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Antibronchospastic activity of MEN10,627, a novel tachykinin NK_2 receptor antagonist, in guinea-pig airways

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Abstract

The antibronchospastic activity against acetylcholine, antigen, histamine plus platelet-activating factor (PAF) or the selective tachykinin neurokinin $(NK)_1$ and NK_2 receptor agonists of the novel tachykinin NK_2 receptor antagonist, MEN10,627 (cyclo(Met-Asp-Trp-Phe-Dap-Leu)cyclo(2β-5β)), was studied in anesthetized guinea-pigs. MEN10,627 (30–100 nmol/kg i.v.) reduced in a dose-dependent manner the bronchospasm induced by the tachykinin NK_2 receptor agonist [βAla^8]neurokinin A-(4–10) and the effect of the highest dose lasted up to 5 h from its administration. Conversely, airway constriction induced by the NK_1 receptor agonist [Sar^9]substance P sulfone or acetylcholine was unaffected by MEN10,627 up to a dose of 3 μmol/kg i.v. In animals sensitized with ovalbumin and pretreated with the endopeptidase inhibitor phosphoramidon, the aerosolized antigen produced a bronchospasm which was inhibited by MEN10,627 (30–100 nmol/kg i.v.) but not by the tachykinin NK_1 receptor antagonist, (±)-CP96,345 ([2*R*,3*R*-*cis*- and [2*S*,3*S*)-*cis*-2-(diphenylmethyl)-*N*-[(2-methoxyphenyl)-methyl]-1-azabicyclo[2.2.2]octan-3-amine]) (3 μmol/kg i.v.). Both MEN10,627 (30–100 nmol/kg i.v.) and (±)-CP96,345 (30–300 nmol/kg i.v.) reduced the PAF-induced hyperresponsiveness to histamine, without affecting the hypotension induced by PAF or the bronchospasm induced by histamine in guinea-pigs not exposed to PAF, showing the involvement of both tachykinin NK_1 and NK_2 receptors in this model. In summary, MEN10,627 behaves as a potent, selective and long-lasting tachykinin NK_2 receptor antagonist in vivo. Further, tachykinin NK_2 receptors could be activated during allergic responses and in the development of airway hyperresponsiveness.

Keywords: Bronchospasm; (±)-CP96,345; MEN10,627; Neurokinin A; PAF (platelet-activating factor); Substance P; Tachykinin

1. Introduction

The mammalian tachykinins are a group of neuropeptides which includes substance P, neurokinin A and B. The biological actions of tachykinins are mediated by three receptors named NK_1, NK_2 and NK_3, whose preferred endogenous ligands are substance P, neurokinin A and B, respectively. Release of tachykinins from capsaicin-sensitive nerve endings (Lundberg and Saria, 1982) has been reported to play an important role in the pathophysiology of airway disease. In fact, both substance P and neurokinin A have been shown to participate in the regulation of smooth muscle tone, mucus secretion and plasma protein extravasation in the airways. The availability of selective tackykinin receptor agonists, such as [Sar^9]substance P sulfone and [βAla^8]neurokinin A-(4–10) for tachykinin NK_1 (Drapeau et al., 1987) and NK_2 (Rovero et al., 1989) receptors respectively, has allowed us to better define the consequences of activation of tachykinin receptors in the airways (Maggi, 1990). Although both tachykinin NK_1 and NK_2 receptors are present in guinea-pig airways (Floch et al., 1994) and their activation produces bronchoconstriction (Ballati et al., 1992), it has been reported that tachykinin NK_2 receptors predominate in animal and human smooth muscle (Manzini, 1994). Intravenous administration of natural tachykinins in anesthetized guinea-pigs elicits remark-

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able bronchoconstriction with neurokinin A being several times more potent than substance P (Manzini et al., 1988), suggesting a preferential involvement of tachykinin NK_2 receptors.

