictor responses in guinea-pigs pretreated with atropine (5.5 μmol/kg) and SR 140333 (1 μmol/kg) (Fig. 5): the effect of capsaicin ensued rapidly and reached the maximum within 15–20 s. The amplitude of the control response, averaging 15 ± 3 mmHg ($n = 6$), did not show significant variations for at least 1 h after administration of the vehicle (Fig. 5).

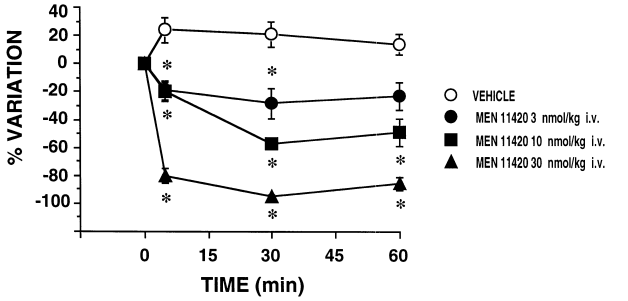

Fig. 5. Inhibitory effect of MEN 11420 on bronchoconstriction induced by i.v. capsaicin in anaesthetized guinea-pigs. Intravenous administration of MEN 11420 dose-dependently inhibited the bronchoconstriction induced by intravenous administration of capsaicin (20 nmol/kg). In all experiments the animals were pretreated with atropine (5.5 μmol/kg i.v.) and SR 140333 (1 μmol/kg i.v.). Each value is mean ± S.E.M. of 5–6 experiments. $* P < 0.05$, significantly different from the respective value of the vehicle group.

MEN 11420 (3–30 nmol/kg i.v., Fig. 5) potently and dose-dependently inhibited the bronchoconstrictor response induced by capsaicin. In fact, a small (about 20%) but statistically significant inhibitory effect of MEN 11420 was already evident at a dose of 3 nmol/kg at 30 min from its administration. A dose of 30 nmol/kg produced a large inhibition (> 80%) of the response to capsaicin for the whole observation period.

3.2.4. Plasma levels of MEN 11420 and MEN 10627 after intravenous administration in the guinea-pig

Table 1 report plasma concentration–time curves and pharmacokinetic parameters obtained after intravenous administration of 1100 and 1300 nmol/kg of MEN 11420 and MEN 10627, respectively (1 mg/kg each).

Plasma levels of MEN 11420 were measurable (> 20 ng/ml) until 360 min after treatment whereas MEN 10627 was detectable only up to 90 min. MEN 11420 showed a

Table 1
Pharmacokinetic parameters measured after intravenous administration of MEN 10627 and MEN 11420 in the guinea-pig

Parameter[a] (units)	MEN 10627	MEN 11420
$t_{1/2}$ α (min)	2.8 ± 0.5	4.3 ± 1.8
$t_{1/2}$ β (min)	23 ± 3	57 ± 2[b]
AUC (μg min/ml)	89 ± 25	384 ± 26[b]
V_d (ml/kg)	447 ± 143	212 ± 6[b]
Cl (ml/min per kg)	14 ± 3	2.6 ± 0.1[b]

Individual concentration–time curves were fitted according to a bi-exponential model. Mean values ± S.E.M. of 5 (MEN 10627) or 3 (MEN 11420) animals are shown.

[a] $t_{1/2}$ α: half-life of early distribution into extracirculatory compartments; $t_{1/2}$ β: half-life for elimination from plasma; AUC: area under the plasma concentration-time curve; V_d: volume of distribution; Cl: systemic clearance.

[b] Significantly different from the value for MEN 10627, $P < 0.05$.

significantly longer half-life than that of MEN 10627 (57 and 23 min, respectively). The area under the plasma concentration-time curve value of MEN 11420 was four times higher than that measured with MEN 10627, whereas the total body clearance and volume of distribution to peripheral compartments were significantly reduced.

4. Discussion

Both tachykinin NK_1 and NK_2 receptors mediate contractions of guinea-pig airways smooth muscle of in vivo and in vitro preparations, yet several studies have shown (see Section 1 for references) that tachykinin NK_2 receptors are predominantly activated by endogenous tachykinins.

The present findings demonstrate that MEN 11420 is a potent and selective antagonist of bronchoconstrictor responses induced by activation of tachykinin NK_2 receptors in guinea-pig airways. MEN 11420 is a glycosylated analog of MEN 10627: as compared to its parent peptide, the introduction of a sugar moiety determined a marked increase in water solubility without producing major changes in its affinity for tachykinin NK_2 receptors and selectivity vs. other molecular targets (Santicioli et al., 1997; Catalioto et al., 1998).

