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Involvement of bradykinin B_1 and B_2 receptors in relaxation of mouse isolated trachea

Liang Li, Kirsi Vaali, Ilari Paakkari & [1]Heikki Vapaatalo

Institute of Biomedicine, Department of Pharmacology and Toxicology, P.O. Box 8, FIN-00014 University of Helsinki, Finland

1 The aim of the present study was to investigate the effects of bradykinin and [des-Arg^9]-bradykinin and their relaxant mechanisms in the mouse isolated trachea.

2 In the resting tracheal preparations with intact epithelium, bradykinin and [des-Arg^9]-bradykinin (each drug, 0.01–10 μM) induced neither contraction nor relaxation. In contrast, bradykinin (0.01–10 μM) induced concentration-dependent relaxation when the tracheal preparations were precontracted with methacholine (1 μM). The relaxation induced by bradykinin was inhibited by the B_2 receptor antagonist, D-Arg^0-[Hyp^3,Thi^5,D-Tic^7,Oic^8]-bradykinin (Hoe 140, 0.01–1 μM) in a concentration-dependent manner whereas the B_1 receptor antagonist, [des-Arg^9,Leu^8]-bradykinin (0.01–1 μM), had no inhibitory effect on bradykinin-induced relaxation. [des-Arg^9]-bradykinin (0.01–10 μM) also caused concentration-dependent relaxation after precontraction with methacholine. The relaxation induced by [des-Arg^9]-bradykinin was concentration-dependently inhibited by the B_1 receptor antagonist, [des-Arg^9,Leu^8]-bradykinin (0.01–1 μM), whereas the B_2 receptor antagonist, Hoe 140 (0.01–1 μM) was without effect.

3 In the presence of the cyclo-oxygenase inhibitor, indomethacin (0.01–1 μM), the relaxations induced by bradykinin and [des-Arg^9]-bradykinin were inhibited concentration-dependently.

4 Two nitric oxide (NO) biosynthesis inhibitors N^G-nitro-L-arginine methyl ester (L-NAME, 100 μM) and N^G-nitro-L-arginine (L-NOARG, 100 μM) had no inhibitory effects on the relaxations induced by bradykinin and [des-Arg^9]-bradykinin. Neither did the selective inhibitor of the soluble guanylate cyclase, 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ, 10 μM) inhibit the relaxations induced by bradykinin and [des-Arg^9]-bradykinin.

5 Prostaglandin E_2 (PGE_2, 0.01–33 μM) caused concentration-dependent relaxation of the tracheal preparations precontracted with methacholine. Indomethacin (1 μM) and ODQ (10 μM) exerted no inhibitory effects on the relaxation induced by PGE_2.

6 The NO-donor, sodium nitroprusside (SNP; 0.01–100 μM) also caused concentration-dependent relaxation of the tracheal preparations precontracted with methacholine. ODQ (0.1–1 μM) concentration-dependently inhibited the relaxation induced by SNP.

7 These data demonstrate that bradykinin and [des-Arg^9]-bradykinin relax the mouse trachea precontracted with methacholine by the activation of bradykinin B_2-receptors and B_1-receptors, respectively. The stimulation of bradykinin receptors induces activation of the cyclo-oxygenase pathway, leading to the production of relaxing prostaglandins. The NO pathway is not involved in the bradykinin-induced relaxation. The relaxation caused by NO-donors in the mouse trachea is likely to be mediated via activation of soluble guanylate cyclase.

Keywords: Bradykinin; [des-Arg^9]-bradykinin; mouse trachea; relaxation; bradykinin receptor; cyclo-oxygenase; nitric oxide synthase inhibitors; soluble guanylate cyclase

Introduction

Kinins are proinflammatory peptides that dilate vessels, increase vascular permeability, contract smooth muscle, and provoke pain. Bradykinin (Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg), a potent mediator of inflammation, has been implicated in asthma (for a review see Farmer, 1991; Barnes, 1992; Trifilieff *et al*., 1993). In asthmatic patients, bradykinin is one of the most potent bronchoconstrictors known (Fuller *et al*., 1987; Dixon & Barnes, 1989). Asthmatic patients also have an elevated kinin concentration in the bronchoalveolar lavage fluid following antigen challenge (Christiansen *et al*., 1987; Baumgarten *et al*., 1992).