In light of the above, the recent characterization of a selective tachykinin NK_2 receptor antagonist, MEN10,627 or cyclo(Met-Asp-Trp-Phe-Dap-Leu)-cyclo(2β-5β) (Maggi et al., 1994), prompted us to evaluate its potential antibronchospastic activity in vivo. To assess its selectivity and efficacy in comparison to the tachykinin NK_1 receptor antagonist, (±)-CP96,345 or [2*R*,3*R*-*cis*- and [2*S*,3*S*)-*cis*-2-(diphenylmethyl)-*N*-[(2-methoxyphenyl)-methyl]-1-azabicyclo[2.2.2]octan-3-amine] (Snider et al., 1991), some spasmogenic stimuli such as the tachykinin NK_1 and NK_2 receptor agonists or acetylcholine were used.

Further, since platelet-activating factor (PAF) infusion-induced airway hyperresponsiveness to histamine as well as the bronchomotor response to antigen in the presence of an endopeptidase inhibitor have been proposed to be modulated by capsaicin-sensitive nerves (Bertrand et al., 1993; Perretti and Manzini, 1993), the antibronchospastic activity of the two tachykinin antagonists, MEN10,627 and (±)-CP96,345, was also evaluated in these experimental models.

2. Materials and methods

2.1. Animals

Male albino guinea pigs (Rodentia, Torre Pallavicina-Bergamo, Italy, 300–350 g) were used. They were housed at constant room temperature (21 ± 1°C) and relative humidity (60%) and with a 12-h light-dark cycle (light on 06.00 a.m.). The animals were deprived of food for 16 h before the experiments, but allowed free access to tap water.

2.2. Measurement of pulmonary insufflation pressure

Guinea-pigs were anesthetized with urethane (17.5 mmol/kg i.p.) and *d*-tubocurarine (3.9 μmol/kg i.v.) was administered to prevent spontaneous respiratory movements. Body temperature was controlled electronically (Marb 8511) and maintained at 34.5°C. The animals were ventilated mechanically through a tracheal cannula by a ventilation pump (Basile 7025) adjusted to a rate of 60 strokes/min. Respiration volume was kept constant by means of a water valve providing an initial basal pressure of 9.4–13.4 mm Hg. Insufflation pressure was measured by attaching a pressure tranducer (Bentley Trantec) to a side-arm of the tracheal cannula. Changes in insufflation pressure were monitored on a Basile Unirecord 7050 polygraph. A polyethylene catheter was inserted in the left jugular vein for drug injection. Drugs were administered in a volume of 1 ml/kg. The basal value of insufflation pressure remained stable for at least 2 h and no significant changes were produced by i.v. saline administration. At the end of the stabilization period (30 min) the guinea-pigs were challenged with i.v. acetylcholine (50 nmol/kg), [Sar^9]substance P sulfone (1 nmol/kg) or [βAla^8]neurokinin A-(4–10) (0.3 nmol/kg). Preliminary experiments showed that at least three reproducible responses to these spasmogens could be evoked at intervals of 30 min in the same animal. The pharmacological modulation of these bronchospastic responses was studied using the following drugs, injected 5 or 30 min before the challenge with the spasmogen: (±)-CP96,345 (3 μmol/kg i.v.) or MEN10,627 (0.03–3 μmol/kg i.v.). In some experiments the animals were challenged several times at intervals of 30, 60, 120, 180 and 300 min with [βAla^8]neurokinin A-(4–10) after MEN10,627 (30–100 nmol/kg i.v.) or its vehicle (10% dimethyl sulfoxide).

