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In vitro effects of HOE 140 in human bronchial and vascular tissue

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Abstract

Bradykinin is a potent inflammatory mediator which may be involved in various airway diseases. A selective and potent antagonist of the bradykinin B_2 receptor has recently been discovered (HOE 140: D-Arg-[Hyp^3,Thi^5,D-Tic^7,Oic^8]bradykinin). The purpose of this study was to evaluate the potency of this compound in isolated human tissue (bronchus, pulmonary artery endothelium, umbilical artery and vein smooth muscle). Bradykinin induced contractions of the isolated human bronchus and umbilical artery and vein (the umbilical vessels were pretreated with indomethacin and L-nitro-arginine to inhibit prostaglandin and nitric oxide synthesis). It provoked an endothelium-dependent relaxation in the human pulmonary artery. HOE 140 was a non-competitive antagonist in human bronchial tissue (pK_B: 8.19 ± 0.30) and a competitive one in vascular tissue (pA_2: 7.97 ± 0.12, 8.16 ± 0.16 and 8.00 ± 0.11 in human pulmonary artery, umbilical artery and vein respectively). The effect of HOE 140 was selective as it did not influence the umbilical vein contractile response to serotonin and histamine. HOE 140 up to 3×10^{-6} M was devoid of residual agonistic activity in the various human preparations studied. Furthermore, although the effects of HOE 140 were fully reversible, in isolated bronchial airways and umbilical veins, HOE 140 (10^{-6} M) still possessed activity 1 h after being washed out in both tissues. Our results indicate that HOE 140 is a potent and potentially long-acting antagonist of the human bradykinin B_2 receptor.

Keywords: HOE 140; Pulmonary artery; Bronchus; Umbilical artery; Umbilical vein; (Human)

1. Introduction

Bradykinin, a nonapeptide generated by the cleavage of kininogen, is a potent mediator of inflammation and may be involved in several airway diseases. It causes bronchoconstriction, pulmonary and bronchial vasodilatation, mucus secretion and microvascular leakage. Bradykinin can activate two different types of receptor, designated as B_1 and B_2, although most of the biological actions seems to be mediated by B_2 receptors (for review: Bhoola et al., 1992). Therefore bradykinin B_2 receptor antagonists could become a new class of drug with therapeutic potential in airway inflammatory diseases.

Bradykinin receptors are widely distributed in human respiratory tissues: nasal mucosa (Baraniuk et al., 1990), bronchial and pulmonary blood vessels, and airways (Mak and Barnes, 1991). In human large airways, the smooth muscle cells are sparsely labelled but a greater labelling could be observed in smaller airways. Molimard et al. (1994a) have also shown that bradykinin contracts small human bronchi (inner diameter ≤ 1 mm), but not the large ones, through bradykinin B_2 receptor stimulation.

Recently HOE 140, a new potent and selective bradykinin B_2 receptor antagonist devoid of residual agonistic activity on a variety of isolated smooth muscle preparations, has been described (Hock et al., 1991). However, to our knowledge its potency has not been evaluated in isolated human lung tissue. This could be of importance considering the species specificity and the subtypes of the bradykinin B_2 receptors (Seguin et

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al., 1992; Regoli et al., 1993; Seguin and Widdoson, 1993; Félétou et al., 1994).

The purpose of this work was to characterize HOE 140 against bradykinin receptors in two types of human lung tissues: bradykinin-induced bronchoconstriction and bradykinin-induced endothelium-dependent relaxation (pulmonary artery). In addition, in order to determine the competitive nature (or not) of the antagonism produced by HOE 140, we selected human tissue according to the following criteria. The biological response studied should be a direct response to the bradykinin-receptor interaction, and the maximum response to the agonist should be obtained (Regoli et al., 1993). Two tissues able to fulfil the required criteria were human umbilical artery and vein (Altura et al., 1972).