The major airway receptors for bradykinin causing bronchoconstriction in asthmatic subjects belong to the B_2 type (Polosa & Holgate, 1990). In the guinea-pig trachea, bradykinin may induce contraction or relaxation by the stimulation of bradykinin B_2 receptors, depending on the tone of the trachea (Rhaleb *et al*., 1992; Trifilieff *et al*., 1992; Da Silva *et al*., 1995). The bradykinin-induced contraction or relaxation is, at least in part, mediated by generation of a prostanoid(s) released by the epithelium (Bramley *et al*., 1990; Schlemper & Calixto, 1994; Da Silva *et al*., 1995). Nitric oxide (NO) has recently been demonstrated to be formed by many tissues (for a review see Moncada & Higgs, 1995). NO may be formed and released by a variety of cells in the lung, including epithelial and endothelial cells, inflammatory cells and smooth muscle cells, and it exerts various functions in the airways (Gaston *et al*., 1994). The bradykinin-induced relaxation in several tissues, such as pig coronary artery (Cowan & Cohen, 1991), rat kidney (Fulton *et al*., 1992) and the guinea-pig trachea (Schlemper & Calixto, 1994; Figini *et al*., 1996) is also mediated by NO. Thus, both the cyclo-oxygenase pathway and NO pathway are involved in the bradykinin-induced relaxation. Bradykinin has different effects on airway smooth muscle in different species. It has rather weak or no effect on cat, dog,

[1] Author for correspondence.

rabbit and rat airways, but causes contraction in ferret isolated trachea (Collier, 1963; Farmer, 1991; Farmer *et al*., 1994). In addition to documenting species variability, the study of pharmacology of murine tissue is increasingly interesting and important because mice are often used in transgenic and knock out studies. The targeted genetic disruption of a B_2 receptor gene in mice eliminates bradykinin action in smooth muscle and neurones (Borkowski *et al*., 1995). This and other transgenic strains may be useful in the future to dissect out the signalling pathways of bradykinin.

We have shown that bradykinin induces relaxation in methacholine-precontracted mouse trachea and its relaxing effect is not mediated via Ca^{2+}-activated K^+ channels (Li *et al*., 1997). However, which subtype of bradykinin receptor is involved in the relaxation of the mouse airways has not been investigated. The present study was, therefore, designed to examine the actions of two different bradykinin receptor agonists, bradykinin and [des-Arg^9]-bradykinin on mouse isolated trachea. Their mechanisms were clarified by using a B_2-receptor antagonist, Hoe 140, and a B_1-receptor antagonist, [des-Arg^9,Leu^8]-bradykinin. In order to see whether cyclo-oxygenase and NO pathways are involved, we also examined the effects of inhibitors of cyclo-oxygenase, NO synthase and soluble guanylate cyclase on the bradykinin- and [des-Arg^9]-bradykinin-mediated responses.

Methods

Tissue preparation

BALB/c mice (8–12 weeks) of either sex were used in the studies. Following intraperitoneal injection of 0.25 ml of pentobarbitone sodium (60 mg kg^{-1}) and thoracotomy, one piece of trachea from each animal was isolated as described previously (Garssen *et al*., 1990). The trachea (3 mm long ring) was then mounted in an organ bath filled with 10 ml of Krebs solution of the following composition (mmol l^{-1}): NaCl 119, $NaHCO_3$ 25, $CaCl_2{\cdot}H_2O$ 1.6, KCl 4.7, KH_2PO_4 1.2, $MgSO_4{\cdot}7H_2O$ 1.2 and glucose 11.1. The solution was maintained at 37°C and bubbled with 96% O_2 and 4% CO_2 gas mixture. The trachea was equilibrated under an optimal resting tension of 400 mg for at least 45 min with replacement of the bath fluid every 15 min. The tone of the tracheal smooth muscle was measured with the computerized complete bath systems by IT1-25 transducers (EMKA Technologies, Paris, France). The experimental procedure was approved by the Animal Experimentation Committee of Institute of Biomedicine, University of Helsinki, Finland.

Experimental procedures

Following the equilibration period, the tracheal preparations were precontracted with methacholine (1 μM). After the contraction evoked had reached a plateau, single relaxation-responses to bradykinin or [des-Arg^9]-bradykinin were obtained. After the tissues had been washed with Krebs solution for 30 min and precontracted again with methacholine, a single relaxation-response to a higher concentration of bradykinin or [des-Arg^9]-bradykinin was obtained. Thus the concentration-response curves to bradykinin or [des-Arg^9]-bradykinin (each drug, 0.01–1 μM) were obtained in a non-cumulative manner in order to avoid the development of tachyphylaxis to the cumulative addition of bradykinin (Figure 1). To test the effect of a B_2 receptor antagonist, non-cumulative concentration-response curves to bradykinin or

