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Modulatory effect of imetit, a histamine H_3 receptor agonist, on C-fibers, cholinergic fibers and mast cells in rabbit lungs in vitro

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Abstract

The pharmacological mechanisms involved in the interactions between C-fibers, cholinergic fibers and mast cells were investigated in tracheally perfused rabbit lungs by measuring the simultaneous release of substance P and histamine in lung effluents. The amounts of substance P and histamine released in lung superfusates were measured by radioimmunoassay (RIA) after administration of capsaicin and carbachol. Capsaicin (10^{-4} M) induced a simultaneous increase in substance P ($273 \pm 56\%$ of baseline) and histamine ($460 \pm 138\%$) release. Similarly, carbachol (10^{-4} M) caused an increase in the release of both substance P ($367 \pm 111\%$) and histamine ($1379 \pm 351\%$). The effect of capsaicin was prevented by pretreating the lungs with the tachykinin NK_1 receptor antagonist SR 140333 (10^{-7} M), and atropine (10^{-6} M). SR 140333 prevented the carbachol-induced release of substance P but not of histamine. Exogenous substance P induced an increase in histamine release ($136 \pm 7\%$) which was significantly greater in lungs perfused with the neutral endopeptidase inhibitor, thiorphan (10^{-5} M) ($272 \pm 35\%$). This effect was prevented by atropine (10^{-6} M). Pretreatment of lungs with imetit (5×10^{-8} M), a selective H_3 receptor agonist, prevented the capsaicin-induced release of both mediators. Imetit also blocked the carbachol-induced release of substance P but not of histamine. Exogenous substance P-evoked histamine release was inhibited by imetit. Therefore, it can be concluded that substance P released through the action of capsaicin can activate cholinergic fibers, leading to cholinoceptor stimulation with subsequent activation of C-fibers and mast cells. While the presence of presynaptic H_3 receptors modulating substance P-induced acetylcholine release was only surmised, the existence of modulating histamine H_3 receptors on C-fibers was confirmed.

Keywords: Histamine H_3 receptor; Lung; (Rabbit); Substance P; Histamine; Mast cell; C-fiber; Cholinergic fiber

1. Introduction

Substance P is a tachykinin localized in C-fibers which belongs to the excitatory non-adrenergic, non-cholinergic nervous system, known for its bronchoconstrictor effects. These fibers innervate airways of several species and are found beneath the epithelium, around blood vessels (Lundberg et al., 1985; Hislop et al., 1990) and close to parasympathetic ganglia and mast cells (Dimitriadou et al., 1994; Domeij et al., 1996). Substance P can be released by various irritants, infectious agents and inflammatory mediators (Saria et al., 1988; Martins et al., 1991a; Hazbun et al., 1993). Substance P activates tachykinin receptors, resulting in an increase in vascular permeability (Rogers et al., 1988; Delaunois et al., 1993), bronchoconstriction (Advenier et al., 1987), vasodilatation (Delay-Goyet et al., 1992) and submucosal secretion (Rogers et al., 1989).

The interactions between C-fibers, cholinergic fibers and mast cells are potentially important in the development of neurogenic inflammation and have been demonstrated in various experimental models. The release of substance P, after stimulation of C-fibers by capsaicin, histamine and methacholine, has been described in tracheally perfused guinea pig lungs (Martins et al., 1991a,b). Similarly, Saria et al. (1988) have shown that substance P is released by histamine and vagal stimulation in vascularly perfused guinea pig lungs. The release of substance P plus thiorphan, following capsaicin stimulation of guinea pig isolated tracheal and bronchial segments, has also been recorded (Manzini et al., 1989). In turn, substance P can induce the release of histamine, as has been demonstrated

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for human pulmonary mast cells (Louis and Radermacker, 1990; Heany et al., 1995; Cross et al., 1996) and for tracheally perfused guinea pig lungs (Lilly et al., 1995). However, the simultaneous determination of substance P and histamine in the same experimental model, in order to investigate the interactions between C-fibers and mast cells, has never been performed.

The pathophysiological consequences of the interactions between C-fibers, cholinergic fibers and mast cells for endothelial permeability and lung mechanics have been studied in vascularly perfused rabbit lungs (Delaunois et al., 1993, 1994a, 1995b, 1996). The protective role of histamine H_3 receptors against acetylcholine- and capsaicin-induced pulmonary edema, under these conditions, has been described (Delaunois et al., 1995a). However, the localization of such receptors on C-fibers, mast cells or cholinergic fibers has not been resolved definitively.

