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Neurokinin A-LI release after antigen challenge in guinea-pig bronchial tubes: influence of histamine and bradykinin

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1 Our aim was to determine if antigen challenge stimulates sensory nerves and provokes the release of tachykinins. The involvement of histamine and bradykinin was studied by using specific receptor antagonists. Capsaicin-induced responses were also examined. Experiments were performed *in vitro* on tracheal and bronchial preparations from ovalbumin-sensitized guinea-pigs.

2 Characterization of ovalbumin-induced contraction, with regard to histamine and bradykinin, was carried out on airway ring preparations in the presence of phosphoramidon. The histamine H_1 receptor antagonist pyrilamine reduced allergen-induced bronchial contractions by about 30%, whereas the bradykinin B_2 receptor antagonist icatibant (Hoe 140) did not significantly affect the response. Combined treatment with pyrilamine (1 μM) and icatibant (0.1 μM) reduced the contractions by about 80%, indicating a synergistic inhibitory action. Tracheal preparations were not significantly affected by treatments, neither were capsaicin-induced contractions.

3 To study the outflow of tachykinins, we used a perfused bronchial-tube preparation, allowing simultaneous measurement of smooth muscle tension and mediator release. Neurokinin A-like immunoreactivity (NKA-LI) and substance P-like immunoreactivity (SP-LI) were determined by radioimmunoassay.

4 The results of the perfusion study showed an increased outflow of NKA-LI into the perfusate in response to ovalbumin (127% of basal) challenge. SP-LI determined in some of the samples showed a much lower amount (40 to 70 times lower) of SP-LI than NKA-LI. Treatment with icatibant and pyrilamine, separately and in combination, significantly reduced the ovalbumin-induced NKA-LI outflow by 38%, 26% and 22%, respectively.

5 Capsaicin-induced outflow (124% of basal) was not significantly affected by treatments (icatibant 121%, pyrilamine 107% and combined treatment 111% of basal). However, when pyrilamine was present the increased outflow was not statistically significant.

6 In conclusion, we found that allergen provocation of guinea-pig bronchi caused an increased outflow of NKA-LI that was reduced by treatment with both pyrilamine and icatibant. These findings demonstrate that the allergen-induced release of histamine and bradykinin stimulate sensory nerves and thereby increase outflow of tachykinins that contribute to the allergic reaction.

Keywords: Neurokinin A; allergen challenge; histamine; bradykinin; pyrilamine; icatibant; guinea-pig airway; tachykinins

Introduction

Antigen challenge of sensitized airways results in bronchoconstriction, mucus production and plasma extravasation due to the action of several different inflammatory mediators (Barnes *et al*., 1988; Barnes, 1996). It has been proposed that the effects of antigen challenge comprise the activities of both inflammatory cells and sensory nerves and interaction between these structures has been suggested (Barnes, 1992a)

Capsaicin, the pungent agent in red pepper, is known to stimulate directly a subset of sensory nerves, probably via specific receptors (Dray, 1992). During activation, the capsaicin-sensitive sensory nerves are able to release the related tachykinins neurokinin A (NKA) and substance P (SP). NKA is a potent bronchoconstrictor (Advenier *et al*., 1987; Martling *et al*., 1987) whereas SP mainly induces plasma extravasation in the airways and the tachykinins have been proposed to play a role in neurogenic inflammation (Barnes, 1992a; Martling, 1987).

Saria *et al*. (1988) found that histamine, a well-documented participant in the allergic reaction, added exogenously to guinea-pig lung released tachykinins by interacting with H_1 receptors. Bradykinin and related kinins are also mediators of inflammation, and there is accumulating evidence that bradykinin can release neuropeptides from capsaicin-sensitive nerves (Geppetti, 1993). Furthermore, bradykinin has been observed to potentiate excitatory NANC (non-adrenergic, non-cholinergic) responses in guinea-pig airways *in vivo* (Miura *et al*., 1994) and *in vitro* (Miura *et al*., 1992), probably via prejunctional bradykinin B_2 receptors. Recently, the involvement of tachykinins and kinins in allergic airways was reviewed (Bertrand & Geppetti, 1996).

Kinins act mainly through interaction with bradykinin B_1 and B_2 receptors (Hall, 1992; Regoli *et al*., 1996), although conflicting data concerning another receptor-subtype in guinea-pigs have been published (Farmer *et al*., 1989; Pruneau *et al*., 1995). Icatibant, the B_2 receptor antagonist used in the present study, was chosen since it also antagonizes bradykinin in guinea-pig trachea (Farmer *et al*., 1989; Pruneau *et al*., 1995). In the present study, we addressed the question of whether antigen challenge of airway preparations stimulates capsaicin-sensitive sensory nerves and thereby increases outflow of tachykinins. We used sensitized guinea-pig bronchial-tube preparations (Lindström & Andersson, 1995) to determine NKA-LI outflow and smooth muscle tension in response to both antigen and capsaicin challenge. Involvement of histamine H_1 receptors and bradykinin B_2 receptors was examined by using the histamine H_1-receptor antagonist pyrilamine and the selective bradykinin B_2 receptor antagonist icatibant (Hock *et al*., 1991).