2.3. Antigen-induced bronchoconstriction

The animals were sensitized by injection of 100 mg/kg i.p. + 100 mg/kg s.c. ovalbumin dissolved in saline and used 2 weeks later. At the time of experiments the animals were anesthetized with sodium pentobarbital (181 μmol/kg i.p.) and surgical procedures and measurement of insufflation pressure were carried out as described before. Sodium pentobarbital was used because of the short length of the experiments. An aerosol of 0.1% ovalbumin for 20 s was produced by an ultrasonic nebulizer (DeVilbiss) and was delivered into the airways through the tracheal cannula of the respirator. The pharmacological modulation of these bronchospastic responses was studied by using MEN10,627 (30–100 nmol/kg i.v.) or (±)-CP96,345 (3 μmol/kg i.v.) given 15 min before the spasmogenic stimulus. To amplify the tachykinin- and to avoid the cholinergic-mediated components of the allergic bronchomotor response, the animals were pretreated with atropine (1.5 μmol/kg i.v.) and the neutral endopeptidase inhibitor phosphoramidon (4.6 μmol/kg i.v.) 15 min before the spasmogenic stimulus, as previously described by Bertrand et al. (1993).

2.4. PAF-induced bronchial hyperresponsiveness to histamine

The guinea-pigs were anesthetized with pentobarbital sodium (181 μmol/kg i.p.) and surgical procedures and measurement of insufflation pressure were carried out as described before. Sodium pentobarbital was used because of the short length of the experiments. In control experiments, histamine (5 nmol/kg) was administered intravenously as a bolus before and

30 min after the infusion of 0.25% bovine serum albumin or PAF (1.1 μmol/kg over 1 h). The total dose of PAF was infused at three increasing rates (5% in 10 min, 20% in 20 min and 75% in 30 min) to prevent cardiovascular collapse (Robertson and Page, 1987). The increase in pulmonary insufflation pressure induced by histamine (5 nmol/kg i.v.) was measured before and 30 min after stopping infusion with PAF or bovine serum albumin. MEN10,627 (3–100 nmol/kg i.v.), (±)-CP96,345 (30–300 nmol/kg i.v.) or 2.2 μmol/kg i.p. of WEB 2086 (3-[4–2-chlorphenyl)-9-methyl-6*H*-thieno[3,2-*f*][1,2,4]triazole-[4,3-*a*][1,4]-diazepin-2-yl]-1-(4-morpholinyl)-1-propanon) was administered 10 min before and concomitantly with PAF or bovine serum albumin infusion, to evaluate the effect of these drugs on PAF-induced bronchial hyperresponsiveness to histamine. In another set of experiments, the effect of pretreatment with the compounds under study was tested on PAF infusion-induced immediate bronchospasm and hypotension. In these experiments the carotid artery was cannulated for measurement of systemic blood pressure.

2.5. Drugs

The following drugs were used: acetylcholine (Serva), atropine sulfate (Merck), [βAla^8]neurokinin A-(4–10) (Peninsula), histamine (Sigma), ovalbumin (grade V; Sigma), PAF (C16, Novabiochem), phoshoramidon (Peninsula), sodium pentobarbital (Sessa), [Sar^9]substance P sulfone (Peninsula), *d*-tubocurarine (Fluka), urethane (Fluka) and WEB 2086 (gift from Boehringer Ingelheim, Germany). MEN10,627 and (±)-CP96,345 were synthesized at the Chemistry Department of Menarini Labs. (Florence, Italy) by conventional solid phase methods. For i.v. injection, all peptides were dissolved in saline and injected in a volume not exceeding 1 ml/kg. For MEN10,627 a stock solution (1 mM) was prepared in dimethyl sulfoxide and then diluted in saline.

2.6. Statistics

All data in the text are expressed as means ± S.E.M. Statistical analysis was performed by means of analysis of variance (ANOVA) followed by Dunnett's test.