Accordingly, the aim of the present study was two-fold: (1) to investigate the interactions between C-fibers, cholinergic fibers and mast cells in tracheally perfused rabbit lungs by measuring the simultaneous release of substance P and histamine in lung effluents and by using a specific tachykinin NK_1 receptor antagonist, SR 140333; and (2) to localize the histamine H_3 receptors on these structures by using a selective histamine H_3 receptor agonist, imetit.

2. Materials and methods

2.1. Tracheally superfused lungs

The following protocol has been approved by the Ethical Committee of the Faculty of Veterinary Medicine of the University of Liège. The animals were fed and kept according to the conditions described by European and Belgian laws relating to laboratory animals.

New Zealand white rabbits (males and females) weighing 2.5–3 kg were anesthetized with a single i.m. injection of ketamine (50 mg kg^{-1}) and buprenorphine (0.1 mg kg^{-1}). The trachea was isolated and cannulated. Pancuronium (0.2 mg kg^{-1}) was administered to the marginal ear vein to prevent reflex movements of the chest as a result of anoxia during the midsternal incision. The animals were ventilated with a small animal respirator (Palmer, Analis, Namur, Belgium) and 2000 U kg^{-1} of heparin was injected into the right ventricle before exsanguination. The lungs were removed from the chest, and numerous small holes were made in the pleural surface with a 25-gauge needle to facilitate the exit of the superfusate, as described by Martins et al. (1991a). The lungs were suspended and superfused via the trachea at a flow rate of 40 ml min^{-1} with a phosphate-buffered physiological solution (PBS), pH 7.5, containing (in mM): 137 NaCl, 1.8 $CaCl_2$, 1.05 $MgCl_2$, 2.68 KCl, 0.6 $NaHCO_3$, 0.13 NaH_2PO_4 and 0.87 Na_2HPO_4. This perfusion buffer was kept at 37°C and passed through a bubble trap before entering the lungs via the tracheal cannula. The lung effluents were collected in polypropylene tubes containing pure acetic acid to yield a final concentration of 5 vol.% and then kept on ice.

2.1.1. Substance P assay

Each perfusate collected was purified and concentrated, using Sep Pak C18 cartridges (Waters, Milford, MA, USA). These cartridges had been conditioned with 20 ml of methanol and washed with 20 ml of distilled water. The samples were then added to the cartridges and washed with 3 ml of distilled water. The peptide was eluted with 8 ml of aqueous solution containing 70% acetonitrile and 0.1% trifluoracetic acid. The eluates were dried under vacuum and kept at -20°C until the assays were performed. With this protocol, the recovery of added substance P in lung effluents was $75 \pm 4.5\%$.

Substance P-like immunoreactivity was determined by radioimmunoassay (RIA). The RIA for substance P was performed as described by Nemmar et al. (1998). The lower limit of detection was 7.4 pM. The assay had 0.1% cross reactivity against neurokinin A, 3% against neurokinin B and no cross-reactivity was recorded against both substance P-(1–7) and substance P-(1–9).

2.1.2. Histamine assay

The histamine content of lung effluents was determined with a commercially available RIA kit (Immunotech, Marseille, France). The lower limit of detection of this assay was 0.2 nM and its specificity was 100% against several endogenous analogues.

2.2. Experimental protocols

Four groups of rabbits were used for this study. Fifteen to twenty minutes after the period during which the lungs were suspended and perfused via the trachea with a PBS, the lung effluents from the numerous small holes made in the pleural surface were collected as three fractions corresponding to three consecutive periods of 3 min: before agonist administration, during continuous perfusion with the agonist, and after perfusion with an agonist-free buffer. All drug concentrations given in the text are final concentrations in perfusates.

The effects of a single dose of capsaicin (10^{-4} M) on the release of substance P and histamine in lung superfusates were studied in six rabbits ($n = 6$). Control values for capsaicin ($n = 5$) were calculated by measuring the amounts of substance P and histamine in lung effluents after administration of the solvent for capsaicin. In order to study the role of the tachykinin NK_1 receptors on the release of substance P and histamine induced by capsaicin, four lungs were perfused with a PBS containing SR 140333 (10^{-7} M) 8 min before the perfusion with capsaicin and during the 3-min stimulation with capsaicin. Other lungs

were pretreated with atropine (10^{-6} M; $n = 5$) to investigate any cholinergic contribution in the response to capsaicin.