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Methods

Animals and sensitization

Experiments were approved in advance by the local ethical review committee on animal experiments (Linköping, Sweden).

Male Dunkin-Hartley guinea-pigs (Sahlins, Malmö, Sweden) weighing 400–700 g were used. The animals were raised under standardized conditions and sensitized by an intraperitoneal injection of 10 μg of ovalbumin, with 100 mg of aluminium hydroxide ($Al(OH)_3$) as adjuvant (Fügner, 1985). The sensitization was performed at least 2 weeks before the experiments.

Preparation

Guinea-pigs were stunned and bled. Bronchi and trachea were then isolated, dissected free from surrounding tissue and immersed in Krebs solution (composition in mM: NaCl 12.2, KCl 4.7, $CaCl_2$ 2.5, $MgCl_2$ 1.2, $NaHCO_3$ 15.4, KH_2PO_4 1.2 and glucose 5.5) equilibrated with 95% O_2 and 5% CO_2, pH 7.4. The epithelium was largely removed from all preparations by mechanical action (by a plastic rod), as previously described (Grundström *et al*., 1992).

Contractility studies

Main bronchi and trachea were prepared and divided into ring segments, approximately 3 mm in length; the segments were mounted on special holders (Grundström *et al*., 1981) and immersed in organ baths containing the modified Krebs solution. The preparations were given an initial tension of 0.5–1.0 g. After equilibration, the preparations were repeatedly contracted with 10 μM acetylcholine until a stable response was recorded; no other drug was present during this provocation. Thereafter, the preparations were washed and equilibrated for an additional 45 min. Drugs, the bradykinin B_2 receptor antagonist icatibant (0.01, 0.1 and 1 μM), the histamine H_1 receptor antagonist pyrilamine (1 μM), and the endopeptidase inhibitor phosphoramidon (10 μM) were present as indicated during the equilibration period and throughout the experiments. After the equilibration period, the preparations were provoked by antigen, i.e. ovalbumin (0.1 mg ml^{-1}). When the response was stable, the preparations were washed repeatedly and allowed to return to baseline tension in the presence of the drugs being used. Thereafter, stimulation with capsaicin (10 μM) was carried out. In a separate set of experiments we compared capsaicin-induced contractions performed with or without any previous ovalbumin-challenge.

The results were calculated as % of the acetylcholine-induced contraction.

Perfusion studies

A method used to measure the muscle tension of a tracheal or bronchial-tube preparation has been described by our research group (Grundström *et al*., 1992; Lindström & Andersson, 1995). In the present study, the modified method that is suitable for the bronchial-tube preparations (Lindström & Andersson, 1995) was used. In short, the trachea and all branches of the bronchi were ligated to create a single unbranched tube actually consisting of both bronchi. The two open (distal) ends were then connected to perfusion cannulae to allow continuous perfusion of the preparation with 37°C Krebs solution. Thereafter, the prepared bronchial tube was mounted in a small closed chamber filled with Krebs solution and changes in smooth muscle tension were estimated by measuring the accompanying changes in chamber pressure (i.e. contraction of the preparation resulted in a decrease in chamber pressure). A pressure transducer (type AE 840, AS Mikroelektronick, Horten, Norway) was used for measurements and results were recorded on a polygraph (Grass model 7). The Krebs solution contained gelatine (0.2%), to prevent the adherence of neuropeptides to the plastic tubes. The endopeptidase inhibitor phosphoramidon (10 μM) was also present to minimize degradation of NKA. Drugs, icatibant (0.1 μM) and pyrilamine (1 μM) were added to the perfusion solution and to the buffer surrounding the preparation 40 min before stimulation. Ovalbumin (1 mg ml^{-1}) was applied in the perfusion solution flowing through the lumen of the preparation. After the ovalbumin challenge, a 45-min wash-out (in the presence of the drugs used) was performed and the preparation was subsequently stimulated with capsaicin (10 μM). In four experiments capsaicin was applied directly without any pre-exposure to ovalbumin.

Tachykinin determination

The perfusate from the bronchial-tube preparation was collected for 10 min immediately before stimulation to obtain basal values and after that for 10 min during stimulation. The perfusate was collected on ice and acetic acid was immediately added to a final concentration of 0.5 M. Isolute-C18 cartridges (IST, by Sorbent AB, V. Frölunda, Sweden) were then used to purify and concentrate the samples (Lindström & Andersson, 1995; Saria *et al*., 1988). Briefly, cartridges were activated with a methanol solution (0.1% trifluoroacetic acid containing 0.06 M sodium chloride and 80 voL % methanol) and equilibrated with TFA solution (0.1% trifluoroacetic acid in water containing 0.06 M sodium chloride). Samples were applied to the cartridges and washed, and thereafter peptides were eluted with the methanol solution. The methanol component in the eluted samples was evaporated before freeze-drying. This treatment resulted in an approximately 86 ± 1.5% recovery of synthetic NKA. Neurokinin A-like immunoreactivity was determined by radioimmunoassay (RIA; Neurokinin A RIA KIT, Peninsula Laboratories, Inc, U.S.A.); the analysis was performed according to the manual provided with the kit. The cross-reactivity of the kit assay to kassinin and neuropeptide K is 100%, to NKB 80%, and to SP < 0.05%, detection range 1–128 pg/tube. Substance P-like immunoreactivity content was determined by RIA in some of the samples (Substance P, RIA KIT, Peninsula Laboratories, Inc, U.S.A.; range; 0.1–64 pg/tube; cross-reactivity to NKA < 0.01%).