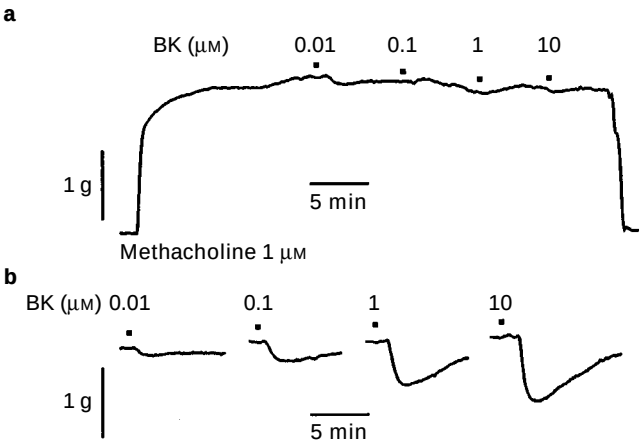

Figure 1 Representative tracing (a) showing the development of tachyphylaxis to bradykinin (BK, 0.01–10 μM) and (b) the relaxation induced by BK (0.01–10 μM) in non-cumulative manner.

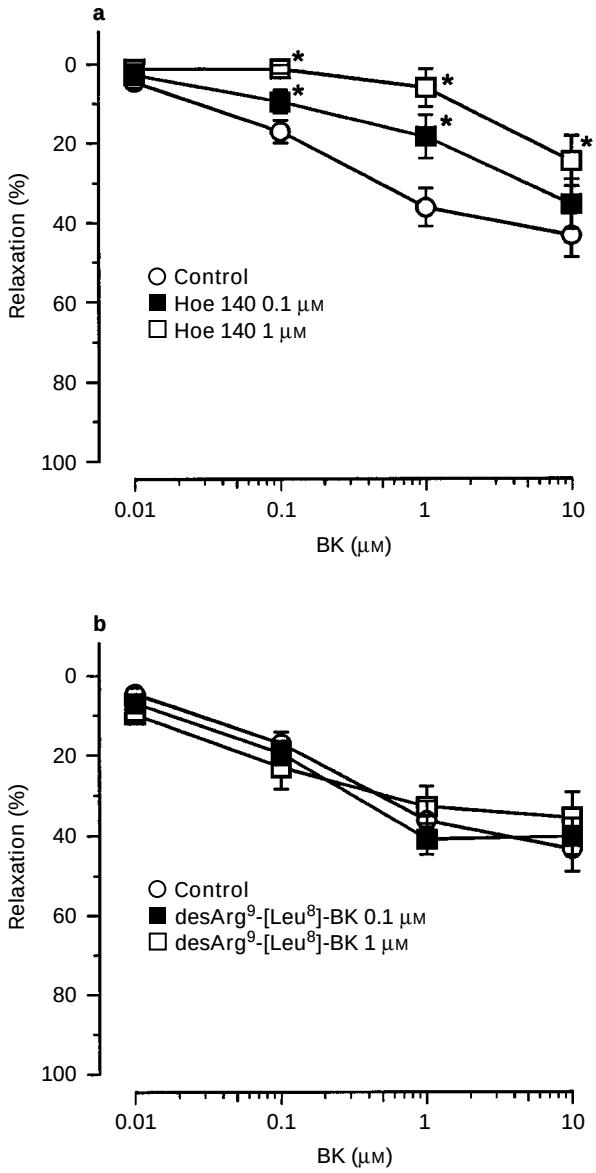

Figure 2 Non-cumulative concentration-response curves to bradykinin (BK, 0.01–10 μM) in the mouse isolated trachea after methacholine (1 μM) precontraction in the absence (control) and presence of (a) Hoe 140 (0.1 μM and 1 μM) or (b) desArg^9-[Leu^8]-bradykinin (0.1 μM and 1 μM). Each point represents mean and vertical lines show s.e.mean ($n=5-6$). Significantly different from control, $^*P<0.05$.

[des-Arg^9]-bradykinin (each drug, 0.01 – 1 μM) were obtained in the absence and presence of Hoe 140 (0.1 – 1 μM), preincubated with the preparations for 20 min. To test the effect of a B_1 receptor antagonist, non-cumulative concentration-response curves to bradykinin or [des-Arg^9]-bradykinin (each drug, 0.01 – 1 μM) were obtained in the absence and presence of [des-Arg^9,Leu^8]-bradykinin (0.1 – 1 μM), preincubated with the preparations for 20 min. To test the effects of inhibition of cyclo-oxygenase, non-cumulative concentration-response curves to bradykinin or [des-Arg^9]-bradykinin (each drug, 0.01 – 1 μM) were obtained in the absence and presence of indomethacin (0.01 – 1 μM), preincubated with the preparations for 20 min. The effects of inhibition of NO synthesis on the bradykinin- or [des-Arg^9]-bradykinin-induced relaxation were investigated in the absence and presence of two NO biosynthesis inhibitors, L-NAME (100 μM) and L-NOARG (100 μM), preincubated with the preparations for 20 min. To see if NO-stimulated production of guanosine 3′:5′-cyclic monophosphate (cyclic GMP) was involved in relaxation, non-cumulative concentration-response curves to bradykinin or [des-Arg^9]-bradykinin were obtained in the absence and presence of an inhibitor of NO-activated soluble guanylate cyclase, ODQ (10 μM), preincubated with the preparations for 20 min.

