

# MEN16132, a Novel Potent and Selective Nonpeptide Kinin B<sub>2</sub> Receptor Antagonist: In Vivo Activity on Bradykinin-Induced Bronchoconstriction and Nasal Mucosa Microvascular Leakage in Anesthetized Guinea Pigs

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## ABSTRACT

We have tested the activity of 4-(S)-amino-5-(4-{4-[2,4-dichloro-3-(2,4-dimethyl-8-quinolyloxymethyl)phenylsulfonamido]-tetrahydro-2H-4-pyranilcarbonyl} piperazino)-5-oxopentyl(trimethyl)ammonium chloride hydrochloride (MEN16132), a novel nonpeptide kinin B<sub>2</sub> receptor antagonist, on bradykinin (BK)-induced inflammatory responses, bronchoconstriction, and hypotension in guinea pigs. After i.v. (1–10 nmol/kg i.v.), intratracheal (i.t.) (10–100 nmol/kg i.t.), or aerosol (0.01–0.1 mM/5 min) administration, MEN16132 inhibited in a dose-dependent manner the bronchoconstriction induced by BK (10 nmol/kg i.v.). MEN16132 was more potent and possessed a longer duration of action as compared with the peptide B<sub>2</sub> receptor antagonist icatibant (HOE140; *H*-D-Arg-Arg-Pro-Hyp-Gly-Thi-Ser-D-Tic-Oic-Arg-OH trifluoroacetate). After i.v. administration, its inhibitory effect on bronchoconstriction lasted more than 8 h at 30 nmol/kg. When administered by i.v. or i.t. routes, the dose completely inhibiting bronchoconstriction also

partially reduced the hypotensive response to BK, whereas after aerosol administration, the inhibitory effect was limited to respiratory level. Intranasal (i.n.) administration of MEN16132 (0.01–0.3 nmol/nostril) reduced, in a dose-dependent and long-lasting manner, the nasal mucosa plasma protein extravasation induced by BK (100 nmol/nostril), and it exerted a complete inhibition at about 30-fold lower dose than icatibant. At 1 nmol/nostril, MEN16132 activity was significant for at least 6 h with no systemic effect measured as inhibition of BK-induced hypotension, and at 10 nmol/nostril, the inhibitory effect lasted for more than 15 h with only a weak effect on hypotension. These findings indicate that in vivo MEN16132 is a potent kinin B<sub>2</sub> receptor antagonist with long duration of action, both after i.v. and local administration. A complete and prolonged inhibition of BK-induced bronchoconstriction or nasal inflammation can be achieved with MEN16132 topical administration (aerosol or i.n.) at doses devoid of systemic effects.

BK, a nonapeptide generated in plasma and tissues by activation of kininogens, exerts its effects through the constitutively expressed B<sub>2</sub> receptor before degradation by enzymatic cleavage. The des-Arg<sup>9</sup> metabolites of BK maintain a biological activity mediated by the B<sub>1</sub> receptor, which is not or barely expressed in normal tissues, but it is up-regulated following inflammation and tissue injury (Leeb-Lundberg et al., 2005).

In humans and guinea pigs, the kinin B<sub>2</sub> receptors are present in lungs (Mak and Barnes, 1991) and nasal airways (Fujiwara et al., 1989; Dear et al., 1996). In guinea pig airways, BK induces bronchoconstriction (Wirth et al., 1993), microvascular leakage (Ichinose and Barnes, 1990; Ricciardolo et al., 1994), and bronchial hyperreactivity (Omini et al., 1989); furthermore, in vitro evidence indicates that BK may stimulate inflammatory cell recruitment (Sato et al., 1996; Koyama et al., 2000). These effects are mediated by the B<sub>2</sub> receptor, also indirectly through the release of cyclooxygenase products and sensory nerve endings stimulation (Ichinose et al., 1990; Nakajima et al., 1994). In humans, BK induces potent bronchoconstriction and cough when inhaled

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**ABBREVIATIONS:** BK, bradykinin; MEN16132, 4-(S)-amino-5-(4-{4-[2,4-dichloro-3-(2,4-dimethyl-8-quinolyloxymethyl)phenylsulfonamido]-tetrahydro-2H-4-pyranilcarbonyl} piperazino)-5-oxopentyl(trimethyl)ammonium chloride hydrochloride; PPE, plasma protein extravasation; i.t., intratracheal; i.n., intranasal; FR173657, (E)-3-(6-acetamido-3-pyridyl-N-[N-[2,4-dichloro-3-[(2-methyl-8-quinolinyl)-oxymethyl]-phenyl]N-methyl-amino-carbonylmethyl] acrylamide; LF16-0687, 1-[[2,4-dichloro-3-[(2,4-dimethylquinolin-8-yl)oxy)methyl]phenyl]sulfonyl-N-[3-[[4-(aminoimino-methyl)phenyl]carbonylamino]propyl]-2(S)-pyrrolidine carboxamide.

in asthmatic patients (Choudry et al., 1989; Polosa and Holgate, 1990) and rhinitis-like symptoms when instilled into the nose (Churchill et al., 1991). Furthermore, BK is generated in human nasal secretions during rhinovirus infections (Shibayama et al., 1996) and allergic rhinitis (Proud et al., 1983; Svensson et al., 1990). On the basis of these findings, a therapeutic potential role of kinin B<sub>2</sub> receptor antagonists has been hypothesized for the treatment of airways inflammatory pathologies associated with hyperresponsiveness to BK, such as chronic bronchial asthma (Akbari et al., 1996), or with the release of BK, such as perennial and seasonal allergic rhinitis (Austin et al., 1994; Turner et al., 2001).