2. Materials and methods

2.1. Tissue preparations

Human tissue, lungs obtained from patients undergoing surgery for lung cancer, excised away from the malignancy, and umbilical cords collected just after delivery, were transported to the laboratory in an ice-cold Krebs solution previously aerated with a mixture of 95% O_2-5% CO_2. Bronchi (inner diameter of 0.5–1 mm), intra-lobar pulmonary arteries, umbilical arteries and veins were dissected free of connective tissue, cut into rings and suspended in organ chambers filled with Krebs solution (37°C gassed with 95% O_2-5% CO_2; pH 7.40) of the following composition (mM): NaCl 118.3, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25 and glucose 11.0. The isolated rings were connected to a force transducer and isometric changes in tension were recorded. In some pulmonary arterial rings and in the umbilical arterial and venous rings, the endothelium was carefully removed by inserting a pair of watchmaker's forceps into the lumen and rolling the rings back and forth on saline wetted paper. Bronchial rings were submitted to an initial load of 1.5 g, pulmonary arterial and umbilical venous rings to 2–4 g, and umbilical arterial rings to 5–7 g.

2.2. Protocols

After an equilibration period of 60 min, human bronchial rings were contracted with acetylcholine 10^{-3} M and relaxed to maximal relaxation with theophylline 3×10^{-3} M. They were then allowed to equilibrate for a further 60 min period during which they were washed with Krebs solution every 15 min. Blood vessel rings were contracted several times with KCl (40 mM) and, after an equilibration period, a reference contractile response to KCl (60 mM) was obtained. Pulmonary arterial rings were precontracted with U46619 (the stable analogue of thromboxane A_2). Umbilical arterial and venous rings were pretreated with indomethacin (5×10^{-6} M) and L-nitro-arginine (10^{-4} M). Experiments were performed in parallel and a single cumulative concentration-response curve for bradykinin was made for each tissue. In pulmonary arteries, papaverine (10^{-4} M) was added at the end of the experiment to obtain maximal relaxation of the tissue. The selective bradykinin B_2 receptor antagonist HOE 140 was used as pretreatment before the application of bradykinin. Bronchial contractions were expressed as a percentage of the maximal effect induced by acetylcholine (10^{-3} M), arterial relaxations as a percentage of the maximum effect of papaverine (10^{-4} M) and umbilical vessel contractions as a percentage of the reference contraction to KCl (60 mM).

In bronchi and umbilical venous rings, in order to evaluate the time of HOE 140 (10^{-6} M) retention, the bradykinin B_2 receptor antagonist was added for 15 min and 40 min respectively and the antagonist was then washed away with Krebs solution every 15 min for 30, 60 or 120 min. Concentration-response curves for bradykinin were made at these different times (Naline et al., 1994)

2.3. Statistical analysis

Data are expressed as means ± S.E.M.; n represents the number of patients from whom tissue was taken. Statistical analysis was performed by analysis of variance and Student's t-test for paired or unpaired data, as appropriate. Probability values of $P < 0.05$ were considered significant. The activity of bradykinin on the various human tissues is indicated by providing the pD_2 (pD_2: $-\log$ of the bradykinin concentration that produces 50% of the maximum effect). If the slope of the regression line log (CR–1) vs. molar concentration of antagonist was not significantly different from unity, a pA_2 value was determined (Arunlakshana and Schild, 1959) with the slope constrained to unity. If the slope was significantly different from unity, the equilibrium dissociation constant (K_B) for non-competitive and/or pseudo-irreversible antagonists was evaluated (Kenakin, 1987). In practice, a double-reciprocal plot of equieffective concentrations of agonist (A) in the absence (1/A) and in the presence (1/A') of the antagonist HOE 140 (B) was constructed, and K_B was derived from the equation: $K_B = [B]/\text{slope}-1$ (Kenakin, 1987).