The effects of a single dose of carbachol (10^{-4} M) on the release of substance P and histamine were studied in five rabbits. Control values for carbachol ($n = 5$) were determined after perfusion with a PBS that did not contain any drug. To assess the role of the tachykinin NK_1 receptors in the release of substance P and histamine induced by carbachol, five lungs were perfused with a PBS containing SR 140333 (10^{-7} M) 8 min before the perfusion with carbachol and during the 3-min stimulation with carbachol.

The effects of substance P (10^{-6} M; $n = 3$) alone and with thiorphan (10^{-5} M; $n = 5$), an inhibitor of neutral endopeptidase enzyme, on the release of histamine were studied. Control values for substance P ($n = 5$) were determined after perfusion with a PBS that did not contain any drug. To determine any cholinergic involvement in the response to exogenous substance P plus thiorphan, the lungs were pretreated with atropine (10^{-6} M; $n = 6$).

In order to study the influence of histamine H_3 receptors on the response to capsaicin, carbachol and exogenous substance P plus thiorphan, different groups of lungs were pretreated with a single dose of imetit (5×10^{-8} M), a histamine H_3 receptor agonist, before and during the administration of capsaicin ($n = 4$), carbachol ($n = 5$) and exogenous substance P plus thiorphan ($n = 4$).

2.3. Drugs

Carbamylcholine chloride, substance P and atropine (all supplied by Sigma, St. Louis, MO, USA) and imetit (Research Biochemical International, Natick, MA, USA) were dissolved in PBS. Capsaicin and thiorphan (both from Sigma) and SR 140333 ((*S*)1-(2-(3-(3,4-dichlorophenyl)-1-(3-*iso*-propoxyphenylacetyl)piperidin-(3-yl)-ethyl)-phenyl-1azoniabicyclo azoniabicyclo(2.2.2)octane chloride; kindly donated by Prof. Advenier, Paris, France), were dissolved in ethanol (0.3 ml) and then diluted in perfusion liquid. The solvents for the drugs had no effect on the release of substance P and histamine.

2.4. Statistics and calculations

The data are expressed as the means ± standard error of the mean (S.E.M). A paired or unpaired Student's *t*-test was used for the comparison of two means. Where necessary, the probability (P) values for significance were corrected by Bonferonni's method to allow multiple comparisons (Wallenoinstein et al., 1980). Differences were considered significant when $P < 0.05$.

For each lung, the substance P and histamine values recorded during the prestimulation period were considered as the baseline and expressed as 100%. The values for substance P and histamine recorded during or after stimulation periods were then expressed as percentages of their corresponding baseline values.

3. Results

3.1. Pharmacological modulation of capsaicin-induced substance P and histamine release

The mean baseline amounts of substance P and histamine recovered from rabbit lung effluents were 5 pM and 2.9 nM, respectively (pooling all the groups). No difference was observed between the baseline values of the different groups. In control lungs for capsaicin, no significant release of substance P (91 ± 8%) or histamine (105 ± 6%) was recorded when only the vehicle was administered.