Statistical analysis

All data are presented as mean $\pm$ s.e. mean. Statistical analysis was performed by use of ANOVA followed by an ad hoc test (Dunett's). All treatments were compared with control values and statistical significance was denoted; *($P < 0.05$), **($P < 0.01$). Student's paired t test was used as indicated.

Drugs

Icatibant (Hoe 140) was a gift from Hoechst AG (Frankfurt am Main, Germany). Capsaicin, neurokinin A, phosphoramidon, ovalbumin, gelatine, acetylcholine chloride and pyrilamine (mepyramine) were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Capsaicin was dissolved in ethanol and then diluted to the final concentration in Krebs buffer (final concentration 0.1% ethanol). Other agents were dissolved in Krebs solution.

Results

Contractility studies

Bronchial preparations Contractile responses are presented as percentages of the acetylcholine-induced contraction recorded before any drug treatment. The mean contraction of the bronchial preparations in response to acetylcholine (10 μM) was 0.65 ± 0.035 g ($n = 67$); no significant difference was observed between treatment groups. Acetylcholine- and antigen-induced contractions showed positive correlation ($r = 0.87$,

$n=10$). Ovalbumin (0.1 mg ml^{-1}) challenge of bronchial-ring preparations resulted in contractions of $137.7\pm15.6\%$ ($n=7$). Phosphoramidon (10 μM) treatment of these epithelium-denuded preparations did not alter the response significantly (Figure 1a), whereas the histamine H_1 receptor antagonist pyrilamine (1 μM) did ($90.28\pm12.9\%$, $n=6$, $P<0.05$). Treatment with pyrilamine in the presence of phosphoramidon resulted in a similar reduction as when pyrilamine was used alone (Figure 1a). Capsaicin-induced responses ($99.8\pm23.1\%$, $n=6$) were not significantly affected by any of the previously mentioned treatments (pyrilamine $87\pm20\%$, $n=5$, and Figure 1b). Although no significant effect of phosphoramidon treatment was noted, we chose to use it throughout this study.

The selective bradykinin B_2 receptor antagonist icatibant, at concentrations of 0.01, 0.1 and 1 μM, did not significantly affect the contractile responses of the bronchial-ring preparations to ovalbumin ($149\pm31\%$, $112\pm32\%$, and $101\pm22\%$, respectively; $n=6$). However, when pyrilamine and icatibant were co-administered, contractions were inhibited. This effect was most pronounced and significant at a concentration of 0.1 μM icatibant (Figure 1a).

Capsaicin-induced contractions were not significantly affected by icatibant, either in the presence or the absence of pyrilamine (Figure 1b).

Tracheal preparations The contractions exhibited by the tracheal-ring preparations in response to antigen and capsaicin challenge were not significantly affected by any treatment performed. Neither was there any significant difference between contractions elicited by capsaicin directly or after ovalbumin-challenge (data not shown).

Perfusion studies

In this series of experiments, phosphoramidon was present at all times, and the concentration of icatibant that gave the most