2.4. Substances

The substances used were: acetylcholine (PCH, Paris, France), bradykinin, indomethacin, L-nitro-arginine, papaverine, serotonin, histamine (Sigma, La

Verpillere, France), U46619 (9,11-dideoxy-11α,9α-epoxymethano prostaglandin $F_{2\alpha}$, Cayman Chemical, Denver, USA), HOE 140 (D-Arg-[Hyp^3,Thi^5,D-Tic^7,Oic^8]bradykinin) (IdRS, Suresnes, France), theophylline sodium anisate (Bruneau, Paris, France). All drugs were dissolved daily in distilled water and then diluted in Krebs solution with the exception of U46619, which was first dissolved in ethanol, and indomethacin, which was prepared in an equimolar concentration of $NaCO_3$.

3. Results

3.1. Isolated bronchus

In isolated bronchi, the addition of bradykinin (10^{-9} to 10^{-4} M) induced a concentration-dependent contraction which represented 60% of the maximal response to acetylcholine (pD_2: 7.25 ± 0.14; n = 15). Incubation with HOE 140 (10^{-9} to 10^{-6} M) for 15 min produced a concentration-dependent shift to the right of the concentration-response curve for bradykinin without inducing any significant changes in the maximal response (Fig. 1). However, the slope of the Schild plot was significantly different from unity (0.54 ± 0.11), suggesting a non-competitive interaction. The calculated pK_B was 8.19 ± 0.30 (n = 14). Incubation with HOE 140 (10^{-9} to 10^{-6} M) for 1 h prior to the addition of bradykinin gave identical results (slope: 0.38 ± 0.49; pK_B: 8.18 ± 0.38; n = 4).

In the small bronchi, a preincubation of 15 min with HOE 140 (10^{-6} M) followed by the washout of the antagonist produced a significant inhibition of the contractions induced by the subsequent administration of bradykinin, up to 1 h after its washout (Fig. 2).