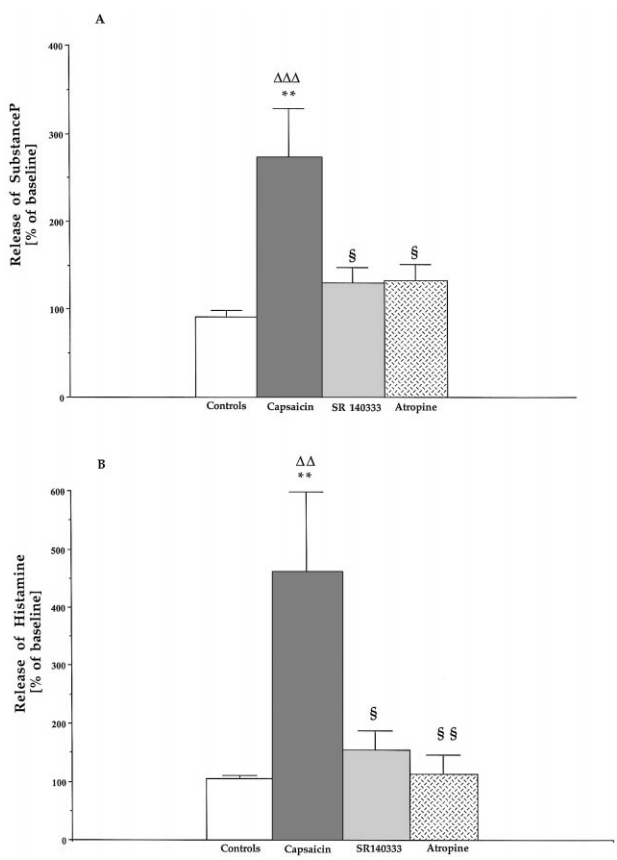

Fig. 1. Effect of capsaicin on release of substance P (A) and histamine (B) from rabbit lung in vitro. The values for substance P and histamine are expressed as percent of baseline, in control lungs ($n = 5$), in lungs treated with capsaicin without ($n = 6$) or with pretreatment with SR 140333 ($n = 4$), or atropine ($n = 5$). The mean baseline values of substance P and histamine were 5 pM and 2.9 nM, respectively (pooling all the groups). No difference was observed between the baseline values of the different groups. * Indicates a value significantly different from the baseline; ** indicates $P < 0.01$. $^{\Delta}$ Indicates a value significantly different from the corresponding value measured in control group; $^{\Delta\Delta}$ indicates $P < 0.01$; $^{\Delta\Delta\Delta}$ indicates $P < 0.001$. § Indicates a value significantly different from the corresponding value measured in capsaicin group ($P < 0.05$); §§ indicates $P < 0.01$.

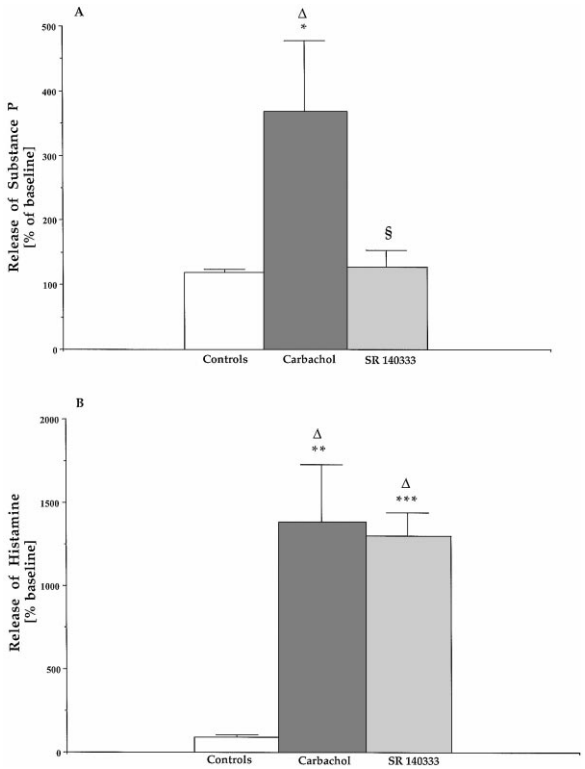

Fig. 2. Effect of carbachol on release of substance P (A) and histamine (B) from rabbit lung in vitro. The values for substance P and histamine are expressed as percent of baseline, in control lungs ($n = 5$), in lungs treated with carbachol without ($n = 5$) or with pretreatment with SR 140333 ($n = 5$). The mean baseline values of substance P and histamine were 5 pM and 2.9 nM, respectively (pooling all the groups). No difference was observed between the baseline values of the different groups. * Indicates a value significantly different from the baseline value ($P < 0.05$); ** indicates $P < 0.01$; and *** indicates $P < 0.001$. $^{\Delta}$ Indicates a value significantly different from the corresponding value measured in control group ($P < 0.05$). § Indicates a value significantly different from the corresponding value measured in carbachol group ($P < 0.05$).

Fig. 1 illustrates the effects of capsaicin (10^{-4} M) on the release of substance P and histamine in lung effluents. Capsaicin induced the simultaneous release of substance P and histamine, $273 \pm 56\%$ and $460 \pm 138\%$ of baseline values ($P < 0.01$), respectively.

Pretreatment of lungs with SR 140333 (10^{-7} M) strongly prevented the capsaicin-induced release of substance P ($131 \pm 17\%$) and histamine ($154 \pm 33\%$) ($P < 0.05$). Atropine also strongly inhibited the release of both mediators. The values recorded after pretreatment with atropine were $133 \pm 19\%$ ($P < 0.05$) and $113 \pm 19\%$ ($P < 0.01$) for substance P and histamine, respectively.

3.2. Pharmacological modulation of carbachol-induced substance P and histamine release

Like capsaicin, carbachol (10^{-4} M) induced the simultaneous release of substance P ($367 \pm 111\%$) ($P < 0.05$) and histamine ($1379 \pm 351\%$) ($P < 0.01$) (Fig. 2). No significant change was observed in the controls for carbachol.

Pretreatment with SR 140333 (10^{-7} M) blocked the release of substance P ($P < 0.05$) but not of histamine (Fig. 2).