Prostaglandin E_2 (PGE_2) and the NO-donor, sodium nitroprusside (SNP) were used as reference tracheal smooth muscle relaxing drugs. Following the equilibration period and after methacholine precontraction of the tracheal preparations, PGE_2 (0.01 – 33 μM) or SNP (0.01 – 100 μM) was added cumulatively at 5 – 7 min intervals. The cumulative concentration-response curves to PGE_2 (0.01 – 33 μM) or SNP (0.01 – 100 μM) was obtained in the absence and presence of

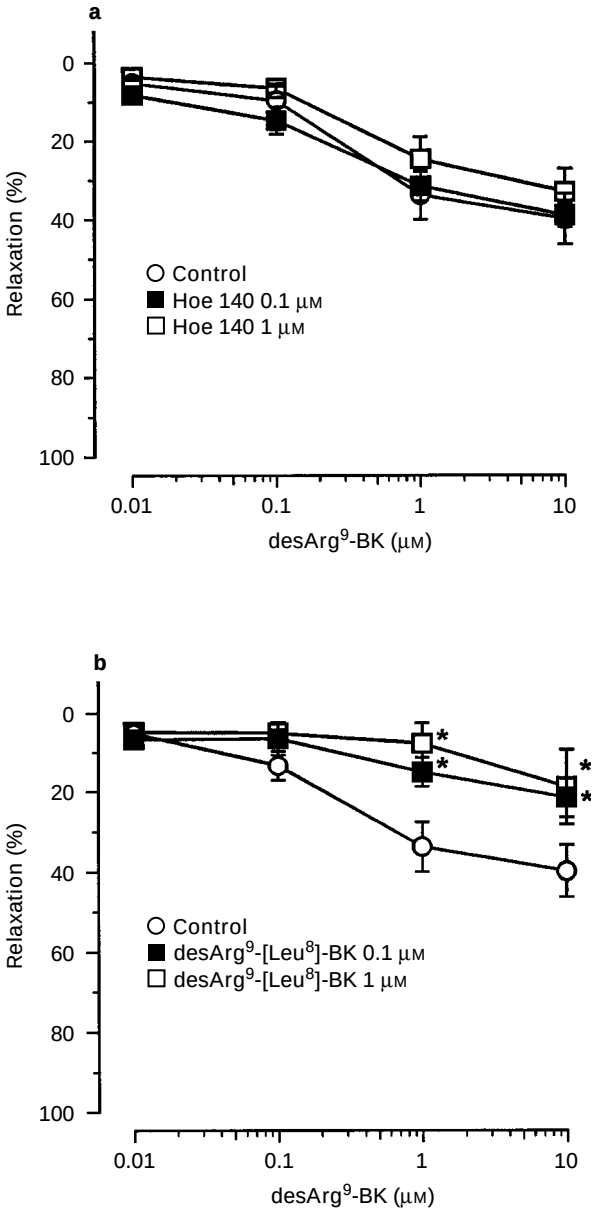

Figure 3 Non-cumulative concentration-response curves to desArg^9-bradykinin (desArg^9-BK, 0.01 – 10 μM) in the mouse isolated trachea after methacholine (1 μM) precontraction in the absence (control) and presence of (a) Hoe 140 (0.1 μM and 1 μM) or (b) desArg^9-[Leu^8]-bradykinin (0.1 μM and 1 μM). Each point represents mean and vertical lines show s.e.mean ($n = 5 – 6$). Significantly different from control, $^*P < 0.05$.