On the other hand, in addition to their proinflammatory activities, kinins also exert protective effects on the cardiovascular system. Several studies have provided evidence suggesting that BK contributes to the antihypertensive and cardioprotective effects of angiotensin-converting enzyme inhibitors (Kuoppala et al., 2000; Campbell et al., 2005). In rat, BK exerts an injury-limiting effect in the ischemic and reperfused heart (Ito et al., 2003; Driamov et al., 2004), and acute cardiovascular effects produced by kinin B<sub>2</sub> receptor antagonists have been observed (Carini et al., 2002). On the basis of these considerations, it would be important to obtain a block of B<sub>2</sub> receptors in the airways without interfering with those at the cardiovascular level.

MEN16132 (Fig. 1) is a new selective nonpeptide antagonist of the kinin B<sub>2</sub> receptor endowed with high affinity for the guinea pig and human receptors (P. Cucchi, S. Meini, and C. Catalani, unpublished data). In this study, we have investigated the ability of the new B<sub>2</sub> receptor antagonist MEN16132 in inhibiting BK-induced bronchoconstriction or PPE in nasal mucosa after i.v. or local administration (aerosol, intratracheal, or intranasal). The peptide B<sub>2</sub> receptor antagonist icatibant has been used as a reference compound. To check the systemic absorption after local administration of MEN16132, its effect on BK-induced hypotension was also determined.

## Materials and Methods

Experiments were performed in male Dunkin Hartley guinea pigs weighing 350 to 400 g (Charles River Italiana, Calco, Italy) in accordance with the principles and guidelines of the Italian Government, the European Union regulations, and the local ethical committee.

**Bronchoconstriction and Hypotension Induced by Intravenous Bradykinin.** Guinea pigs were anesthetized with urethane (1.5 g/kg s.c.). The left jugular vein and the carotid artery were cannulated for drugs administration and blood pressure recording, respectively. The pressure signal was recorded by means of a pressure transducer. The animals were mechanically ventilated (10

ml/kg room air, 50 strokes/min), and pulmonary insufflation pressure was measured by a transducer connected to the tracheal cannula. The pressure transducers (Transpac IV; Abbott Labs, Pomezia, Italy) were connected to a Mac Lab/8S ML 780 data acquisition system (ADInstruments Pty Ltd., Castle Hill, Australia). The body temperature was kept constant at 37°C by a thermoregulated heating lamp. A dose-response curve to i.v. BK was first determined to select a dose suitable for the evaluation of B<sub>2</sub> receptor antagonist activity.

After a 15-min stabilization period from the surgical operation, two control responses to BK (10 nmol/kg i.v.) were obtained, whereupon MEN16132, icatibant, or their vehicles were administered by i.v. route, or into the airways, through i.t. instillation or aerosol delivery in the afferent limb of the respiratory pump for 5 min by means of an ultrasonic nebulizer (Micrò, Markos-Air Liquide; Markos-Mefar, Bovezzo, Italy). The vehicle received by the control group was saline, for i.v. and aerosol administration, and saline with 30% dimethylsulfoxide for i.t. administration. The challenge with i.v. BK was performed 5 min after the antagonists or the vehicle administration, then repeated every 30 min up to 210 min. In another series of experiments, aiming to check the duration of action of i.v. MEN16132 on BK-induced bronchoconstriction, the observation period was prolonged to 8 h. BK (10 nmol/kg i.v.) was administered every 1 h to avoid possible tachyphylaxis.

To check the possibility of antagonist systemic absorption after intranasal application, MEN16132 or its vehicle (saline, 30  $\mu$ l/nostril) were instilled in both nostrils of unanesthetized guinea pigs. After 15 min, the animals were anesthetized with urethane (1 g/kg i.p.). The trachea and carotid artery were cannulated for mechanical ventilation and blood pressure recording, respectively, as described above; 45 min after antagonist administration, BK (10 nmol/kg i.v.) was given and repeated twice at 30-min interval.

**Nasal Mucosa Microvascular Leakage Induced by Intranasal Bradykinin.** MEN16132, icatibant, or their vehicle were instilled in a volume of 30  $\mu$ l in both nostrils of unanesthetized guinea pigs. After 10 min, the animals were anesthetized with sodium pentobarbital (40 mg/kg i.p.), the jugular vein was cannulated for drug administration, and the animals were mechanically ventilated through a tracheal cannula (10 ml/kg room air, 50 strokes/min). Guinea pigs were pretreated (5 min before) with captopril (1 mg/kg i.v.); then, 30 min after antagonist, Evans blue (30 mg/kg i.v.) and BK (100 nmol/nostril) or its vehicle (saline, 30  $\mu$ l/nostril) were administered. A dose-response curve to i.n. BK was first obtained to select a dose suitable for evaluation of the antagonists activity.

After 15 min, the chest was opened, a cannula was inserted into the left ventricle, and the circulation was perfused with 100 ml of saline containing heparin (180 IU/ml) to flush out the dye; the right atrium was opened to allow the expulsion of the perfusion medium. The nasal mucosa was removed and weighed. The Evans blue extravasated from the microcirculation was quantified by measuring the optical density of the formamide extracts (for 6 h at 60°C) at 630-nm wavelength with a spectrophotometer (CERES UV900C; Bio-Tek Instruments, Winooski, VT). To determine the time course of MEN16132 inhibition on BK-induced nasal mucosa PPE, the agonist was administered i.n. 3, 6, 9, or 15 h after i.n. antagonist.

**Drugs and Chemicals.** MEN16132 and icatibant were synthesized at the Chemistry Departments of Menarini Ricerche, Florence and Pomezia (Italy). Bradykinin (NeoMPS, Strasbourg, France), formamide (Merck, Darmstadt, Germany), captopril, dimethylsulfoxide, Evans blue, sodium pentobarbital, and urethane (Sigma-Aldrich, St. Louis, MO) were purchased.