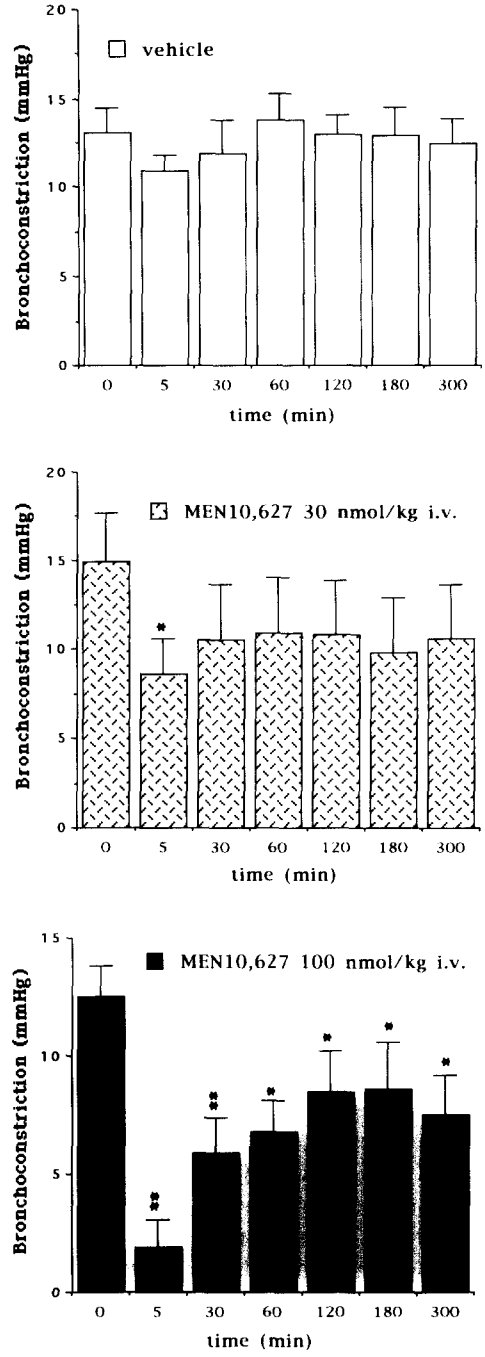

Fig. 1. Effect of MEN10,627 (30–100 nmol/kg i.v.) or its vehicle on bronchospasm induced by repeated (up to 5 h from drug administration) challenge with 0.3 nmol/kg i.v. of [βAla^8]neurokinin A-(4–10). * $P < 0.05$ and ** $P < 0.01$ as compared to respective time 0 group; $n = 4$–6 for all groups.

3. Results

3.1. Effects of the tachykinin receptor antagonists, (±)-CP96,345 and MEN10,627, on the increase in insufflation pressure induced by selective tachykinin receptor agonists and acetylcholine

As shown in Fig. 1 (upper panel) the injection of [βAla^8]neurokinin A-(4–10) in vehicle-pretreated animals produced a reproducible response at intervals of 30, 60, 120, 180 and 300 min from the first administration of spasmogen. MEN10,627 (30–100 nmol/kg i.v.) reduced the bronchomotor response to [βAla^8]neurokinin A-(4–10) in a dose-dependent manner (Fig. 1), the peak of the effect being at 5 min after drug injection (Fig. 1). The antibronchospastic effect of 100

nmol/kg i.v. of MEN10,627 lasted up to 5 h from its administration (Fig. 1; lower panel). (±)-CP96,345, at a dose (3 μmol/kg i.v.) that markedly reduced the response to [Sar^9]substance P sulfone (Table 1), did not affect the bronchospasm induced by [βAla^8]neurokinin A-(4–10) (9.7 ± 0.1 mm Hg of controls vs. 10.1 ± 0.2 and 9.7 ± 0.2 mm Hg in (±)-CP96,345-treated animals at 5 and 30 min after drug administration; $n = 4$ for all groups).

MEN10,627 up to a dose of 3 μmol/kg i.v. did not affect the bronchospastic response produced by stimulation of the NK_1 receptor through the agonist [Sar^9]substance P sulfone (Table 1) or acetylcholine (17.6 ± 2.4 mm Hg of controls vs. 18.3 ± 3.6 mm Hg of MEN10,627-treated at 30 min after its administration). The bronchospasm induced by acetylcholine (15.6 ± 3.8 mm Hg; $n = 4$) was not affected (21.7 ± 5.2 mm Hg; $n = 4$) by treatment with (±)-CP96,345 (3 μmol/kg i.v. injected 30 min before the challenge).