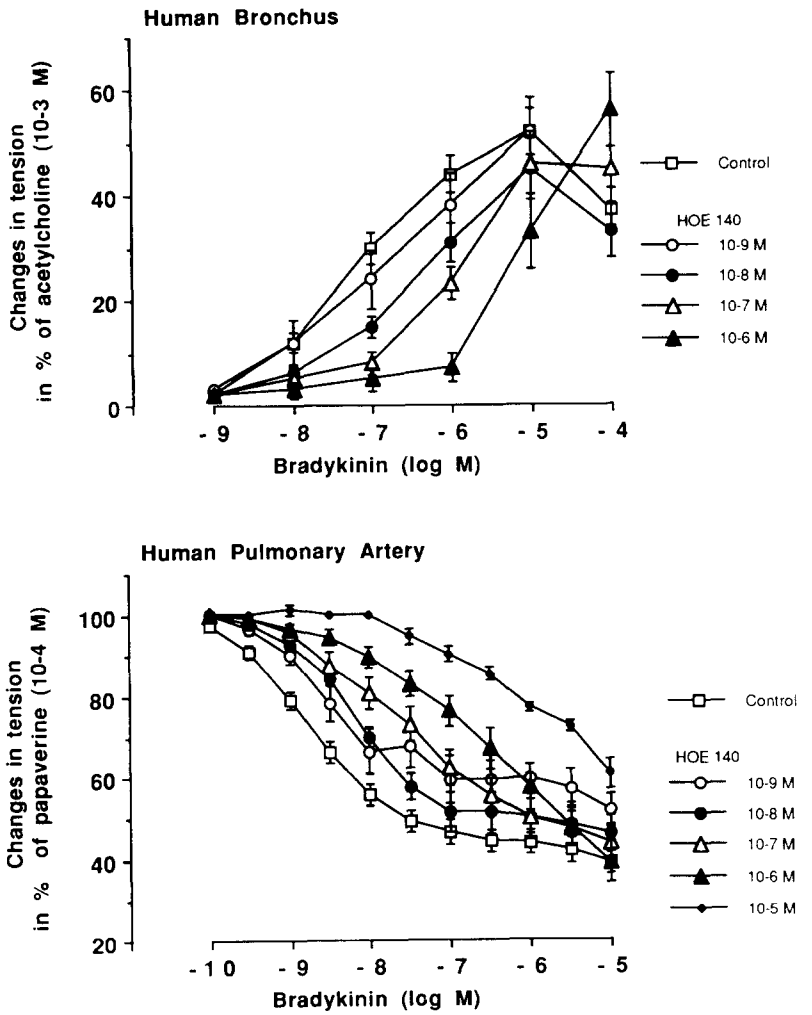

Fig. 1. Cumulative concentration-response curves for bradykinin in the absence or presence of HOE 140 in isolated human bronchi (upper panel, n = 3–15) and in isolated human pulmonary arteries contracted with U 46619 (lower panel, n = 3–12). Data are shown as means ± S.E.M. Control: open square; HOE 140 (10^{-9} M): open circle; HOE 140 (10^{-8} M): filled circle; HOE 140 (10^{-7} M): open triangle; HOE 140 (10^{-6} M): filled triangle; HOE 140 (10^{-5} M): filled diamond.

3.2. Isolated pulmonary artery

In U46619 (10^{-9} to 10^{-8} M)-precontracted pulmonary arterial rings, the addition of bradykinin (10^{-10} to 10^{-5} M) evoked an endothelium-dependent relaxation (pD_2: 8.59 ± 0.08; $n = 12$). Incubation with HOE 140 (10^{-9} to 10^{-5} M) for 40 min produced a concentration-dependent shift to the right of the concentration-response curves for bradykinin without inducing any significant changes in the maximal response (Fig. 1). The inhibitory effect was significant for concentrations of HOE 140 equal to or higher than 10^{-8} M. The slope of the Schild plot was only marginally different from unity (0.80 ± 0.12); the calculated pA_2 was 7.97 ± 0.12 (slope constrained to unity).

3.3. Isolated umbilical vessels

Isolated umbilical arteries and veins exhibited spontaneous activity (slow waves). Pretreatment with indomethacin (5×10^{-6} M) and L-nitro-arginine (10^{-4} M) markedly decreased this spontaneous activity. In the treated vessels, bradykinin (10^{-10} to 10^{-5} M) produced a concentration-dependent contraction which represented 150% of the reference contraction to KCl (pD_2: 8.00 ± 0.23; $n = 10$ and 8.75 ± 0.17; $n = 12$ for umbilical arteries and veins respectively). In both vessels, pretreament with HOE 140 for 40 min produced rightward shifts of the concentration-response curves for bradykinin. The maximum responses to the kinin were not affected by HOE 140 (Fig. 3). In both cases