**Evaluation of Data.** The bronchoconstriction was calculated as amplitude of the response over the basal value of insufflation pressure and the hypotension as the difference of the diastolic pressure before and after the BK challenge. For each different timepoint, the effects of the antagonists were expressed as percent inhibition of the responses to BK obtained before drugs administration. The data were compared by means of factorial two-way analysis of variance

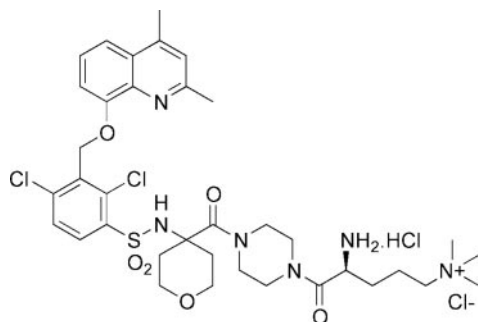


Fig. 1. Chemical structure of MEN16132.

followed by Fisher's least-significant difference test. The differences were considered statistically significant at a level of  $P < 0.05$ .

For the dose-response curve in bronchoconstriction experiments, to quantify the potency and duration of antagonists effect with a single value, the total inhibition during 210 min was expressed as the summation of the percent inhibition recorded at each time and then as percentage of maximal inhibitory effect achievable. Data were analyzed by sigmoidal nonlinear regression fit by Prism 4.0 (GraphPad Software Inc., San Diego, CA) to determine  $ID_{50-\Sigma}$  as dose inhibiting the 50% of agonist-produced maximal effect.

For the PPE experiments, the levels of Evans blue were expressed in nanogram per milligram of tissue. Statistical analysis was performed by one-way analysis of variance followed by Dunnett's multiple comparison test, and the differences were considered significant at a level of  $P < 0.05$ .

## Results

**Effect of Systemic and Local Administration of MEN16132 on BK-Induced Bronchoconstriction and Hypotension.** The i.v. administration of BK (0.01–100 nmol/kg,  $n = 16$ , data not shown) induced a dose-dependent bronchoconstriction and hypotension. The dose of 10 nmol/kg i.v., producing an increase in pulmonary insufflation pressure of  $10.3 \pm 1.4$  mm Hg ( $+110 \pm 14\%$  of the basal value) and  $21 \pm 3$  mm Hg decrease in diastolic blood pressure ( $54 \pm 5\%$  inhibition,  $n = 8$ ), was selected to test the inhibitory activity of  $B_2$  receptor antagonists. Both MEN16132 and icatibant exerted a dose-dependent inhibition of BK-induced bronchoconstriction and hypotension when administered by the i.v. route (Figs. 2 and 3).

The lowest effective dose of MEN16132 on bronchoconstriction was 3 nmol/kg i.v., reaching about 50% inhibition within 5 min with a recovery to 20% inhibition within 60 min. At 10 and 30 nmol/kg i.v., the block became complete for 150 and 210 min, respectively (Fig. 2A). The  $ID_{50-\Sigma}$  was  $4.6 \pm 1.9$  nmol/kg i.v. (Fig. 4A). MEN16132 (30 nmol/kg i.v.), 8 h after administration, still showed a strong ( $88 \pm 4\%$ ,  $n = 4$ ) inhibition of BK-induced bronchoconstriction. In control animals, no significant tachyphylaxis was observed, the BK response being about 75% of the basal value at the end of the experiment ( $n = 4$ , data not shown).

MEN16132 achieved a complete inhibition of BK-induced hypotension at 5 min from administration, at a dose of 30 nmol/kg i.v., but subsequently a time-dependent recovery of the BK response was observed; the lowest effective dose on BK-induced hypotension (producing 40% inhibition) was 10 nmol/kg i.v. (Fig. 2B).

Icatibant produced a transient but deep (about 80%) inhibition of BK-induced bronchoconstriction at 10 nmol/kg i.v. At 30 nmol/kg i.v., icatibant produced a complete inhibition of BK-induced bronchoconstriction, but this effect showed a strong tendency to reduce after 30 min from administration. A substantial recovery of the bronchoconstrictor response to BK was still evident at 300 nmol/kg i.v. (Fig. 3A). The calculated  $ID_{50-\Sigma}$  for icatibant was  $56.1 \pm 18.7$  nmol/kg i.v. (Fig. 4A). Icatibant exerted about 80, 40, and 20% inhibition of BK-induced hypotension at the doses of 300, 10, and 3 nmol/kg i.v., respectively. Also, this effect was subjected to time-dependent recovery of basal response, which was even more prominent than for bronchoconstriction (Fig. 3B). After application into the airways by i.t. route, MEN16132 and icatibant were able to inhibit the BK-induced bronchoconstriction in a dose-dependent manner (Figs. 2 and 3).

MEN16132 produced a complete inhibition of bronchoconstriction at the dose of 100 nmol/kg i.t. At the lower doses, 10 and 30 nmol/kg i.t., about 40 and 80% inhibition was observed, respectively, with an  $ID_{50-\Sigma} = 18 \pm 2$  nmol/kg (Fig. 4B). Independently from the dose, the inhibitory effect lasted for at least 210 min (Fig. 2C). MEN16132 (10–100 nmol/kg i.t.) produced a significant inhibitory effect ( $55 \pm 13\%$ ) on BK-induced hypotension only at the highest dose tested, and at 210 min, the recovery of control response was almost complete (Fig. 2D).

Icatibant, at 30, 100, and 300 nmol/kg i.t., inhibited by about 40, 80, and 100% the BK-induced bronchoconstriction, respectively; a slow recovery of BK response was observed starting at 30 min from antagonist administration (Fig. 3C). The  $ID_{50-\Sigma}$  was  $130 \pm 21$  nmol/kg (Fig. 4B). At 100 and 300 nmol/kg i.t., icatibant exerted similar inhibitory effect on BK-induced hypotension. A maximal inhibition of about 45% was recorded at 5 min from its administration, but the effect disappeared within 150 min. At the lowest dose, 30 nmol/kg i.t., only a weak (20–30%) and transient inhibitory effect was observed (Fig. 3D). Aerosol administration of MEN16132 or icatibant produced a dose-dependent inhibition of BK-induced bronchoconstriction without affecting the hypotension (Figs. 2 and 3).