3.3. Pharmacological modulation of exogenous substance P-induced release of histamine

Exogenous substance P induced a low but significant release of histamine ($136 \pm 7\%$ of baseline value) ($P < 0.05$) (Fig. 3). Following pretreatment of the lungs with thiorphan (10^{-5} M), the release of histamine induced by exogenous substance P was significantly enhanced ($272 \pm 35\%$ of baseline) ($P < 0.001$). Pretreatment with atropine (10^{-6} M) markedly blocked the release of histamine induced by substance P plus thiorphan ($115 \pm 16\%$ of baseline) ($P < 0.001$) (Fig. 3).

3.4. Pharmacological modulation of capsaicin-, carbachol- and exogenous substance P-induced release of mediators by imetit

Fig. 4 illustrates the effects of imetit on the capsaicin- and carbachol-induced release of substance P and histamine, and on the exogenous substance P-evoked histamine release. Pretreatment of the lungs with imetit prevented the capsaicin-induced release of both substance P

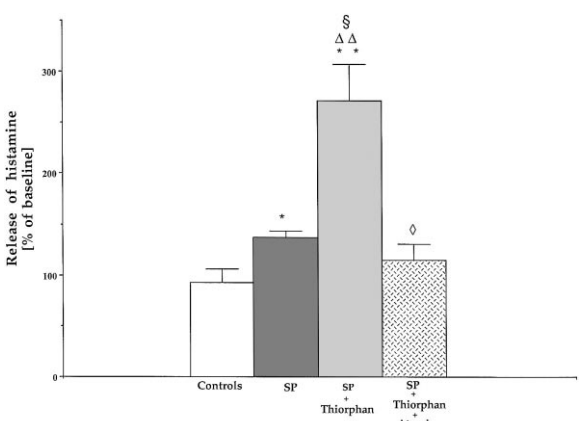

Fig. 3. Effect of substance P on release of histamine from rabbit lung in vitro. The values for histamine are expressed as percent of baseline, in control lungs ($n = 5$), in lungs treated with substance P without ($n = 3$) or with pretreatment with thiorphan ($n = 5$) and atropine ($n = 6$). The mean baseline values of histamine were 2.9 nM (pooling all the groups). No difference was observed between the baseline values of the different groups. * Indicates a value significantly different from the baseline value ($P < 0.05$); ** indicates $P < 0.001$. $^{\Delta}$ Indicates a value significantly different from the corresponding value measured in control group; $^{\Delta\Delta}$ indicates $P < 0.001$. § Indicates a value significantly different from the corresponding value measured in substance P group ($P < 0.001$). $^{\Diamond}$ Indicates a value significantly different from the corresponding value measured in substance P plus thiorphan group ($P < 0.001$).