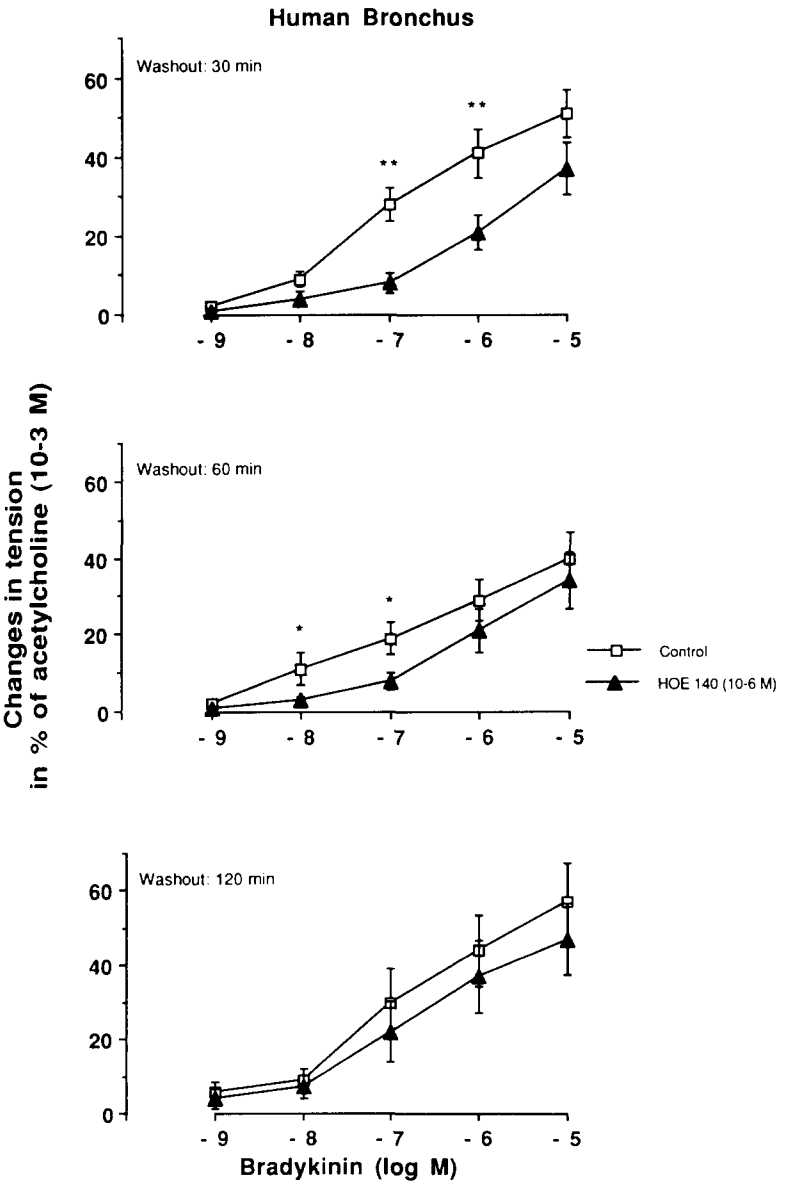

Fig. 2. Isolated human bronchi. Cumulative concentration-response curves for bradykinin in the absence or after a 15-min incubation with HOE 140 followed by a washout period of 30 min (upper panel), 60 min (middle panel) or 120 min (lower panel). Data are shown as means ± S.E.M. ($n = 6$–8). Control: open square; pretreatment with HOE 140 (10^{-6} M): filled triangle.