The inhalation of aerosolized 0.1 mM solution of MEN16132 for 5 min reduced by about 80% the BK-induced bronchoconstriction for the whole experimental period, whereas 0.01 or 0.03 mM solution of MEN16132 produced inhibitory effects of about 20 and 40%, respectively (Fig. 2E). The corresponding value of  $ID_{50-\Sigma}$  was  $0.07 \pm 0.03$  mM (Fig. 4C). Aerosolized MEN16132 did not exert any significant inhibitory effect on the BK-induced hypotension at all concentrations (0.01–0.1 mM) tested (Fig. 2F).

After inhalation of aerosolized 0.3, 1, or 3 mM solution of icatibant for 5 min, a maximal inhibition on BK-induced bronchoconstriction of about 40, 60, and 80% was achieved in the first 30 min, respectively. An almost complete recovery of the basal response to BK was observed at the end of the experimental period (Fig. 3E). The  $ID_{50-\Sigma}$  was  $1.4 \pm 0.3$  mM aerosol for 5 min (Fig. 4C). The effect of aerosolized icatibant on BK-induced hypotension did not exceed 30% inhibition at the highest concentration tested (Fig. 3F).

**Effect of Intranasal MEN16132 on Topical BK-Induced Nasal Mucosa PPE.** The i.n. instillation of BK (50–200 nmol/nostril,  $n = 5$ ) induced Evans blue PPE in the nasal mucosa in a dose-dependent manner. The effect produced by 50 nmol/nostril ( $35 \pm 1$  ng/mg tissue) was about 30% of the maximal response observed at 100 nmol/nostril ( $62 \pm 5$  ng/mg tissue), the basal value being  $24 \pm 2$  ng/mg tissue in the control group. The dose of 100 nmol/nostril was selected to test the activity of the  $B_2$  receptor antagonists. At about 30 min from their administration, MEN16132 and icatibant, at 0.3 and 10 nmol/nostril, respectively, abolished the BK-induced PPE; at 0.03 and 3 nmol/nostril for MEN16132 and icatibant, a significant inhibition of BK response by about 55 and 70%, respectively, was still observed (Fig. 5).

MEN16132 (0.3 nmol/nostril), at 3 h from its i.n. administration, produced a still significant inhibitory effect (60%). At this time, the i.n. application of MEN16132 (1 and 3 nmol/nostril) exerted a complete inhibition of BK-induced nasal mucosa PPE; this inhibitory effect was maintained at about 80% after 6 h. At 10 nmol/nostril,