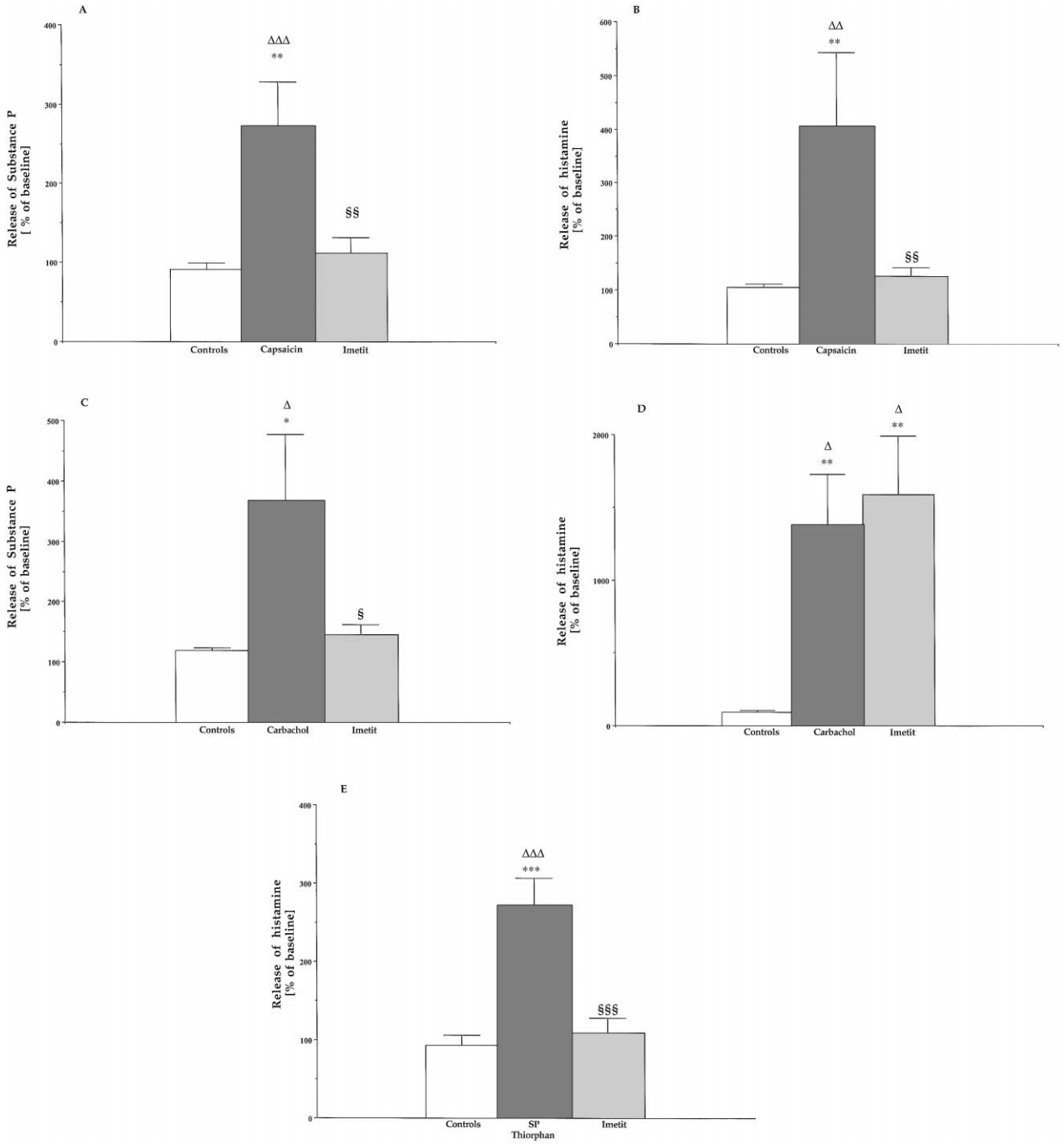

Fig. 4. Effect of imetit on the simultaneous release of substance P and histamine, expressed as percent of baseline, induced by capsaicin (A and B), carbachol (C and D) or substance P plus thiorphan (E). The mean baseline values of substance P and histamine were 5 pM and 2.9 nM, respectively (pooling all the groups). No difference was observed between the baseline values of the different groups. * Indicates a value significantly different from the baseline value ($P < 0.05$); ** indicates $P < 0.01$; and *** indicates $P < 0.001$. $^{\Delta}$ Indicates a value significantly different from the corresponding value measured in control group ($P < 0.05$); $^{\Delta\,\Delta}$ indicates $P < 0.01$; $^{\Delta\,\Delta\,\Delta}$ indicates $P < 0.001$. § Indicates a value significantly different from the corresponding value measured in capsaicin group, carbachol group and substance P plus thiorphan group ($P < 0.05$); §§indicates $P < 0.01$; §§§indicates $P < 0.001$.

($114 \pm 14\%$) ($P < 0.01$) and histamine ($125 \pm 11\%$) ($P < 0.01$). Imetit also blocked the carbachol-induced release of substance P ($146 \pm 17\%$) ($P < 0.05$) but not of histamine ($1592 \pm 404\%$). The substance P-induced release of histamine was prevented by pretreatment of the lungs with imetit ($109 \pm 19\%$ of baseline) ($P < 0.001$).

4. Discussion

The present study was designed to explore the interactions between C-fibers, cholinergic fibers and mast cells in tracheally perfused rabbit lungs, and to localize the histamine H_3 receptors involved in these interactions.

Substance P is a tachykinin localized in the airway sensory nerves of various species. Substance P-immunoreactive nerves are found under and within the airway epithelium, around blood vessels and within airway smooth muscle. Stimulation of sensory nerves results in various responses, including vasodilation, increased vascular permeability, gland secretion, airway smooth muscle contraction and coughing (see the work of Maggi (1995) for review).