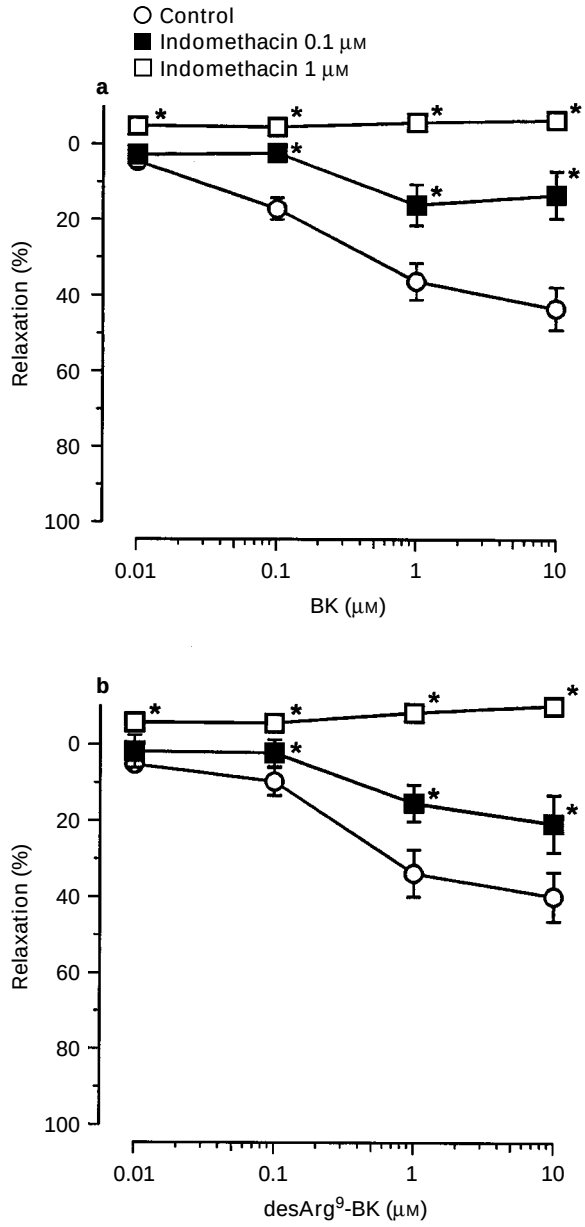

Figure 4 Non-cumulative concentration-response curves to (a) bradykinin (BK, 0.01 – 10 μM) or (b) desArg^9-bradykinin (desArg^9-BK, 0.01 – 10 μM) in the mouse isolated trachea after methacholine (1 μM) precontraction in the absence (control) and presence of indomethacin (0.1 μM and 1 μM). Each point represents mean and vertical lines show s.e.mean ($n = 5 – 6$). Significantly different from control, $^*P < 0.05$.

indomethacin (1 μM) or ODQ (0.1–1 μM), in order to see the effects of inhibition of cyclo-oxygenase or NO-activated soluble guanylate cyclase on both agonist-induced relaxations. The relaxing responses to bradykinin and other agonists were expressed as percentage of relaxation of submaximal contraction induced by methacholine (1 μM).

Statistical analysis

The results are expressed as mean ± s.e.mean of the indicated number of experiments. Statistical analysis of the results was performed by analysis of variance (ANOVA) followed by Duncan's multiple range test. Differences were considered significant when $P<0.05$.

Drugs

Drugs from the following sources were used: methacholine (acetyl-β-methylcholine chloride), bradykinin, [des-Arg^9]-bradykinin, [des-Arg^9,Leu^8]-bradykinin, indomethacin and N^G-nitro-L-arginine methyl ester (L-NAME, Sigma Chemical Co., St. Louis, MO, U.S.A.), 1 H-[1,2,4]oxadiazolo[4,3,-a]quinoxalin-1-one (ODQ, Tocris Cookson Ltd., Bristol, U.K.), D-Arg^0[Hyp^3,Thi^5,D-Tic^7,Oic^8]bradykinin (Hoe 140, Hoechst AG, Frankfurt, Germany), N^G-nitro-L-arginine (L-NOARG, Alexis Corporation, Läufelfingen, Switzerland), pentobarbitone sodium, (Grinsted Products, Grinsted, Denmark), prostaglandin E_2 (Schering AG, Berlin, Germany), sodium