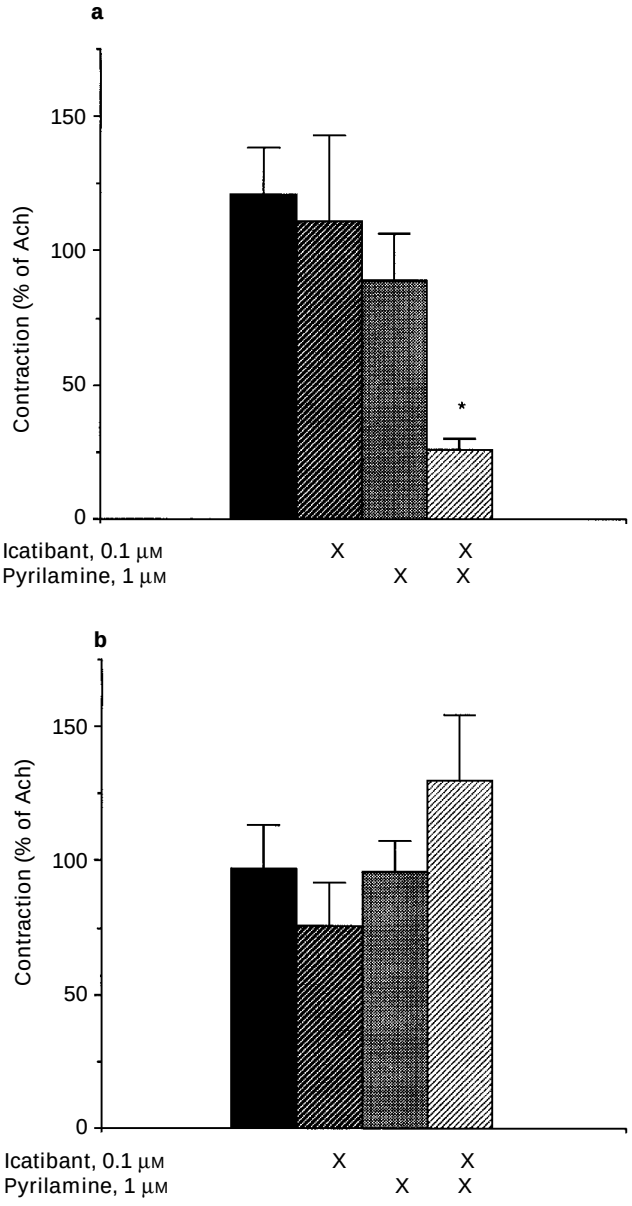

Figure 1 Contractions of bronchial-ring preparations challenged with (a) ovalbumin (0.1 mg ml^{-1}) and (b) capsaicin (10 μM) and the modulatory action of icatibant and pyrilamine on the induced responses. Phosphoramidon (10 μM) was present in all experiments. Each column represents mean$\pm$s.e.mean, $n=6-7$. $^*P<0.05$ compared with control (solid column).

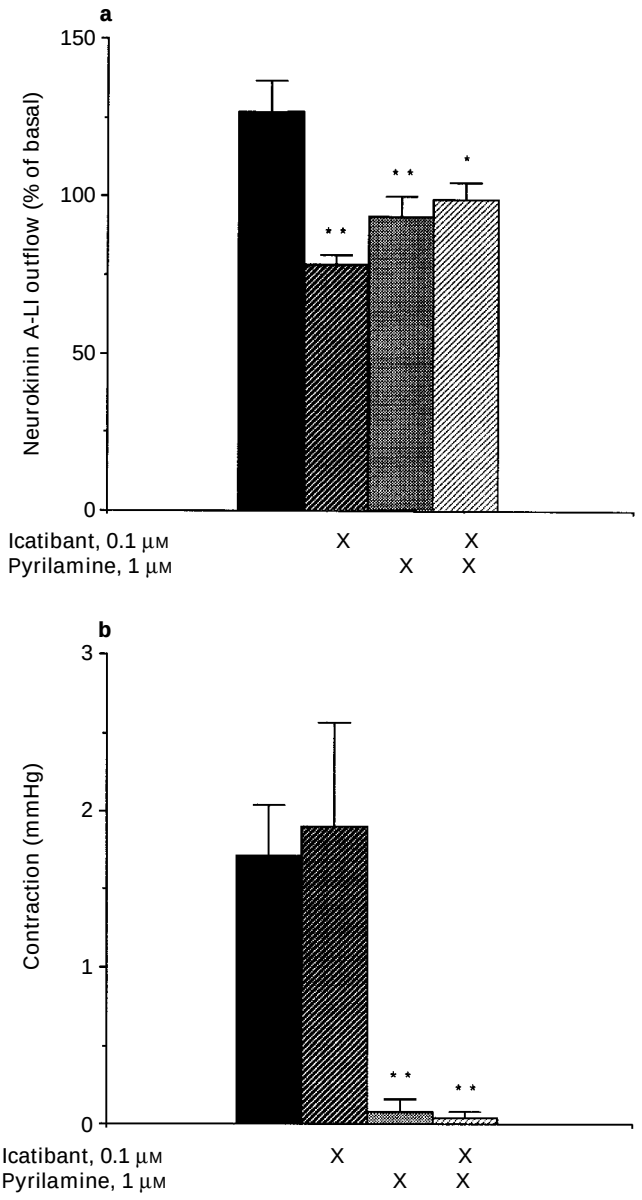

Figure 2 Bronchial-tube preparations challenged with ovalbumin (1 mg ml^{-1}). Effects of icatibant and pyrilamine on (a) NKA-LI outflow and (b) contractile responses. Phosphoramidon (10 μM) was present in all experiments. Each column represents mean$\pm$s.e.mean, $n=6$. $^*P<0.05$ and $^{**}P<0.01$, compared with control (solid column).

pronounced response in the bronchial-ring preparation studies (i.e. 0.1 μM) was used.

NKA-LI was determined in the perfusate collected before antigen challenge (basal) and during the challenge. Results in the figures are expressed as % of basal outflow of NKA-LI.

Antigen stimulation of the bronchial tubes significantly increased the amount of NKA-LI in the perfusate (basal, 1300 ± 59 and challenged, 1626.6 ± 110 pg g^{-1} dw (dry weight,); $P<0.05$, Student's paired t test) (Figure 2a). This increased outflow was inhibited by the treatments performed (Figure 2a). Basal levels (sampled before ovalbumin-challenge) of NKA-LI were not affected by icatibant treatment (1303 ± 91 pg g^{-1} dw) while pyrilamine and combined treatment significantly decreased basal values (965 ± 113, $P<0.05$ and 674 ± 71, $P<0.01$, pg g^{-1} dw, respectively). No significant difference between basal values sampled before ovalbumin and capsaicin-challenge was observed except when icatibant was present alone (basal before capsaicin 1142 ± 109 pg g^{-1} dw, $P<0.01$ Student's paired t test).