As shown here, negligible differences exist in the affinities of MEN 11420 and MEN 10627 for tachykinin NK_2 receptors producing contraction of the guinea-pig isolated bronchus: both compounds act as pure competitive receptor antagonists in this preparation. If any, MEN 10627 is slightly more potent than MEN 11420 as tachykinin NK_2 receptor antagonist in the guinea-pig isolated bronchus and it is also a bit more effective in inhibiting the atropine-resistant bronchoconstriction produced by electrical field stimulation. In contrast to the in vitro data, MEN 11420 is distinctly more potent than MEN 10627 and its duration of action largely outlasts that of the parent non-glycosylated peptide as an antibronchoconstrictor in vivo. As an example, a dose of 1000 nmol/kg i.v. of MEN 10627 was required to produce an almost total suppression of the response to [βAla^8]neurokinin A-(4–10) followed by a significant inhibitory effect lasting for at least 1 h, while a comparable profile of antibronchoconstrictor action was produced by MEN 11420 at a ten-fold lower i.v. dose (100 nmol/kg, Fig. 3). This remarkable difference in the in vivo potency/duration of action of the two compounds is likely to be related to a longer persistence of MEN 11420 in plasma which, in turn, can be at least partly explained by its greater resistance to degradation as compared to MEN 10627 (Catalioto et al., 1998). Moreover, it appears conceivable that the more lipophilic compound, MEN 10627, could be distributed in vivo in a way which is unfavourable for the expression of its full effectiveness as tachykinin NK_2 receptor antagonist in the smooth muscle of the tracheobronchial tree.

It is particularly interesting to compare the activities of MEN 11420 and MEN 10627 with that of the non peptide, SR 48968. It is well established that both peptide and non-peptide tachykinin receptor antagonists exhibit marked species-related differences in their affinities for tachykinin NK_2 receptors (Maggi et al., 1993): this behaviour has not yet been explained at the molecular level, but species-related variations in the aminoacid sequence of the tachykinin NK_2 receptor protein likely contribute to determining these differences. SR 48968 is more potent than either MEN 11420 or MEN 10627 in blocking tachykinin NK_2 receptors in the guinea-pig isolated airways. An accurate estimate of the affinity of SR 48968 for tachykinin NK_2 receptors is difficult because of the marked time-dependency of its antagonist action, which is evident in some preparations, (Emonds-Alt et al., 1992; Advenier et al., 1992) including the guinea-pig bronchus. Assuming that a 120 min contact time had been sufficient to attain equilibrium, and assuming a competitive interaction with tachykinin NK_2 receptors, as supported by radioligand binding/displacement data (Emonds-Alt et al., 1992, 1993b), an apparent pK_B of 9.57 and 9.0 after 15 min has been calculated in this study for SR 48968 at tachykinin NK_2 receptors in guinea-pig bronchus: this value compares to an about 10 fold lower affinity estimate for MEN 11420 and MEN 10627. We have reported previously (Patacchini et al., 1994) that the time-dependency of the antagonism exerted by SR 48968 at guinea-pig tachykinin NK_2 receptors is due to the essentially irreversible kinetics of the binding of this compound on the receptors. In the same study we showed that MEN 10627 produces fully reversible and competitive antagonism (Patacchini et al., 1994). In the present study we checked whether the antagonism of MEN 11420 were time-dependent, by prolonging the incubation from 15 min to 120 min. As stated before for MEN 10627, MEN 11420 does not produce time-dependent antagonism at guinea-pig tachykinin NK_2 receptors: thus it may be considered a simple competitive and reversible antagonist in this tissue. The time-dependency of action of SR 48968 was in some way evident also in vivo, since doses of the drug producing full inhibition of, e.g., the bronchoconstrictor response to vagal stimulation (30–100 nmol/kg i.v.) achieved their maximal inhibitory effect at about 60–90 min from i.v. administration, (Fig. 4).

The slow, time-dependent onset of the antibronchoconstrictor effect of SR 48968 could not be considered as a favourable characteristic of this compound if tachykinin NK_2 receptor antagonists had to be used as symptomatic antibronchoconstrictors in humans: in that case, a rapid onset of action would be desirable and a rapid inhibitory effect can be obtained with relatively high doses of SR 48968. No data on the in vivo kinetic/distribution of SR 48968 are available: it appears that the slow-onset blockade of tachykinin NK_2 receptor may influence the relatively poor in vivo performance of SR 48968 as compared

to MEN 11420, despite the fact that MEN 11420 is about 10 fold less potent than SR 48968 at guinea-pig bronchus tachykinin NK_2 receptors in vitro.

It is interesting to note that, in vivo, the three antagonists were all effective in inhibiting the bronchoconstriction mediated by endogenous tachykinins (atropine-resistant response to vagal stimulation) at doses lower than those effective in blocking the bronchoconstriction produced by administration of the tachykinin NK_2 receptor agonist, [βAla^8]neurokinin A-(4–10). The mechanisms responsible for this difference could be multiple. On the one hand, it has been proposed that subtypes of tachykinin NK_2 receptors exists in the guinea-pig bronchus, one of them being located prejunctionally to modulate tachykinin release from sensory nerves (Renzetti et al., 1992). However, no structural evidence for the existence of tachykinin NK_2 receptor subtypes is available at present, and the results of another study have excluded the existence of NK_2 autoreceptors on sensory nerves in guinea-pig lung (Lou et al., 1993). On the other hand, vagal stimulation determines the release of several mediators in addition to tachykinins, most notably the release of calcitonin gene-related peptide (Martling et al., 1988): these additional mediators could, directly or indirectly, influence bronchomotor tone in such a way that the antibronchoconstrictor effect of tachykinin NK_2 receptor antagonist becomes more effective.

In conclusion the present findings demonstrate that MEN 11420 is a potent, selective and long lasting inhibitor of bronchoconstrictor responses produced by activation of tachykinin NK_2 receptors in guinea-pig. In vivo MEN 11420 is significantly more potent than its more lipophilic parent compound, MEN 10627, and this characteristic likely originates from a more favourable kinetic profile.

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