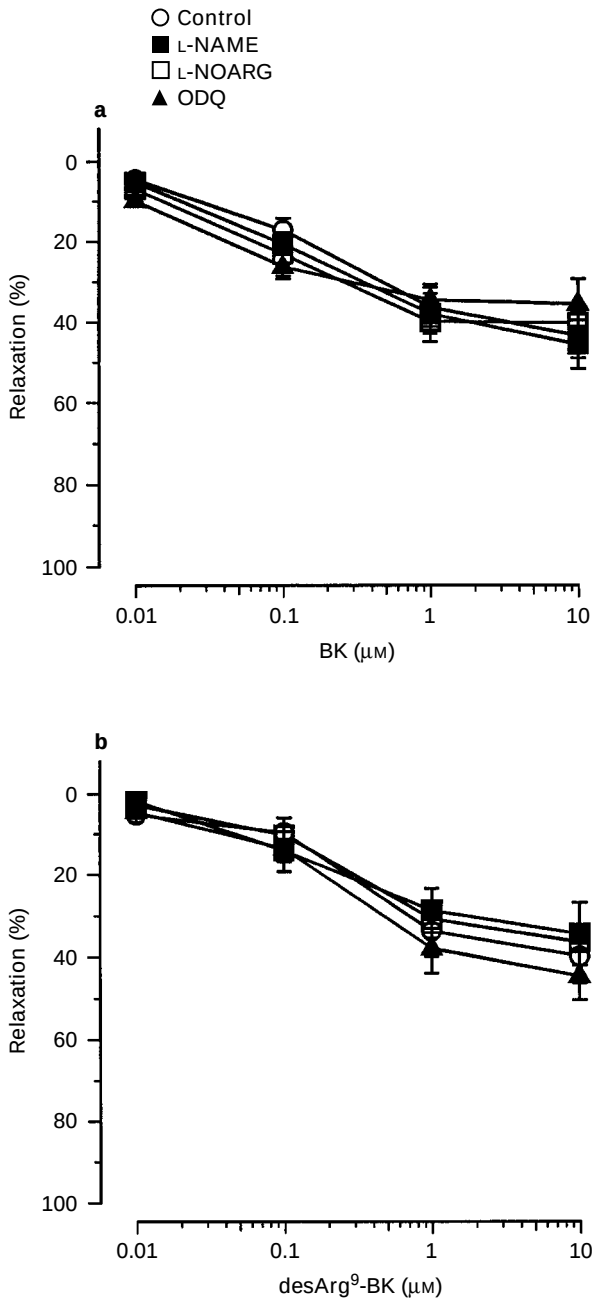

Figure 5 Non-cumulative concentration-response curves to (a) bradykinin (BK, 0.01–10 μM) or (b) desArg^9-bradykinin (desArg^9-BK, 0.01–10 μM) in the mouse isolated trachea after methacholine (1 μM) precontraction in the absence (control) and presence of L-NAME (100 μM), L-NOARG (100 μM) or ODQ (10 μM). Each point represents mean and vertical lines show s.e.mean (n = 5–6).

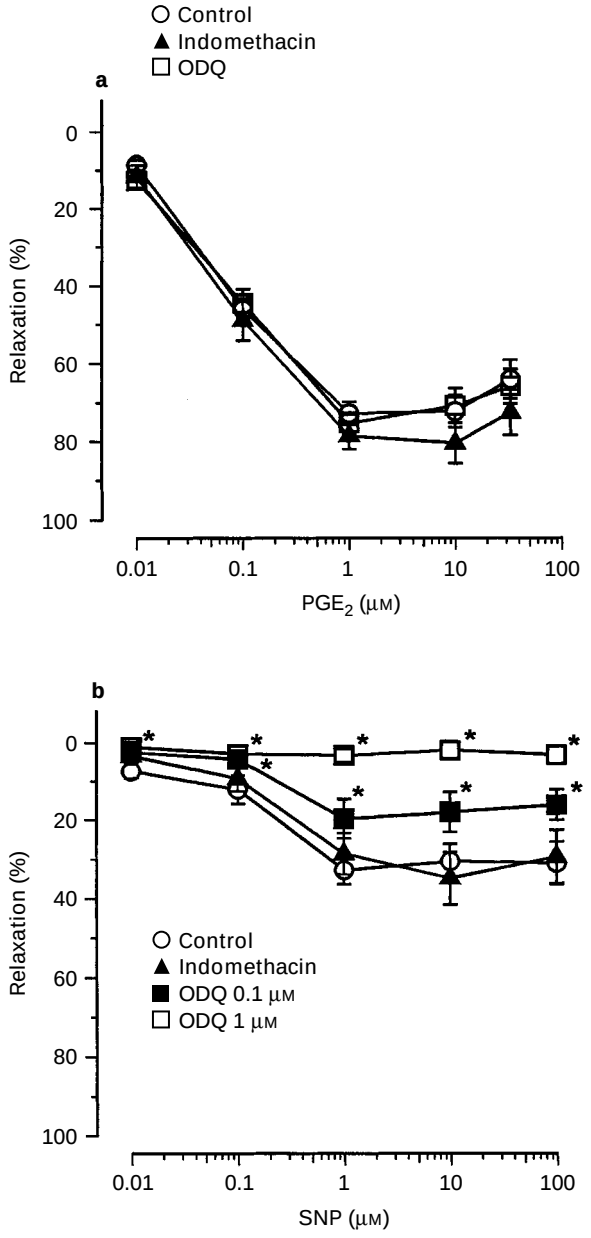

Figure 6 (a) Cumulative concentration-response curves to PGE_2 (0.01–33 μM) in the mouse isolated trachea after methacholine (1 μM) precontraction in the absence (control) and presence of indomethacin (1 μM) and ODQ (10 μM). (b) Cumulative concentration-response curves to SNP (0.01–100 μM) in the mouse isolated trachea after methacholine (1 μM) precontraction in the absence and presence of ODQ (0.1 μM and 1 μM) or indomethacin (1 μM). Each point represents mean and vertical lines show s.e.mean ($n = 5–6$). Significantly different from control, $^*P<0.05$.