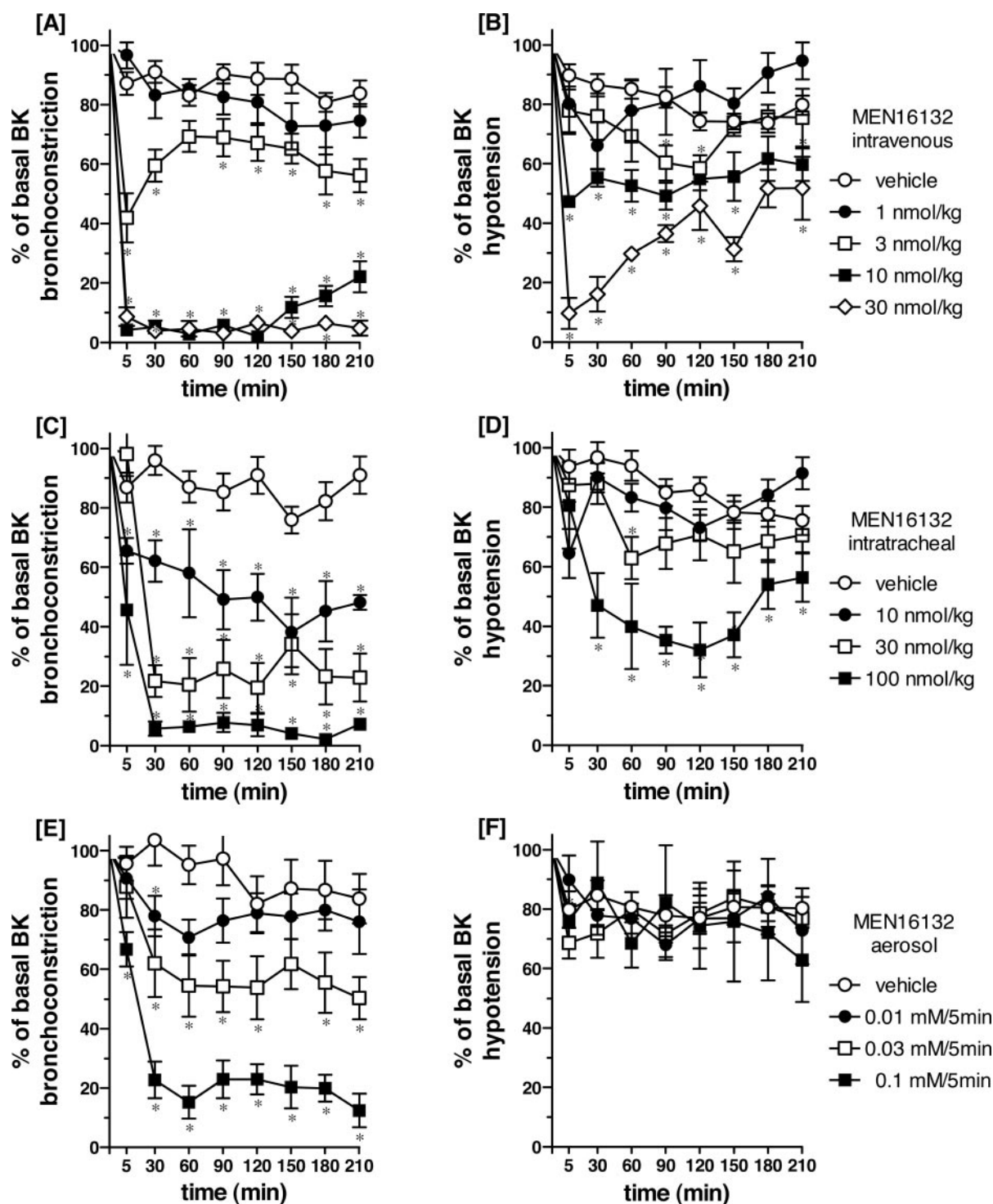


**Fig. 2.** Effect of i.v. [A and B; 1 (●), 3 (□), 10 (■), and 30 (◇) nmol/kg], i.t. [C and D; 10 (●), 30 (□), and 100 (■) nmol/kg], or aerosol [E and F; 0.01 (●), 0.03 (□), and 0.1 (■) mM/5 min] administration of MEN16132 on bronchoconstriction (left panels, A, C, and E) and hypotension (right panels, B, D, and F) induced by BK (10 nmol/kg i.v.). Results are expressed as percentage of basal response to BK recorded before treatment. Vehicles (○) are: i.v., saline, 100  $\mu$ l/kg; i.t., saline containing 30% dimethylsulfoxide, 100  $\mu$ l/kg; and aerosol, saline, for 5 min. Each value is the mean  $\pm$  S.E.M. of five to seven experiments. \*,  $P < 0.05$  significantly different from the respective value in the vehicle group.

MEN16132 blocked the PPE for 6 h; a small recovery was observed after 9 h; however, at 15 h, the inhibitory effect was still about 60% (Fig. 6).

**Effect of Intranasal MEN16132 on BK-Induced Hypotension.** The local inhibitory effect exerted by MEN16132 (1 nmol/nostril) on nasal mucosa PPE was not

accompanied by systemic effects since no inhibition of hypotension induced by BK (10 nmol/kg i.v.) was observed. At the highest dose tested (10 nmol/nostril), MEN16132 showed only a weak systemic effect, exerting about 25% inhibition of BK-induced hypotension after 75 min from antagonist administration (Fig. 7).