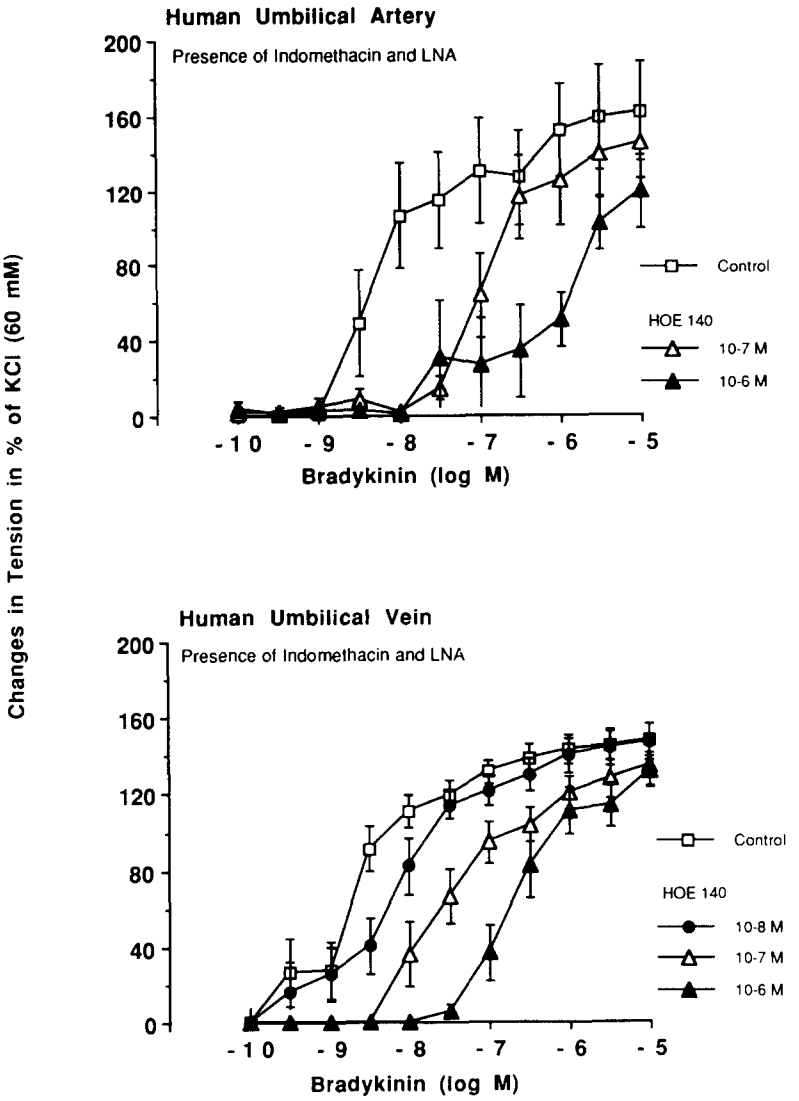

Fig. 3. Cumulative concentration-response curves for bradykinin in the absence or presence of HOE 140 in isolated human umbilical artery (upper panel) and in isolated human umbilical vein (lower panel). Presence of indomethacin (5×10^{-6} M) and L-nitro-arginine (10^{-4} M). Data are shown as means ± S.E.M. ($n = 10$). Control: open square; HOE 140 (10^{-8} M): filled circle; HOE 140 (10^{-7} M): open triangle; HOE 140 (10^{-6} M): filled triangle.

the slopes of the Schild plots were not significantly different from unity (0.82 ± 0.33, $n = 8$ and 1.06 ± 0.14, $n = 10$ in arteries and veins respectively). The calculated pA_2 values were 8.16 ± 0.16 and 8.00 ± 0.11, in arteries and veins respectively.

Serotonin (10^{-9} to 10^{-4} M) and histamine (10^{-9} to 10^{-4} M) produced a concentration-dependent contraction of the isolated umbilical vein. HOE 140 (10^{-6} M) did not influence the contractile responses to both agonists (serotonin: ED_{50}: 8.12 ± 0.24 and 8.16 ± 0.13 respectively, in control and in the presence of HOE 140, $n = 5$; histamine: ED_{50}: 6.91 ± 0.11 and 6.86 ± 0.11 respectively, in control and in the presence of HOE 140, $n = 5$).

In the umbilical vein, a preincubation of 40 min with HOE 140 (10^{-6} M) followed by the washout of the antagonist produced a significant inhibition of the contractions induced by the subsequent administration of bradykinin, up to 1 h after its washout (Fig. 4).

3.4. Residual agonistic activity

HOE 140, up to 3×10^{-6} M, did not show any agonistic activity in any of the tissues studied.

4. Discussion

Our results confirm that bradykinin induces a significant and potent contraction of human small bronchi and an endothelium-dependent relaxation in pulmonary arteries, both through activation of the

bradykinin B_2 receptor subtype. The smooth muscle contractile response of the umbilical vessels can also be attributed to bradykinin B_2 receptor stimulation as HOE 140, a selective bradykinin B_2 receptor antagonist, inhibited the various responses (Hock et al., 1991).

In human bronchial smooth muscle, the contractile response to bradykinin is solely due to bradykinin B_2 receptor activation, as bradykinin B_1 receptor agonist and antagonist are not able to produce a contraction or to antagonize the effect of bradykinin (Molimard et al., 1994a). In this tissue, the effects of bradykinin were antagonized significantly by HOE 140 in a non-competitive manner. However, in human pulmonary arteries, and in umbilical arteries and veins, the inhibition of the response to bradykinin appeared to be competitive. In order to characterize the competitive nature of the HOE 140 antagonism, we selected a tissue according to the following criteria. The biological response studied should be a direct response to the bradykinin-receptor interaction, and the maximum response to the agonist should be obtained (Regoli et al., 1993). This is not the case in bronchus, as the contraction involves the release of arachidonic acid derivatives and thromboxane A_2 (Molimard et al., 1994a,b) or in pulmonary arteries, as the endothelium is the obligatory relay. Two tissues which fulfilled the required criteria were human umbilical arteries and veins (Altura et al., 1972). In umbilical veins and arteries, the contraction to bradykinin could reasonably be attributed to a direct stimulation of vascular smooth muscle receptors as prostaglandin syn-