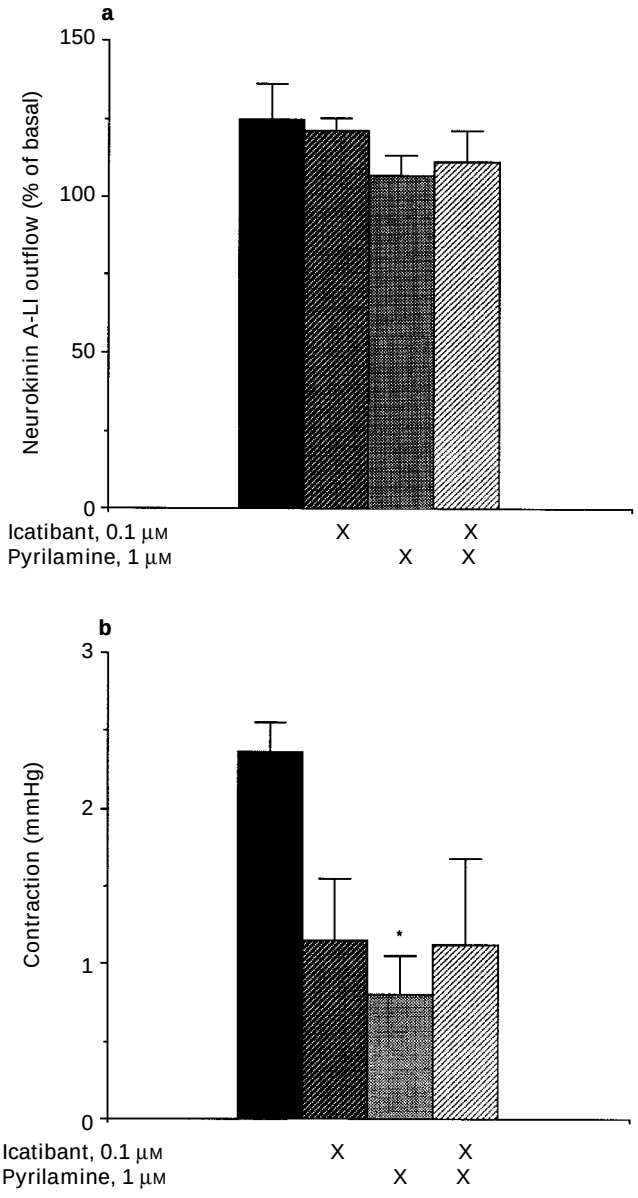

Figure 3 Bronchial-tube preparations challenged with capsaicin (10 μM). Effects of icatibant and pyrilamine on (a) NKA-LI outflow and (b) contractile responses. Phosphoramidon (10 μM) was present in all experiments. Each column represents mean $\pm$ s.e.mean, $n=6$. *$P<0.05$, compared with control (solid column).

Capsaicin challenge provoked a similar liberation of NKA-LI as ovalbumin-challenge (basal, 1572 ± 161 and challenged, 1925.6 ± 174 pg g^{-1} dw, $P<0.05$, Student's paired t test) (Figure 3a), but no significant difference between treatments was noted (Figure 3a). However, when pyrilamine was present the increased outflow was not statistically significant.

No significant difference between NKA-LI outflow, when capsaicin-challenge was performed directly or after ovalbumin-challenge, was noted (data not shown).

SP-LI was analysed in twelve of the NKA samples and a comparison of these two tachykinins revealed about 40 to 70 times more NKA-LI than SP-LI in the samples.

Contractions elicited by ovalbumin challenge of bronchial tubes were not affected by treatment with icatibant (0.1 μM), whereas pyrilamine (1 μM) alone effectively reduced the contractions and addition of icatibant did not result in any further decrease (Figure 2b). Only exposure to pyrilamine significantly decreased the capsaicin-induced contractions (Figure 3b).

Discussion

In the present study, characterization of the response to antigen and capsaicin stimulation, with regard to histamine and bradykinin, was performed in bronchial and tracheal preparations from sensitized guinea-pigs. We found that contractions induced by antigen challenge of bronchial ring preparations could be reduced by pyrilamine treatment but were not affected by exposure to icatibant. In contrast, when pyrilamine and icatibant were administered together the contractions of the bronchial but not the tracheal ring-preparations were reduced significantly. These results indicate that the antagonists used in our experiments act synergistically to inhibit the antigen-induced contractions of the bronchial-ring preparations. The varying distribution of excitatory and inhibitory NANC nerves in guinea-pig airways (Grundström, 1986) and other morphological differences may explain the divergent results we obtained with bronchial and tracheal preparations. The results also emphasize that findings obtained on tracheal preparations are not always applicable to lower airways. Capsaicin, a stimulus known to have a direct effect on sensory nerves, probably via specific receptors (Dray, 1992), induced contractions of the airway ring preparations that were not significantly affected by any of the applied treatments.