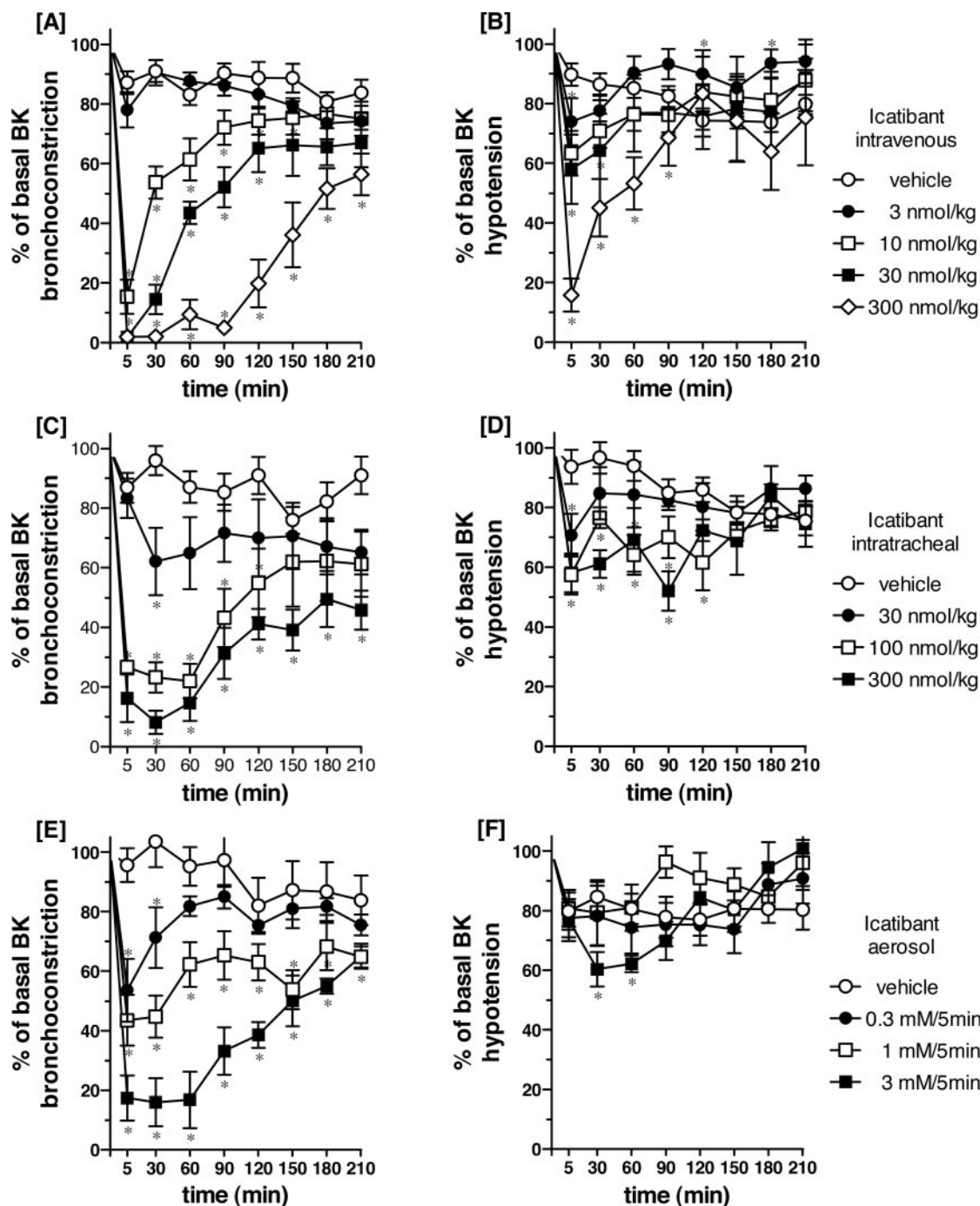


**Fig. 3.** Effect of i.v. [A and B; 3 (●), 10 (□), 30 (■), and 300 (◇) nmol/kg], i.t. [C and D; 30 (●), 100 (□), and 300 (■) nmol/kg], or aerosol [E and F; 0.3 (●), 1 (□), and 3 (■) mM/5 min] administration of icatibant on bronchoconstriction (left panels, A, C, and E) and hypotension (right panels, B, D, and F) induced by BK (10 nmol/kg i.v.). Results are expressed as percentage of basal response to BK recorded before treatment. Vehicles (○) are: i.v., saline, 100  $\mu$ l/kg; i.t., saline containing 30% dimethylsulfoxide, 100  $\mu$ l/kg; and aerosol, saline, for 5 min. Each value is the mean  $\pm$  S.E.M. of five to seven experiments. \*,  $P < 0.05$  significantly different from the respective value in the vehicle group.

## Discussion

MEN16132 is a new potent and selective nonpeptide kinin  $B_2$  receptor antagonist whose structure was designed for optimal pharmacological profile starting from known nonpeptide ligands (LF16-0335C; Pruneau et al., 1999a). The key structural features of MEN16132 are the conformationally constrained 4-amino-4-carboxytetrahydropyran and the basic charged *N*- $\delta$ -trimethyl-ornithine moiety (Fig. 1). The combination of the above elements led to high affinity and

potency at the human  $B_2$  receptor expressed in Chinese hamster ovary cells, with a  $pK_i$  value of  $10.5 \pm 0.05$ , and to a strong selectivity, its affinity at human  $B_1$ -transfected receptor showing  $pK_i$  value  $< 5$  (P. Cucchi, S. Meini, and C. Catalani, unpublished data). Furthermore, MEN16132 is characterized by a potent and long-lasting inhibitory activity on BK-induced responses in the guinea pig airways, with poor systemic effects after local administration.

After i.v. administration, MEN16132 resulted about 3-fold

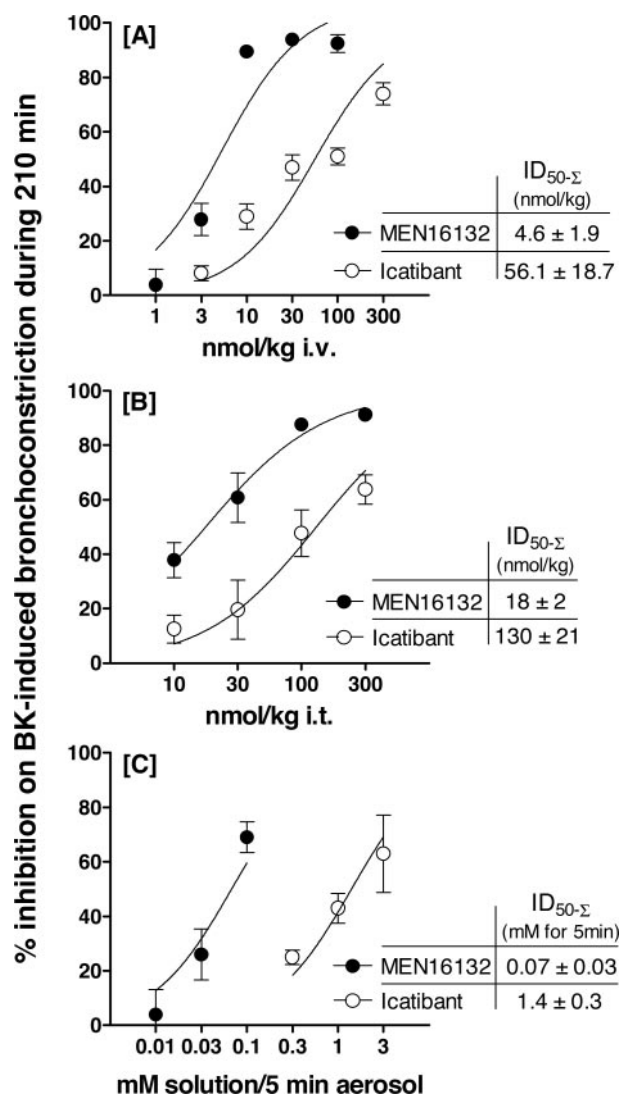


**Fig. 4.** Dose-response curves of MEN16132 and icatibant inhibitory effect on BK-induced bronchoconstriction after i.v. (A), i.t. (B), and aerosol (C) administration. Each value represents the total inhibitory effect during 210 min calculated as summation of the percent inhibition at various timepoints, then percentualized to the maximal inhibitory effect achievable. Data are the mean of five to seven experiments.

more potent than icatibant in inhibiting the BK-induced bronchoconstriction. These data are in agreement with the affinity data of the two antagonists ( $pK_B = 10.1$  and  $9.5$  for MEN16132 and icatibant, respectively) for guinea pig B<sub>2</sub> receptors (Meini et al., 2000; P. Cucchi, S. Meini, and C. Catalani, unpublished data). However, if we consider the total inhibitory effect on bronchoconstriction during 210 min, the corresponding ID<sub>50-Σ</sub> value is about 10-fold lower for MEN16132 than icatibant, a difference greater than expected on the basis of their relative in vitro affinity for guinea pig B<sub>2</sub> receptors. This finding cannot be explained by a different type of competition since both compounds exerted an insurmountable antagonism (Meini et al., 2000; P. Cucchi, S. Meini, and C. Catalani, unpublished data), but it could be attributed to a better metabolic/kinetic profile of the nonpeptide versus peptide structure of the two antagonists. We have verified that icatibant was degraded when incubated with guinea pig liver or lung homogenates (Tramontana et al., 2001), whereas preliminary results on metabolic stability of