The interactions between cholinergic fibers, C-fibers and mast cells appear potentially important in the development of neurogenic inflammation and bronchoconstriction. Morphological studies demonstrated a close relationship between C-fibers and mast cells (Alving et al., 1991; Dimitriadou et al., 1994). The relationship between nerves and mast cells seems to be bi-directional since mast cell products are able to stimulate a nervous reflex (Undem et al., 1991), while neuropeptides, in turn, can induce mast cell degranulation (Benyon et al., 1987). Interactions between C-fibers and cholinergic fibers have also been demonstrated. In guinea pigs, methacholine and vagal stimulation can induce the release of substance P (Saria et al., 1988; Martins et al., 1991a). Simultaneously with substance P, calcitonin gene-related peptide (CGRP) could also be released after C-fiber stimulation and act on mast cells (Foreman, 1993).

The baseline value for substance P recovered (0.33 pM g^{-1} min^{-1}) in our experimental model is similar to that described for guinea pig lungs (0.22 pM g^{-1} min^{-1}) (Martins et al., 1991b). The release of substance P following capsaicin (10^{-4} M) and carbachol (10^{-4} M) was 273% and 367% of the baseline value, respectively. These values are also in agreement with the data of Martins et al., 1991a,b, who described an increase of 300% and 200% after capsaicin (10^{-7} mol) and methacholine (10^{-8} mol) stimulation. The high doses of capsaicin and carbachol used in our study were selected on the basis of work showing their efficacy in rabbit lungs (Delaunois et al., 1995b). While capsaicin 10^{-4} M can induce pulmonary edema, lower concentrations failed to induce any effect. In our preparation, no responses were recorded when lower concentrations of capsaicin were used. Carbachol, 10^{-4} M, yielded a maximum effect. The baseline value for histamine recovered (0.24 nM g^{-1} min^{-1}) in our experimental model was lower than that described for guinea pig lungs (1.13 nM g^{-1} min^{-1}) (Lilly et al., 1995). The release of histamine following capsaicin (10^{-4} M) and substance P (10^{-6} M) was 460% and 272% of the baseline value, respectively. Lilly et al. (1995) described an increase of 1130% and 3045% after capsaicin (10^{-9} mol) and substance P (10^{-6} mol) stimulation.

In rabbits, capsaicin-induced pulmonary edema is blocked by atropine and SR 140333, a tachykinin NK_1 receptor antagonist, indicating a role of acetylcholine and tachykinins in this effect. Pulmonary edema induced by acetylcholine administration also being blocked by the tachykinin NK_1 receptor antagonist SR 140333, it was suspected that substance P, released from C-fibers, could act on the tachykinin NK_1 receptors present on cholinergic fibers, with the subsequent release of acetylcholine (Delaunois et al., 1995b). In turn, the latter activates the C-fibers via muscarinic receptors (Delaunois et al., 1994a). The data now obtained are in agreement with this hypothesis and confirm that substance P is really released in the rabbit lung upon the action of capsaicin and parasympathetic drugs (Figs. 1 and 2). They also confirm the determinant role of endogenous acetylcholine in the release of substance P in rabbit lungs, since capsaicin-induced substance P release was inhibited by atropine (Fig. 1). Moreover, as previously demonstrated in guinea pig lungs by Martins et al. (1991a), substance P could exert a positive feed back on its own release, as suggested by the blockade of carbachol-induced substance P release by the tachykinin NK_1 receptor antagonist SR 140333 (Fig. 2). A similar positive feed back by CGRP has also seen with the rabbit lung (Delaunois et al., 1994b).

In the rabbit, the interactions between nervous fibers and mast cells seem to depend on the target tissue. Indeed,