nitroprusside (SNP, F. Hoffmann-La Roche, Ltd., Basel, Switzerland). Unless otherwise stated, all drugs were prepared daily in ultrapure water (MilliQ, Millipore Corp., Bedford, MA, U.S.A.) just before the experiments and protected from light. Stock solutions of 10 mM bradykinin, [des-Arg^9]-bradykinin and [des-Arg^9,Leu^8]-bradykinin were stored at -20°C until use. Indomethacin was dissolved in absolute ethanol at 10 mM. ODQ was prepared in dimethyl sulphoxide (DMSO) at 10 mM.

Results

Bradykinin- and [des-Arg^9]-bradykinin-induced relaxation

At resting tone, the mouse trachea failed to respond to bradykinin and [des-Arg^9]-bradykinin (each drug, 0.01–10 μM). However, bradykinin (0.01–10 μM) induced concentration-dependent relaxations when the tracheal preparations were precontracted with methacholine (1 μM). The B_2 receptor antagonist, Hoe 140 (0.01–1 μM), antagonized the relaxation induced by bradykinin in a concentration-dependent manner, whereas the B_1 receptor antagonist (0.01–1 μM), [des-Arg^9,Leu^8]-bradykinin, had no effect (Figure 2). [Des-Arg^9]-bradykinin (0.01–10 μM) also induced concentration-dependent relaxation after precontraction with methacholine (1 μM). The B_1 receptor antagonist, [des-Arg^9,Leu^8]-bradykinin, antagonized the relaxation induced by [des-Arg^9]-bradykinin in a concentration-dependent manner, whereas Hoe 140 did not inhibit [des-Arg^9]-bradykinin-induced relaxation (Figure 3).

Effects of indomethacin, L-NAME, L-NOARG and ODQ, on bradykinin- and [des-Arg^9]-bradykinin-induced relaxation

Indomethacin, L-NAME, L-NOARG did not affect the response to methacholine, but ODQ elevated the response to methacholine by about 10%. In the presence of the cyclo-oxygenase inhibitor, indomethacin (0.01–1 μM), the relaxations induced by bradykinin and [des-Arg^9]-bradykinin were inhibited concentration-dependently. After pretreatment with indomethacin (1 μM) both bradykinin and [des-Arg^9]-bradykinin caused a slight contraction (Figure 4). Two inhibitors of NO biosynthesis, L-NAME (up to 100 μM) and L-NOARG (up to 100 μM) did not modify either the bradykinin- or [des-Arg^9]-bradykinin-induced relaxation. The selective inhibitor ODQ (up to 10 μM) also had no inhibitory effect on bradykinin- and [des-Arg^9]-bradykinin-induced relaxations (Figure 5).

Effects of indomethacin and ODQ on other agonist-induced relaxations

PGE_2 (0.01–33 μM) caused a concentration-dependent relaxation of the tracheal preparations precontracted with methacholine. The maximal relaxation induced by PGE_2 was 73%. Indomethacin (up to 1 μM) and ODQ (up to 1 μM) did not prevent the relaxation induced by PGE_2 (Figure 6a). SNP (0.01–100 μM) also relaxed the tracheal preparations precontracted with methacholine (1 μM) in a concentration-dependent manner. The maximal relaxation induced by SNP was 32%. Indomethacin (up to 1 μM) did not inhibit SNP-induced relaxation. In contrast, ODQ (0.1–1 μM) concentration-dependently reduced the relaxation induced by SNP. In the presence of ODQ (1 μM), the SNP-induced relaxation was abolished (Figure 6b).