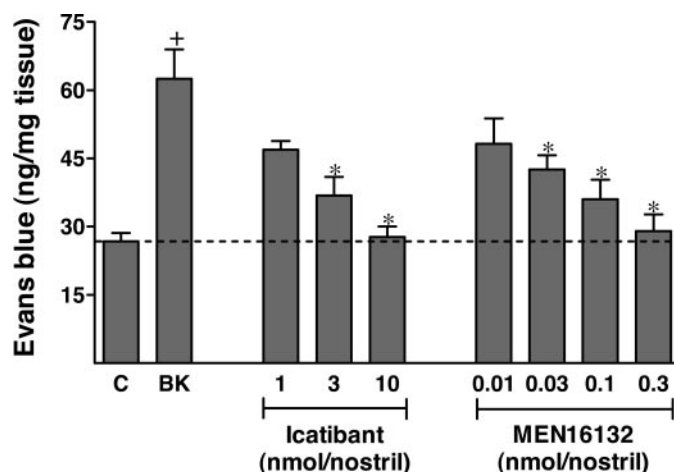


**Fig. 5.** Dose-dependent effect of MEN16132 (0.01–0.3 nmol/30  $\mu$ l nostril) or icatibant (1–10 nmol/30  $\mu$ l nostril) i.n. instillation on nasal mucosa PPE induced by topical application of BK (100 nmol/nostril). Antagonists or the vehicle [saline, 30  $\mu$ l/nostril, for control (C) and BK groups] were instilled in the nose of unanesthetized guinea pigs. Data are mean  $\pm$  S.E.M. of 5 to 10 experiments. \*,  $P < 0.05$ ; +,  $P < 0.05$  significantly different from BK and C groups, respectively.

MEN16132 indicate the absence of metabolites in plasma, urine, and bile and unchanged plasmatic levels up to 180 min from its administration (A. Lecci, personal communication). The interesting profile of MEN16132, in terms of long duration of action, is confirmed by the evidence that a dose of 30 nmol/kg is able to block the bronchoconstriction by BK up to 8 h from i.v. administration.

The nonpeptide B<sub>2</sub> receptor antagonists FR173657 (Griesbacher et al., 1997) and LF16-0687 (Pruneau et al., 1999b) showed a rapid decay of their inhibitory effect on BK-induced bronchoconstriction after i.v. or i.t. administration (Tramontana et al., 2001). Therefore, among the nonpeptide B<sub>2</sub> receptor antagonists, the long-lasting activity is a peculiar feature of MEN16132.

The BK-induced hypotension is less affected by the B<sub>2</sub> receptor antagonists than bronchoconstriction. A comparable inhibition of hypotensive response and bronchoconstriction was reached with a dose 3- and 30-fold higher for MEN16132 and icatibant, respectively. These data are encouraging in terms of the opportunity to achieve a selective block of BK-induced responses at bronchial level, reducing possible systemic adverse effects due to the blockade of the protective cardiovascular effects of BK. In fact, an important drawback for the clinical use of kinin B<sub>2</sub> receptor antagonists has been hypothesized because BK exerts beneficial effects on the cardiovascular system via B<sub>2</sub> receptors. Some evidence suggests that BK contributes to the antihypertensive and cardioprotective effects of angiotensin-converting enzyme inhibitors as a consequence of the block of BK major degrading enzyme in plasma (Gainer et al., 1998; Campbell et al., 2005). Moreover, BK exerts a protective effect against the reperfusion-induced injuries in ischemic heart (Ito et al., 2003; Driamov et al., 2004), and the kinin B<sub>2</sub> receptor antagonists can produce acute cardiovascular effects such as hypertension (Carini et al., 2002). The effects of BK at cardiovascular and respiratory level are produced by the B<sub>2</sub> receptor activation. Based on the insensitivity to B<sub>2</sub> antagonists, the existence of B<sub>3</sub> kinin receptor subtype in guinea pig trachea has been suggested (Farmer et al., 1989), although it has been not confirmed by