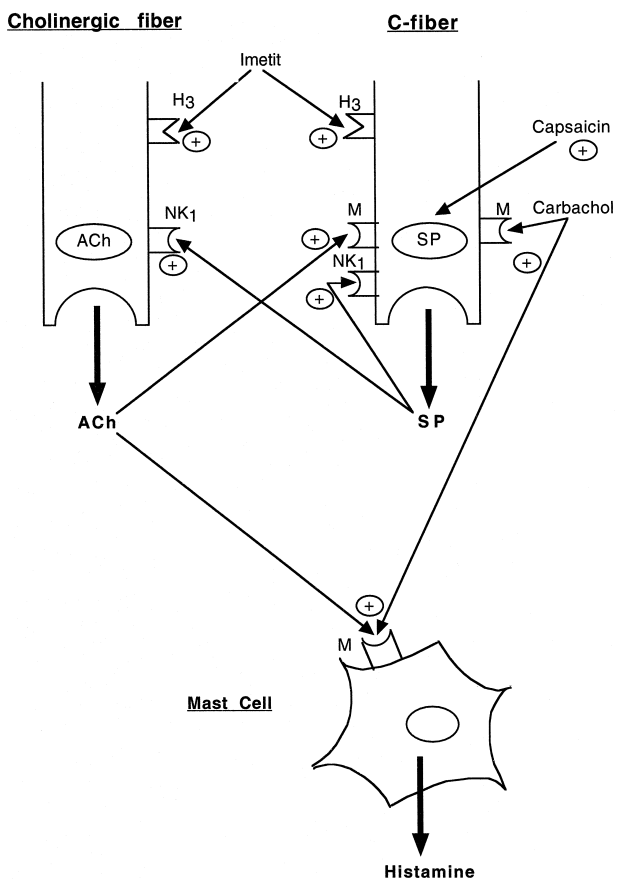

Fig. 5. Scheme of proposed mechanisms explaining the interactions between cholinergic fibers, C-fibers and mast cells in tracheally perfused rabbit lungs and the localization of histamine H_3 receptors. SP released by capsaicin can activate cholinergic fibers, leading to cholinoceptor stimulation with subsequent activation of the C-fibers and mast cells.

in vascularly perfused lung preparations, both exogenous and endogenous substance P-induced pulmonary edema through a direct action on mast cells (Delaunois et al., 1995b). In contrast, in the airways, substance P can contract smooth muscle cells independently of mast cells activation, while acetylcholine acts through degranulation of mast cells and through a direct effect on smooth muscle (Delaunois et al., 1996). The absence of any effect of the tachykinin a NK_1 receptor antagonist on the carbachol-induced histamine release (Fig. 2) and the fact that atropine can markedly reduce the substance P-induced histamine release (Fig. 3) argue in favour of an effect of muscarinic agonists on mast cells. However, the activation of another system cannot be excluded. Release of histamine and serotonin from cerebral arteries and abdominal and thoracic mast cells by muscarinic agonists, has also been demonstrated for both rabbit and rat (Insel and Kennedy, 1978; Reynier-Rebuffel et al., 1992). The direct effect of substance P on mast cells seen in rabbit and guinea pig lung (Delaunois et al., 1993; Lilly et al., 1995), and in isolated human pulmonary mast cells (Heany et al., 1995; Cross et al., 1996) was not detected in our experimental model (Fig. 3). However, one cannot exclude the ability of substance P to activate mast cells when higher concentrations of substance P are administered. This hypothesis was not tested due to the large amount of substance P necessary to obtain concentrations higher than 10^{-6} M in our preparation.

Recently, Delaunois et al. (1995a) demonstrated that histamine H_3 receptor activation by imetit, a well-known histamine H_3 receptor agonist with marked selectivity and high potency (Garbarg et al., 1992), can protect the lungs against acetylcholine and capsaicin-induced pulmonary edema via a prejunctional modulatory effect on C-fibers. The inhibition of carbachol- and capsaicin-induced substance P release by imetit (Fig. 4) confirms the presence of H_3 receptors on C-fibers.

The presence of such receptors on mast cells and cholinergic fibers was also proposed to explain the protective effect of imetit against exogenous substance P. However, the localization of H_3 receptors was not clearly determined (Delaunois et al., 1995a). The cascade of interactive events described above, and the absence of any effect of imetit on carbachol-induced histamine release in the present work (Fig. 4), seem to suggest that the activation of H_3 receptors, possibly located on mast cells, does not lead to the inhibition of muscarinic agonist-induced histamine release. However, the presence of H_3 receptors in this area cannot be completely excluded, as was suggested by Ichinose and Barnes (1990) for guinea pig lungs. The inhibitory effect of imetit against substance P-induced histamine release in rabbit lung (Fig. 4) could also be explained by activation of H_3 receptors on mast cells. However, since our data show an indirect action of substance P on mast cells through the activation of cholinergic fibers, the inhibition of substance P-induced histamine release by imetit (Fig. 4) argues in favour of the presence of H_3 receptors on cholinergic fibers. Such a cholinergic localization of histamine H_3 receptors has also been described for guinea pig ileum by Ichinose and Barnes (1989) and for human airways by Clapham and Kilpatrick (1992).

In light of all these results, it can be concluded that the isolated tracheally perfused rabbit lung is an appropriate in vitro model for the investigation of interactions between C-fibers, cholinergic fibers and mast cells. The following mechanisms, which are illustrated in Fig. 5 can be suggested. Substance P released through the action of capsaicin can activate cholinergic fibers, leading to cholinoceptor stimulation with subsequent activation of the C-fibers and mast cells. While the presence of presynaptic H_3 receptors modulating substance P-induced acetylcholine release was only surmised, the existence of modulating H_3 receptors on C-fibers was confirmed.

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