It has been proposed that interplay occurs between sensory nerves and inflammatory cells and the results of the ring-preparation experiments could indicate this. We have evaluated the hypothesis that mediators released in response to allergen provocation stimulate sensory nerves and elicit increased outflow of tachykinins. This was accomplished by using a bronchial tube preparation that allowed simultaneous measurement of smooth muscle tension and mediator release. We found that antigen and capsaicin provoked an increased outflow of NKA-LI and SP-LI, these tachykinins are known to be co-stored in sensory nerves. Our finding of substantially larger amounts of NKA-LI than of SP-LI is indirectly supported by others (Ellis & Undem, 1994; Ellis, 1995). However, sample treatment in our study was aimed at maximizing NKA recovery, so the contribution of SP to the response must also be considered. Histamine and/or bradykinin could be a link between inflammatory cells and the nervous system, since both have been shown to stimulate sensory nerves (Saria *et al*., 1988; Geppetti, 1993). For instance, it has been proposed that bradykinin could activate NANC nerves by interacting with B_2 receptors (Dixon & Barnes, 1989; Ichinose & Barnes, 1990) and icatibant has been found to decrease the contractile response induced by aerosolized ovalbumin *in vivo* (Ricciardolo *et al*., 1994).

We found that although NKA-LI outflow in response to ovalbumin-challenge was substantially decreased by icatibant treatment, no effect on contractions could be measured. Also, antigen-induced contractions were almost abolished by treatment with pyrilamine and no further inhibition was seen when icatibant was added. This is in agreement with our previous

results on epithelium-denuded tracheal tube preparations, showing a predominant contractile effect of histamine (Lindström *et al*., 1992). During conditions when the role of histamine is less distinct, e.g. in human airways, the effect of decreased sensory nerve activity could be more pronounced.

Discrepancies in the results between ring and tube preparations may be due to differences in the experimental conditions: in the tubes, the luminal and serosal side of the preparation were separated, perfusion was continuous, and the challenge was made intraluminally; in the ring preparations, luminal, serosal and cut surfaces were exposed to the challenge and released mediators may accumulate in the vicinity of the preparation.

Inconsistency between *in vivo* (Ricciardolo *et al*., 1994) and our *in vitro* results may reflect differences in *de novo* synthesis of kinins, although generation of kinins in airway preparations has been demonstrated (Farmer *et al*., 1994). It has also been shown in both guinea-pig (Arakawa *et al*., 1992; Lach *et al*., 1994) and human (Molimard *et al*., 1994) airways that bradykinin exerts part of its effect through a pathway that is sensitive to indomethacin (a cyclo-oxygenase inhibitor). Prostanoid synthesis may originate from the airway epithelium (Lindström *et al*., 1992), which could in part explain the divergent results obtained with our epithelium-denuded preparations. In our study, treatment with phosphoramidon did not affect contractile responses induced by either antigen or capsaicin. This may be due to reduced NEP (neutral endopeptidase) activity as a consequence of the epithelium removal; comparable results have previously been found by Frossard *et al*. (1989).

In guinea-pigs, tachykinins cause bronchoconstriction mainly by a direct action on bronchial smooth muscle, but a recent study suggests that they may also release histamine (Lilly *et al*., 1995). In agreement with these results, we found that pyrilamine had an inhibitory effect on capsaicin-induced contractions in bronchial-tube preparations. Results concerning the modulatory action of pyrilamine and icatibant on capsaicin-induced responses are difficult to interpret since they are small and variable.

Our finding that pyrilamine reduced basal outflow of NKA-LI suggests that histamine could exert a sensitizing effect on sensory nerves.

Although, icatibant treatment did not affect basal NKA-LI outflow, a pronounced effect on the antigen-induced reaction was seen. NKA-LI outflow was significantly decreased below the basal level and the inhibition appeared to be prolonged since basal values sampled before capsaicin-challenge were lower than those sampled before ovalbumin-challenge. Inflammatory mediators may act on several prejunctional receptors to modulate the release of neurotransmitters (Barnes, 1992b) and an alteration of the balance between inhibitory and facilitating signals may contribute to our results.

Conflicting results have been obtained with regard to the contributions tachykinins make to the allergen-induced reaction (Manzini *et al*., 1987; Ingenito *et al*., 1991; Lai, 1991; Warth *et al*., 1995). In a recent study it was implied that NK_2 receptors are not particularly important for the acute antigen-elicited bronchospasm (Mizuguchi *et al*., 1996). However, in this study the animals were pretreated with an H_1 receptor antagonist and our results showed that pyrilamine reduces NKA-LI outflow.

In conclusion, we have shown that antigen challenge of guinea-pig airways results in an increased outflow of NKA-LI that is modulated by antagonists of both histamine H_1 and bradykinin B_2 receptors.