3.2. Effects of the tachykinin receptor antagonists, (±)-CP96,345 and MEN10,627, on insufflation pressure induced by aerosolized antigen

The aerosol of a low concentration (0.1%) of ovalbumin caused a small increase in insufflation pressure that was slow in onset with a peak at 4 min (4.2 ± 1.6 mm Hg; $n = 5$) and disappeared almost completely after 20 min. A significantly greater response (11.9 ± 2.5 mm Hg; $n = 6$), as compared to that of vehicle-treated animals, with a peak at 2 min and lasting 20 min, was observed in the presence of phosphoramidon (4.6 μmol/kg i.v., 15 min before; see Fig. 2). Pretreatment with MEN10,627 (30–100 nmol/kg i.v.) significantly reduced the increase in insufflation pressure induced by antigen during the first 5 min (Fig. 2, upper panel). Conversely, (±)-CP96,345 (3 μmol/kg i.v.) decreased the antigen-induced bronchospasm at the onset of the response (1 min), leaving unaffected the other part of the bronchospasm recorded over 20 min (Fig. 2, lower panel). In all experiments the animals were pretreated with atropine to avoid the cholinergic component of

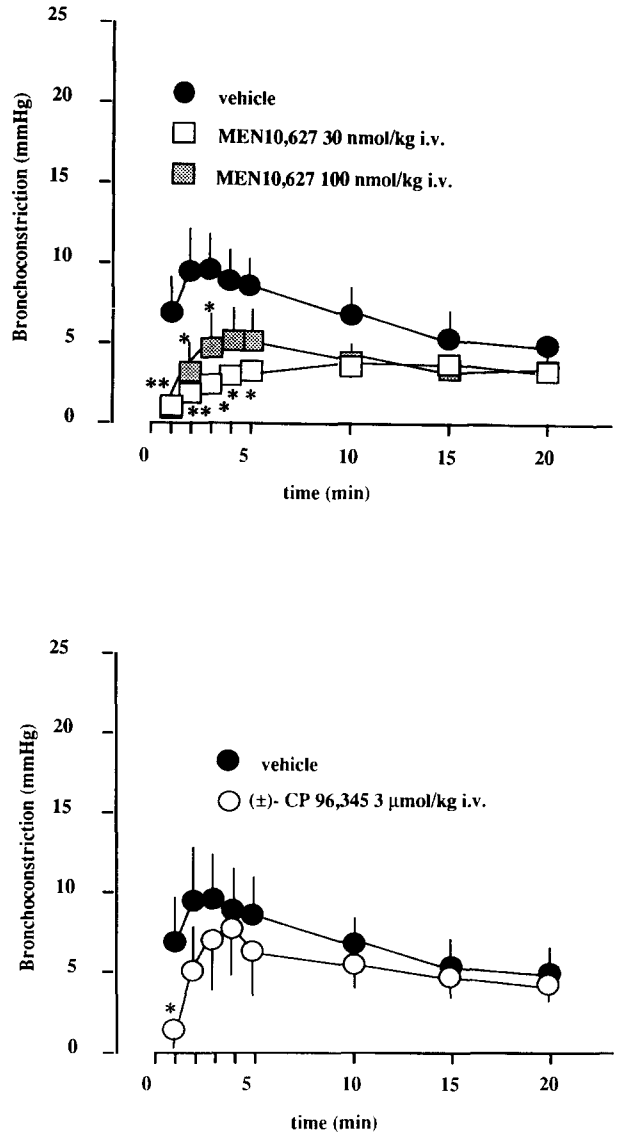

Fig. 2. Effect of MEN10,627 (30–100 nmol/kg i.v.; upper panel) or (±)-CP96,345 (3 μmol/kg i.v.; lower panel) on insufflation pressure induced by 0.1% ovalbumin in anesthetized and sensitized guinea-pigs pretreated with atropine (1.5 μmol/kg i.v.) and phosphoramidon (4.6 μmol/kg i.v.). * $P < 0.05$ and ** $P < 0.01$ as compared to controls treated with the vehicle (solid circles); $n = 4$–6 for all groups.