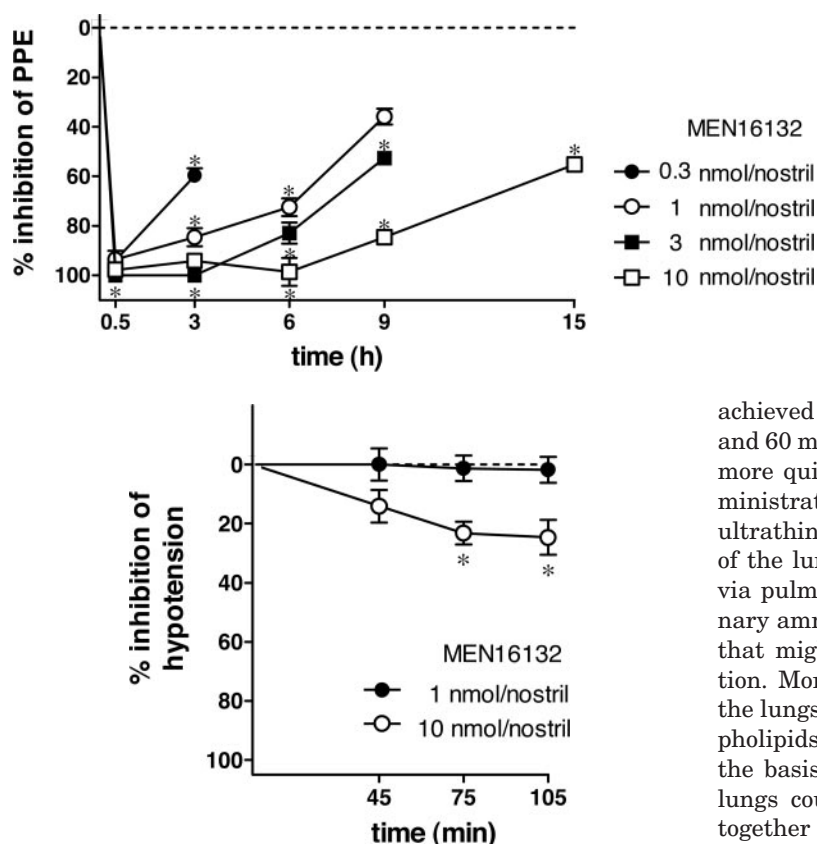


**Fig. 7.** Effect of MEN16132 i.n. administration on hypotension induced by i.v. administration of BK (10 nmol/kg) in guinea pigs. MEN16132 [1 (●) and 10 (○) nmol/nostril] or its vehicle (saline, 30  $\mu$ l/nostril) for the control group were instilled in the nose before anesthesia. BK was given at 45 min from i.n. administration of MEN16132 or the vehicle and repeated twice at 30-min interval. Data are mean  $\pm$  S.E.M. of six to eight experiments. \*,  $P < 0.05$  significantly different from control (vehicle) group.

molecular cloning of  $B_2$  receptor protein variants (for review, see Regoli et al., 1998). In our experimental conditions, the i.t. administration of MEN16132 produces a complete and long-lasting inhibition of bronchoconstriction; therefore, there is no evidence to hypothesize a role for the kinin  $B_3$  receptor in BK-induced bronchoconstriction in guinea pig. Since at present there is no evidence of the existence of different kinin  $B_2$  receptor subtypes in the same species, the only possibility to exert airway-selective effects consists of limiting the systemic absorption of kinin  $B_2$  receptor antagonist by local administration.

This study demonstrated that MEN16132 is effective after local administration both in the lower airways, by intratracheal or aerosol administration, and in the upper airways after intranasal application, with absent or reduced systemic effects. Aerosolized MEN16132 showed an  $ID_{50-\Sigma}$  value, for the total anti-bronchoconstrictor effect, 20-fold lower than icatibant, against a 10-fold difference observed for i.v. and i.t. administration. The increased antagonist potency of MEN16132 versus icatibant, after aerosol delivery, could be likely ascribed to a greater stability of MEN16132 to local metabolism.

Interestingly, icatibant reaches its maximal inhibition of bronchoconstriction after 5 min from intratracheal or aerosol administration, whereas the peak effect of MEN16132 is recorded at about 30 min; likewise, icatibant and MEN16132

**Fig. 6.** Time course of MEN16132 inhibitory effect on BK-induced nasal mucosa PPE. MEN16132 [0.3 (●), 1 (○), 3 (■), and 10 (□) nmol/nostril] or its vehicle (saline, 30  $\mu$ l/nostril) were instilled in the nose of unanesthetized guinea pigs. After 0.5, 3, 6, 9, or 15 h, the animals were anesthetized, and BK (100 nmol/nostril) was administered intranasally. The effect of MEN16132 was reported as percent inhibition of control BK-induced increase of Evans blue in nasal mucosa at each timepoint, normalized to the response obtained in vehicle-treated animals. Data are mean  $\pm$  S.E.M. of four to eight experiments. \*,  $P < 0.05$  significantly different from control group.

achieved the maximal inhibitory effect on hypotension after 5 and 60 min, respectively. Therefore, the peptide antagonist is more quickly absorbed at systemic level following local administration as compared with nonpeptide antagonist. The ultrathinness of the alveolar epithelium is a peculiar feature of the lung that can facilitate systemic delivery of peptides via pulmonary administration. MEN16132, being a quaternary ammonium compound, has an intrinsic positive charge that might limit the systemic absorption following inhalation. Moreover, cationic lipophilic drugs can accumulate in the lungs consequently to their alignment with anionic phospholipids in the surfactant (Upton and Doolette, 1999). On the basis of these considerations, we can suppose that the lungs could act as a reservoir for MEN16132; this effect, together with metabolic stability, could explain the long-lasting inhibition of BK-induced bronchoconstriction following topical administration.

In a clinical study on inhibition of responses to nasal provocation with BK, icatibant at 650 nmol/nostril has shown a short duration of action that could severely limit its utility in chronic rhinitis treatment (Proud et al., 1995). MEN16132, as observed in BK-induced bronchoconstriction after aerosol administration, exerts a long-lasting inhibition of BK-induced nasal mucosa PPE, being 30-fold more potent than icatibant and maintaining an almost complete inhibitory effect up to 9 h at 10 nmol/nostril dose. This high potency, associated with a long duration of action, could be a favorable characteristic for clinical studies, i.e., in rhinitis and in bronchoconstriction related to asthma.

To check the possibility of systemic absorption after local intranasal application of MEN16132, we have investigated the degree of inhibition on BK-induced hypotension. Considering the highest dose tested (10 nmol/nostril), which produces a strong and long-lasting inhibitory effect on BK-induced nasal mucosa PPE, and assuming that all the compound is absorbed at the same time, we could obtain an available dose in the blood of 20 nmol/animal. This dose is very close to the full active i.v. dose of 30 nmol/kg, corresponding to 10 to 15 nmol/animal. However, we have observed only weak inhibitory effects on hypotension induced by i.v. BK, indicating poor systemic absorption. Notably, at the dose of 1 nmol/nostril, there is a full antagonism of BK-induced PPE in nasal mucosa and no inhibition of BK-induced hypotension. Therefore, MEN16132 can be considered a selective  $B_2$  receptor antagonist suitable for local administration in the airways and devoid of significant systemic effects.

In conclusion, MEN16132 when topically applied into the

airways, exerts a more potent and long-lasting inhibitory action on BK-induced responses as compared with the other nonpeptide and peptide kinin B<sub>2</sub> receptor antagonists; furthermore, at fully effective doses, the systemic absorption of MEN16132 is negligible. Therefore, this molecule shows good pharmacokinetic and pharmacodynamic properties for its potential use in the treatment of chronic airways diseases involving the proinflammatory activity of BK.

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