We propose that histamine and bradykinin released during the allergic reaction stimulate sensory nerves and provoke the release of potent tachykinins. If that mechanism of action also exists in man, then combined treatment with histamine and bradykinin antagonists could be of great therapeutic value in the treatment of allergic asthma.

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References

ADVENIER, C., NALINE, E., DRAPEAU, G. & REGOLI, D. (1987). Relative potencies of neurokinins in guinea pig and human bronchus. *Eur. J. Pharmacol*., **139,** 133–137.

ARAKAWA, H., KAWIKOVA, I., LOFDAHL, C.G. & LOTVALL, J. (1992). Bradykinin-induced airway responses in guinea pig–effects of inhibition of cyclooxygenase and thromboxane synthetase. *Eur. J. Pharmacol*., **229,** 131–136.

BARNES, P.J. (1992a). Neurogenic inflammation and asthma. *J. Asthma*, **29,** 165–180.

BARNES, P.J. (1992b). Modulation of neurotransmission in airways. *Physiol. Rev*., **72,** 699–729.

BARNES, P.J. (1996). Pathophysiology of asthma. *Br. J. Clin. Pharmacol*., **42,** 3–10.

BARNES, P.J., CHUNG, K.F. & PAGE, C.P. (1988). Inflammatory mediators in asthma. *Pharmacol. Rev*., **40,** 49–84.

BERTRAND, C. & GEPPETTI, P. (1996). Tachykinin and kinin receptor antagonists: therapeutic perspectives in allergic airway disease. *Trends Pharmacol. Sci*., **17,** 255–259.

DIXON, M. & BARNES, P.J. (1989). Bradykinin-induced bronchoconstriction: inhibition by nedocromil sodium and sodium cromoglycate. *Br. J. Clin. Pharmacol*., **27,** 831–836.

DRAY, A. (1992). Mechanism of action of capsaicin-like molecules on sensory neurons. *Life Sci*., **51,** 1759–1765.

ELLIS, J.L. (1995). Neurokinin receptors subserving bronchoconstriction. *Can. J. Physiol. Pharmacol*., **73,** 923–926.

ELLIS, J.L. & UNDEM, B.J. (1994). Inhibition by capsazepine of resiniferatoxin- and capsaicin-induced contractions of guinea pig trachea. *J. Pharmacol. Exp. Ther*., **268,** 85–89.

FARMER, S.G., BROOM, T. & DESIATO, M.A. (1994). Effects of bradykinin receptor agonists, and captopril and thiorphan in ferret isolated trachea: evidence for bradykinin generation in vitro. *Eur. J. Pharmacol*., **259,** 309–313.

FARMER, S.G., BURCH, R.M., MEEKER, S.N. & WILKINS, D.E. (1989). Evidence for pulmonary bradykinin B_3 receptor. *Mol. Pharmacol*., **36,** 1–8.

FROSSARD, N., RHODEN, K.J. & BARNES, P.J. (1989). Influence of epithelium on guinea pig airway responses to tachykinins: role of endopeptidase and cyclooxygenase. *J. Pharmacol. Exp. Ther*., **248,** 292–298.

FÜGNER, A. (1985). *Pulmonary and Antiallergic Drugs*. pp. 123–190. New York: Wiley.

GEPPETTI, P. (1993). Sensory neuropeptide release by bradykinin–mechanisms and pathophysiological implications. *Regul. Pept*., **47,** 1–23.

GRUNDSTRÖM, N. (1986). Pharmacological characterization of the innervation of airway smooth muscle with special reference to modulation via prejunctional alpha-2-adrenoceptors. *Dissertation, Department of Pharmacology, Linköping University, Linköping, Sweden*.

GRUNDSTRÖM, N., ANDERSSON, R.G.G. & WIKBERG, J.E.S. (1981). Pharmacological characterization of the autonomous innervation of the guinea pig tracheo-bronchial smooth muscle. *Acta Pharmacol. Toxicol*., **49,** 150–157.

GRUNDSTRÖM, N., LINDSTRÖM, E.G., AXELSSON, K.L. & ANDERSSON, R.G.G. (1992). Epithelial modulation of allergen and drug effects in guinea pig airways. *J. Appl. Physiol*., **72,** 1953–1959.

HALL, J.M. (1992). Bradykinin receptors: Pharmacological properties and biological roles. *Pharmacol. Ther*., **55,** 131–190.

HOCK, F.J., WIRTH, K., ALBUS, U., LINZ, W., GERHARDS, H.J., WIEMER, G., HENKE, S., BREIPOHL, G., KONIG, W., KNOLLE, J. & SCHÖLKENS, B.A. (1991). Hoe 140 a new potent and long acting bradykinin-antagonist: in vitro studies. *Br. J. Pharmacol*., **102,** 769–773.

ICHINOSE, M. & BARNES, P.J. (1990). Bradykinin-induced airway microvascular leakage and bronchoconstriction are mediated via a bradykinin B_2 receptor. *Am. Rev. Respir. Dis.*, **142,** 1104–1107.

INGENITO, E.P., PLISS, L.B., MARTINS, M.A. & INGRAM, R.H. (1991). Effects of capsaicin on mechanical, cellular, and mediator responses to antigen in sensitized guinea pigs. *Am. Rev. Respir. Dis.*, **143,** 572–577.