Table 1
Effect of tachykinin NK_1 ((±)-CP96,345) or NK_2 (MEN 10,627) receptor antagonists on bronchospasm induced by 1 nmol/kg i.v. of [Sar^9]substance P sulfone

Treatment	Increase in insufflation pressure (mm Hg)		
	Before	After 5 min	After 30 min
MEN10,627 (3 μmol/kg i.v.)	9.2 ± 1.7	7.6 ± 1.9	12.1 ± 1.8
(±)-CP96,345 (3 μmol/kg i.v.)	7.8 ± 1.1	0.9 ± 0.4 [a]	1.6 ± 0.7 [a]

Results are expressed as means ± S.E.; $n = 4$ for all groups. [a] $P < 0.01$ as compared to the respective groups challenged before the treatments.

the antigen response. Whatever this component is, the effect of both (±)-CP96,345 and MEN10,627 was strictly related to their tachykinin antagonism, both being ineffective toward acetylcholine bronchospasm (see above).

3.3. Effects of the tachykinin receptor antagonists, (±)-CP96,345 and MEN10,627, on PAF-induced hyperresponsiveness to histamine

A bolus injection of histamine (5 nmol/kg i.v.) elicited an increase in pulmonary insufflation pressure of 2.3 ± 1.1 mm Hg and 5.7 ± 1.1 mm Hg, before and

30 min after bovine serum albumin ($n = 6$). In contrast, the difference in pulmonary insufflation pressure before and 30 min after a slow infusion of PAF was 11.1 ± 1.7 mm Hg ($n = 5$, $P < 0.01$ as compared to bovine serum albumin). The PAF receptor antagonist WEB 2086 (2.2 μmol/kg i.p.) as well as MEN10,627 (30–100 nmol/kg i.v.) or (±)-CP96,345 (30–300 nmol/kg i.v.), slowly infused 10 min before and concomitantly with the infusion of PAF, markedly reduced the PAF-induced airway hyperresponsiveness to histamine (Fig. 3, panel A). At a lower dose (3 nmol/kg i.v.), MEN10,627 did not prevent the PAF-induced airway hyperresponsiveness, the increase in insufflation pressure being 7.2 ± 2.4 mm Hg ($n = 4$; Fig. 3, panel A). MEN10,627 (100 nmol/kg i.v.) or (±)-CP96,345 (100 nmol/kg i.v.) had no significant effect on the responsiveness to histamine after bovine serum albumin infusion (values of pulmonary insufflation pressure in mm Hg were: 3.4 ± 1.1 for vehicle, 3.9 ± 1.3 for MEN10,627 and 3.6 ± 1.3 for (±)-CP96,345; $n = 3$ for all groups). During the infusion with PAF there was an immediate and transient bronchoconstriction which was reduced by WEB 2086 (2.2 μmol/kg i.p.) or (±)-CP96,345 (30–300 nmol/kg i.v.) but not by the highest dose of MEN10,627 (100 nmol/kg i.v.), as shown in Fig. 3, panel B. The slow infusion of PAF also induced a fall in mean arterial blood pressure of 30.1 ± 7.0 mm Hg ($n = 5$), an effect which was unaffected by MEN10,627 (100 nmol/kg i.v.) or (±)-CP96,345 (30–300 nmol/kg i.v.) but markedly reduced by WEB 2086 (2.2 μmol/kg i.p.), as shown in Fig. 3, panel C.