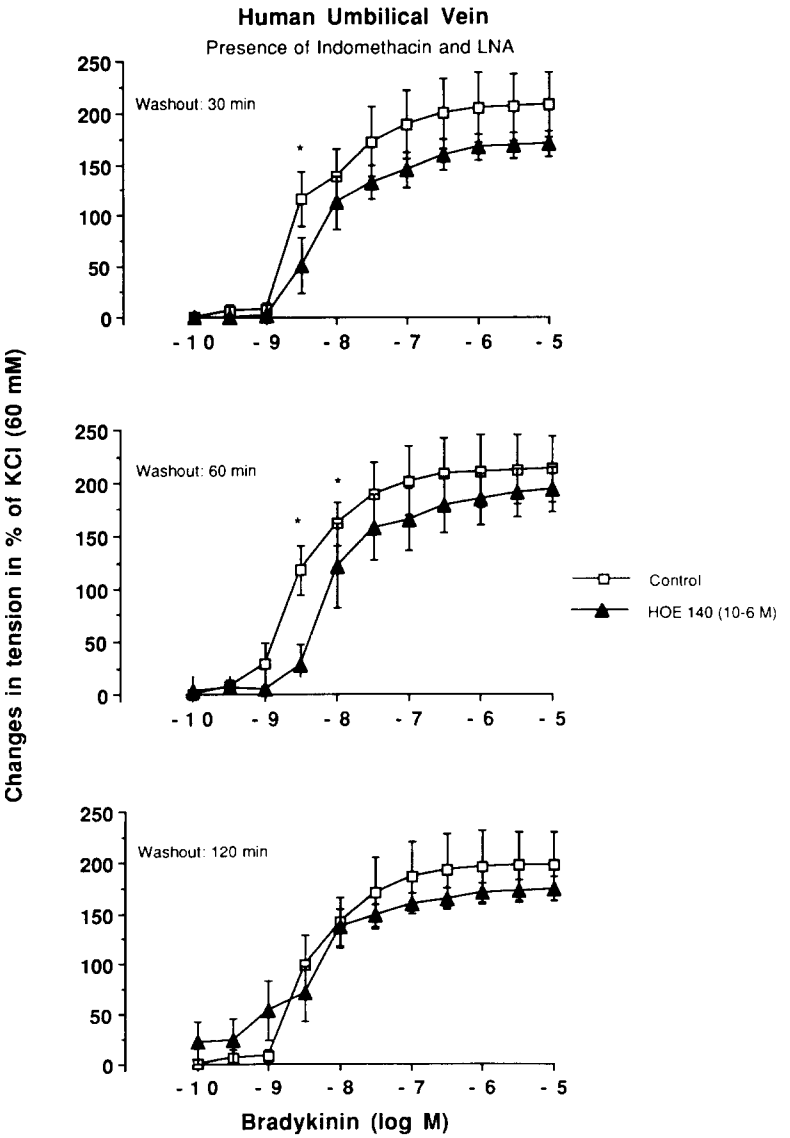

Fig. 4. Isolated human umbilical vein in the presence of indomethacin (5×10^{-6} M) and L-nitro-arginine (10^{-4} M). Cumulative concentration-response curves for bradykinin in the absence or after a 40-min incubation with HOE 140 followed by a washout period of 30 min (upper panel), 60 min (middle panel) or 120 min (lower panel). Data are shown as means ± S.E.M. ($n = 6$). Control: open square; pretreatment with HOE 140 (10^{-6} M): filled triangle.

thesis was inhibited by indomethacin. In these conditions the maximal responses to bradykinin were not depressed by HOE 140, the rightward shifts were parallel and the slopes of the Schild plots were not significantly different from unity. Furthermore the effect of HOE 140, at least in the umbilical vein, was fully reversed by washing out and the antagonist did not show any agonistic activity in the various tissues studied. In the umbilical vein, the effects of HOE 140 were specific, confirming previous reports obtained in functional studies (Lembeck et al., 1991; Rhaleb et al., 1992) or in binding (unpublished personal observations).