LACH, E., TRIFILIEFF, A., MOUSLI, M., LANDRY, Y. & GIES, J.P. (1994). Bradykinin-induced contraction of guinea pig lung in vitro. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **350,** 201–208.

LAI, Y.L. (1991). Endogenous tachykinins in antigen-induced acute bronchial responses of guinea pigs. *Exp. Lung. Res.*, **17,** 1047–1060.

LILLY, C.M., HALL, A.E., RODGER, I.W., KOBIZIK, L., HALEY, K.J. & DRAZEN, J.M. (1995). Substance P-induced histamine release in tracheally perfused guinea pig lungs. *J. Appl. Physiol.*, **78,** 1234–1241.

LINDSTRÖM, E.G. & ANDERSSON, R.G.G. (1995). Morphine modulates contractile responses and neurokinin A-LI release elicited by electrical field stimulation or capsaicin in guinea pig bronchial-tube preparation. *Am. J. Respir. Crit. Care. Med.*, **151,** 1175–1179.

LINDSTRÖM, E.G., GRUNDSTROM, N., HAMMARSTROM, S. & ANDERSSON, R.G.G. (1992). Relationship between histamine, lipoxygenase and cyclooxygenase products in antigen-induced contraction in guinea-pig tracheal tube preparations. *Pharmacol. Toxicol.*, **71,** 216–220.

MANZINI, S., MAGGI, C.A., GEPPETTI, P. & BACCIARELLI, C. (1987). Capsaicin desensitization protects from antigen-induced bronchospasm in conscious guinea-pigs. *Eur. J. Pharmacol.*, **138,** 307–308.

MARTLING, C.R. (1987). Sensory nerves containing tachykinins and CGRP in the lower airways: functional implications for bronchoconstriction, vasodilation and protein extravasation. *Acta Physiol. Scand.*, **563,** 1–57.

MARTLING, C.R., THEODORSSON-NORHEIM, E. & LUNDBERG, J.M. (1987). Occurrence and effects of multiple tachykinins: substance P, neurokinin A and neuropeptide K in human lower airways. *Life Sci.*, **40,** 1633–1643.

MIURA, M., BEL VISI, M.G. & BARNES, P.J. (1992). Effect of bradykinin on airway neural responses invitro. *J. Appl. Physiol.*, **73,** 1537–1541.

MIURA, M., BELVISI, M.G. & BARNES, P.J. (1994). Modulation of nonadrenergic noncholinergic neural bronchoconstriction by bradykinin in anesthetized guinea pigs in vivo. *J. Pharmacol. Exp. Ther.*, **268,** 482–486.

MIZUGUCHI, M., FUJIMURA, M., AMEMIYA, T., NISHI, K., OHKA, T. & MATSUDA, T. (1996). Involvement of NK_2 receptors rather than NK_1 receptors in bronchial hyperresponsiveness induced by allergic reaction in guinea-pigs. *Br. J. Pharmacol.*, **117,** 443–448.

MOLIMARD, M., MARTIN, C.A.E., NALINE, E., HIRSCH, A. & ADVENIER, C. (1994). Contractile effects of bradykinin on isolated human small bronchus. *Am. J. Respir. Crit. Care. Med.*, **149,** 123–127.

PRUNEAU, D., LUCCARINI, J.M., DEFRENE, E., PAQUET, J.L. & BELICHARD, P. (1995). Pharmacological evidence for single bradykinin B_2 receptor in the guinea pig. *Br. J. Pharmacol.*, **116,** 2106–2112.

REGOLI, D., CALO, G., RIZZI, A., BOGONI, G., GOBEIL, F., CAMPOBASSO, C., MOLLICA, G. & BEANI, L. (1996). Bradykinin receptors and receptor ligands (with special emphasis on vascular receptors). *Regul. Pept.*, **65,** 83–89.

RICCIARDOLO, F.L.M., NADEL, J.A., GRAF, P.D., BERTRAND, C., YOSHIHARA, S. & GEPPETTI, P. (1994). Role of kinins in anaphylactic-induced broncoconstriction mediated by tachykinins in guinea-pigs. *Br. J. Pharmacol.*, **113,** 508–512.

SARIA, A., MARTLING, C.-R., YAN, Z., THEODORSSON-NORHEIM, E., GAMSE, R. & LUNDBERG, J.M. (1988). Release of multiple tachykinins from capsaicin-sensitive sensory nerves in the lung by bradykinin, histamine, dimethylphenyl piperazinium, and vagel nerve stimulation. *Am. Rev. Respir. Dis.*, **137,** 1330–1335.

WARTH, M.D.T.N., MALDONADO, E.A.L., FERNEZLIAN, S.M., LEME, A.S., PERINI, A., SALDIVA, P.H.N. & MARTINS, M.A. (1995). Neurokinin depletion attenuates pulmonary changes induced by antigen challenge in sensitized guinea pigs. *Am. J. Physiol.-Lung Cell M. Ph.*, **12,** L781–L788.

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