4. Discussion

The aim of the present study was to investigate the antibronchospastic activity of a novel tachykinin NK_2 receptor antagonist, MEN10,627. This compound has been reported to possess in vitro a marked selectivity and affinity for tachykinin NK_2 receptors as compared to the other tachykinin receptors (Maggi et al., 1994). The present findings show that MEN10,627 is at least 30 times more active in vivo than MEN10,376, a linear peptide tachykinin NK_2 receptor antagonist, previously characterized in our research laboratories (Maggi et al., 1991; Ballati et al., 1992), and is equipotent on a molar basis to the non-peptide antagonist SR48,968 (Bertrand et al., 1993). The tachykinin NK_2 receptor antagonistic effect of MEN10,627 is also associated with long-lasting in vivo activity since MEN10,627 was effective up to 5 h from its administration. A similar long-lasting in vivo tachykinin NK_2 receptor blockade has been found in the inhibition of contraction of urinary bladder and duodenum induced by MEN10,627 (Maggi et al., 1994). Thus, the cyclization of the molecule might confer an enhanced defense against

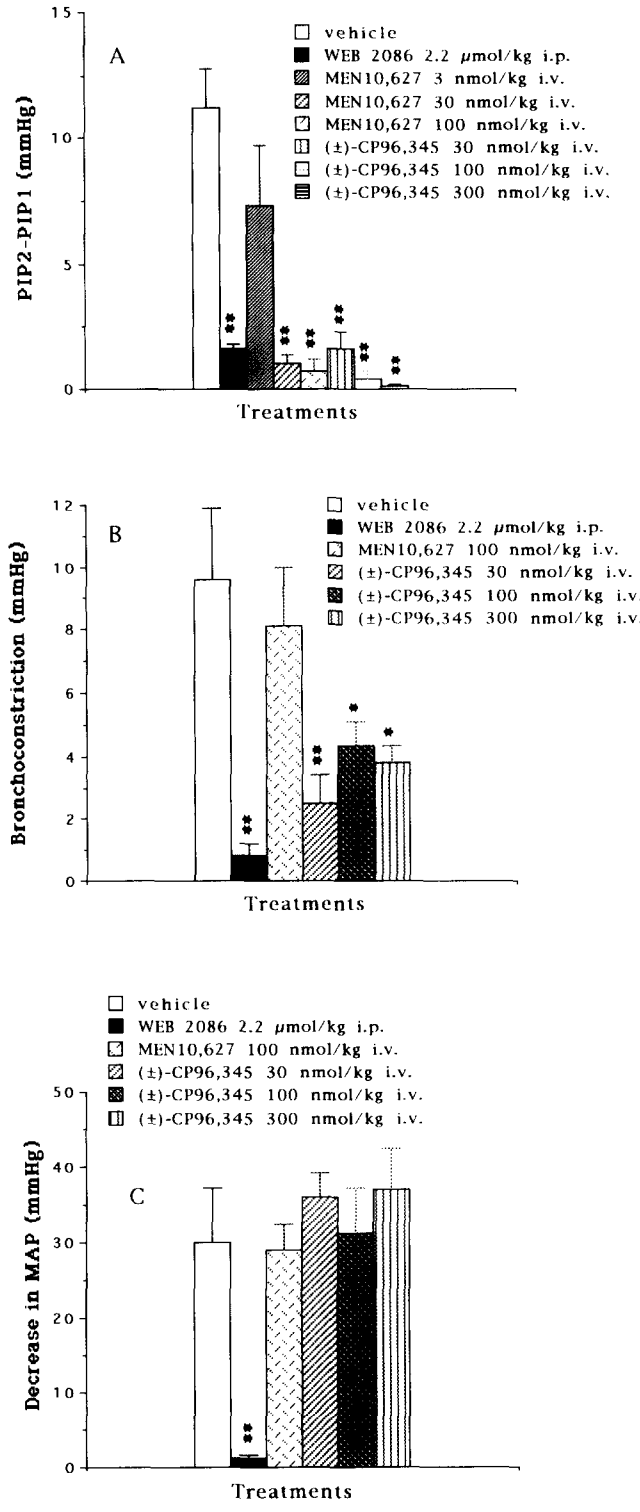

Fig. 3. Effect of WEB 2086 (2.2 μmol/kg i.p.), MEN10,627 (3–100 nmol/kg i.v.) or (±)-CP96,345 (30–300 nmol/kg i.v.) on bronchial hyperresponsiveness to histamine (5 nmol/kg i.v.), registered as pulmonary insufflation pressure (PIP2 − PIP1 = difference between values before and after PAF infusion, panel A), immediate bronchoconstriction (panel B) and decrease in mean arterial pressure (MAP, panel C) after a slow infusion of PAF (1.1 μmol/kg i.v. over 1 h). * $P < 0.05$ and ** $P < 0.01$ as compared to vehicle group; $n = 4$–6 for all groups.

the metabolism which the classical peptidic antagonists are usually subjected to (see Maggi et al., 1993 for a review).