The different nature of the antagonism produced by HOE 140 in the bronchial and vascular tissue (non-competitive and competitive respectively) does not necessarily imply that the bradykinin B_2 receptors studied belong to two different subtypes. Indeed the contraction of the human bronchus is due to the indirect action of bradykinin on the effector cell (i.e. smooth muscle) as it involves the release of prostaglandins and thromboxane A_2 (Molimard et al., 1994a,b). This, or a difference in the transduction process (G-protein coupling...), may explain the difference in the slopes of the Schild plots (Regoli et al., 1993). Furthermore, apparently competitive and non-competitive interactions of HOE 140 on the same receptor have been described according to the parameters measured (short circuit current and intracellular Ca^{2+} in HCA-7 colony 29 cells; Cuthbert et al., 1992).

The affinity of HOE 140 for the bradykinin receptor in the various tissues studied, evaluated either with the pA_2 or the pK_b, was very similar (7.97, 8.16, 8.00, 8.19). These values are in agreement with the pA_2 value obtained for human ileum (8.36) but somewhat lower than the value for the bladder (8.81) (Rhaleb et al., 1992). Interestingly these values are 15–150 times lower than the binding affinity constants obtained for cell lines expressing the human recombinant bradykinin B_2 receptor (65 and 420 pM; Hess et al., 1992 and Eggerickx et al., 1992). The presence in the physiological solutions of monovalent and divalent cations, essential for functional studies, may explain the discrepancies between binding and functional data obtained with isolated organs, as these cations reduce bradykinin binding (Manning et al., 1986; Trifilieff et al., 1992). These pA_2 values were also lower than the pA_2 value obtained in a typical assay for the bradykinin B_2 receptor: the rabbit jugular vein (9.2 and 9.0; Rhaleb et al., 1992 and Félétou et al., 1994). Interestingly, in human blood vessels the bradykinin potency was similar to that reported for rabbit jugular vein ($pD_2 = 8.59$, 8.00, 8.75 respectively in human pulmonary and umbilical artery and umbilical vein vs. 8.48–7.76 in rabbit jugular vein; Regoli et al., 1990; Félétou et al., 1994). Thus, a different diffusion of the peptides to the receptor compartment is unlikely to explain the differences in the potency of HOE 140 observed between human and rabbit tissue. Furthermore, in rabbit jugular vein, the non-competitive nature of the antagonism induced by HOE 140 was evidenced by a marked depression of the maximum response; all these differences might be due to the species specificity of the bradykinin B_2 receptor.

In isolated human bronchi and umbilical vein, HOE 140 had a long duration of action, as it was still active 1 h after having been washed out, though the effects were nevertheless fully reversible. The duration of action of HOE 140 is also species-dependent; in various isolated rabbit preparations, the effect of HOE 140 is virtually not abolished by washing out (Lembeck et al., 1991 and unpublished personal observations). However, in the same isolated human bronchus preparation, the duration of action of HOE 140 is much shorter than that of the long-acting β_2-adrenoceptor agonist salmeterol (Naline et al., 1994).

In conclusion and in agreement with previous works (Mak and Barnes; 1991; Molimard et al., 1994a), the present study shows that bradykinin receptors present on human lung airways, pulmonary arterial endothelial cells and umbilical arterial and venous smooth muscle cells are of the B_2 subtype and that HOE 140 is a potent and potentially long-acting antagonist of the human bradykinin B_2 receptor. As HOE 140 is currently under clinical trial for airway diseases, this work may help to analyse forthcoming results.

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