Discussion

The present study confirms our earlier results that bradykinin induces relaxation of the mouse trachea (Li *et al*., 1997) and describes for the first time the involvement of the bradykinin B_2 and B_1-receptors in this relaxation. It was found that bradykinin and [des-Arg^9]-bradykinin produced no contractile effects under basal conditions. However, when a contraction was induced by methacholine, both bradykinin and [des-Arg^9]-bradykinin caused a significant and concentration-dependent relaxation. In the precontracted tracheal preparations, the bradykinin-induced relaxation was inhibited in a concentration-dependent manner by the B_2-receptor antagonist, Hoe 140 (Hock *et al*., 1991) but not the B_1-receptor antagonist, desArg^9-[Leu^8]-bradykinin, indicating that B_2-receptors are involved in the bradykinin-induced relaxation. Generally the B_1-receptors are not expressed in animal airway tissues and the B_2-receptors mediate most of the biological effects of kinins (Farmer, 1991). However, in the present study the [des-Arg^9]-bradykinin-induced relaxation was inhibited in a concentration-dependent manner by the B_1-receptor antagonist, desArg^9-[Leu^8]-bradykinin but not the B_2-receptor antagonist, Hoe 140, indicating that B_1-receptors are involved in the [des-Arg^9]-bradykinin-induced relaxation. Because the genes encoding the mouse B_2-receptor (Ma *et al*., 1994) and B_1-receptor have been cloned and lipopolysaccharide treatment induces B_1 receptor transcripts in the mouse lung and other tissues (Pesquero *et al*., 1996), it is possible that both B_2 and B_1-receptors are expressed in the murine airways. Taken together, both B_2 and B_1-receptors are likely to be involved in the relaxation of the mouse precontracted trachea.

In the precontracted tracheal preparations, the effects of bradykinin and [des-Arg^9]-bradykinin were completely and concentration-dependently inhibited by the cyclo-oxygenase inhibitor, indomethacin, suggesting that the cyclo-oxygenase pathway is involved in the bradykinin- and [des-Arg^9]-bradykinin-induced relaxation. In addition, we found that in the mouse isolated trachea, indomethacin did not inhibit the concentration-dependent relaxation induced by PGE_2. This further demonstrates that the inhibitory actions of indomethacin on the bradykinin- and [des-Arg^9]-bradykinin-induced relaxation are due to the inhibition of cyclo-oxygenase involved in the biosynthesis of relaxing prostanoids, but not due to interference with prostaglandin receptor coupling mechanisms.

The NO-mediated pathway is not involved in the bradykinin- and [des-Arg^9]-bradykinin-induced relaxation since neither L-NAME (Rees *et al*., 1990) nor L-NOARG (Moore *et al*., 1990), two inhibitors of nitric oxide synthase, reduced the bradykinin- and [des-Arg^9]-bradykinin-induced relaxation. This finding is further strengthened by the fact that ODQ, a selective inhibitor of NO-activated soluble guanylate cyclase, had no inhibitory effect on the bradykinin- or [des-Arg^9]-bradykinin-induced relaxation. ODQ has recently been shown to act as a selective inhibitor of soluble guanylate cyclase without any effect on NO (Garthwaite *et al*., 1995; Moro *et al*., 1996). ODQ also inhibits SNP-induced relaxations in the rabbit anococcygeus muscle, and reduces the basal and NO stimulated production of cyclic GMP (Cellek *et al*., 1996).

Indomethacin had no inhibitory effect on the SNP-induced relaxation, suggesting that cyclo-oxygenase pathway is not involved. However, ODQ concentration-dependently inhibited the relaxation of mouse trachea induced by SNP without

affecting that induced by PGE_2, indicating that the NO-donor-induced relaxation was mediated via soluble guanylate cyclase.

In the guinea-pig trachea, the bradykinin-induced relaxation is mediated by the simultaneous release of relaxing prostanoids (Bramley *et al*., 1990; Schlemper & Calixto, 1994; Da Silva *et al*., 1995) and NO (Schlemper & Calixto, 1994; Figini *et al*., 1996). In contrast, our findings indicate that in the mouse trachea the bradykinin- and [des-Arg^9]-bradykinin-induced relaxations are entirely dependent on arachidonic acid metabolites without the involvement of the NO pathway.

In conclusion, the mouse isolated trachea is a new preparation in which bradykinin and [des-Arg^9]-bradykinin produce relaxation probably mediated via bradykinin B_2- and B_1-receptors, respectively, possibly located in the epithelium or/and in the smooth muscle. The stimulation of B_2- and B_1-receptors seems to result in activation of the cyclo-oxygenase pathway, and the synthesis of a smooth muscle relaxing prostanoid(s), e.g. PGE_2. NO does not appear to be involved in the bradykinin- and [des-Arg^9]-bradykinin-induced relaxation. Moreover, these findings in the mouse trachea further support the hypothesis that the overall responses of mammalian airways to bradykinin and related kinins, contraction or relaxation, differ from species to species and, possibly from physiological to pathological states.

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