We found that both MEN10,627 and (±)-CP96,345 completely blocked the increase in insufflation pressure induced by the respective selective tachykinin receptor agonists, being completely ineffective toward the other tachykinin receptor agonists or acetylcholine. The analysis of these results confirms that in guinea-pig both tachykinin NK_1 and NK_2 receptors mediate an independent bronchoconstriction (Ballati et al., 1992) and emphasizes the selective antibronchospastic activity of MEN10,627. The two antagonists were then tested against the bronchomotor response of antigen in the presence of an endopeptidase inhibitor.

MEN10,627, but not (±)-CP96,345, potently inhibited antigen-induced bronchospasm in the presence of an inhibitor of neutral endopeptidase. In fact, tachykinin receptor antagonists did not affect the bronchospasm evoked by a large dose of ovalbumin (Bertrand et al., 1993), suggesting the predominant involvement of other mediators (e.g. histamine, leukotrienes) in the maximal response to antigen. Conversely, the bronchospasm induced by a lower dose of ovalbumin was greatly enhanced by phosphoramidon, unmasking a potent tachykinin-mediated component (tachykinin NK_2 receptor mediated) in the response to antigen. An inhibition of neutral endopeptidase activity associated with involvement of sensory nerves has been described in respiratory viral infection (Piedimonte et al., 1990), toluene diisocyanate asthma (Sheppard et al., 1988) and cigarette smoke (Dusser et al., 1988).

In view of the potent antibronchospastic effect of both tachykinin NK_1 and NK_2 receptor antagonists under study we decided to investigate the bronchomotor response of PAF-induced hyperresponsiveness to i.v. histamine. We showed previously that depletion of the airway tachykinin content induced by high doses of capsaicin abolished the increased bronchoconstrictive sensitivity to histamine after PAF infusion (Perretti and Manzini, 1993). The present findings demonstrate that tachykinins acting on both NK_1 and NK_2 receptors are involved in this model of airway hyperresponsiveness. PAF has been reported to produce an infiltration of inflammatory cells (Spina et al., 1991) releasing cytotoxic material which may damage and expose the sensory fibers and lead to a local release of tachykinins. The PAF receptor antagonist WEB 2086 markedly reduced the immediate bronchoconstriction, hypotension and hyperresponsiveness to histamine induced by PAF. Conversely, MEN10,627 selectively affected the enhanced bronchospastic response to histamine following PAF but not vehicle infusion, suggesting that tachykinins released after PAF infusion are largely involved in this phenomenon. Unlike MEN10,627, (±)-CP96,345 reduced the immediate bronchoconstriction induced by PAF alone and these effects seem, at least in part, to influence its reduction of the hyperresponsiveness to histamine induced by the phospholipid. (±)-CP96,345, at variance with its tachykinin NK_1 receptor blockade, is known to possess a non-stereoselective calcium antagonistic effect (Schmidt et al., 1992), which in association to its anti-inflammatory properties (Lei et al., 1992) could be involved in its anti-hyperresponsiveness action.

In summary, MEN10,627 behaves as a potent, selective and long-lasting tachykinin NK_2 receptor antagonist in vivo. It potently inhibits tachykinin NK_2 receptor agonist- and antigen-induced bronchoconstriction. In addition, it exerts a protective effect on PAF-induced hyperresponsiveness to i.v. histamine. These latter bronchospastic responses are also mediated by the release of tachykinins from sensory nerves. If a similar mechanism exists in humans, such as described by Ichinose et al. (1992), MEN10,627 may play a potential therapeutic role in airway diseases.

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