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Modulation of acetylcholine, capsaicin and substance P effects by histamine H_3 receptors in isolated perfused rabbit lungs

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Abstract

The modulatory role of histamine H_3 receptors in pulmonary oedema induced by acetylcholine, capsaicin and by exogenous substance P was investigated in isolated, ventilated rabbit lungs. Endothelial permeability was evaluated by measuring the capillary filtration coefficient (Kf,c). Acetylcholine (10^{-8} to 10^{-4} M), substance P (10^{-10} to 10^{-6} M), capsaicin (10^{-4} M) and 5-hydroxytryptamine (5-HT) (10^{-4} M) induced an increase in the Kf,c. Carboperamide, a novel histamine H_3 receptor antagonist, induced a significant leftward shift of the concentration-response curve to acetylcholine and also enhanced the effect of capsaicin on the Kf,c, while it had no significant effect on the response to substance P and 5-HT. Imetit, a new histamine H_3 receptor agonist, strongly inhibited the effects of acetylcholine and capsaicin. Imetit also strongly protected the lung against substance P effects but did not prevent the 5-HT-induced increase in the Kf,c. Carboperamide completely blocked the inhibitory effect of Imetit on the acetylcholine response. (*R*)-α-Methylhistamine, an other histamine H_3 receptor agonist, had the same protective effect against acetylcholine response as Imetit. We conclude that histamine H_3 receptors could protect the lung against acetylcholine- and capsaicin-induced oedema via a prejunctional modulatory effect on the C-fibres. However, since the response to exogenous substance P was also inhibited by histamine H_3 receptor stimulation, the presence of such receptors at a postsynaptic level, probably on mast cells, was also suggested.

Keywords: Histamine H_3 receptor; Lung, rabbit; C-fiber; Acetylcholine; Neuropeptide; Edema

1. Introduction

Histamine is an inflammatory mediator which can play an important role in the respiratory tract and which is involved in the pathogenesis of asthma and other airway diseases (Barnes et al., 1988). The classical effects of histamine on vascular or airway smooth muscle are mediated by two subtypes of receptors. Histamine can induce bronchoconstriction and microvascular leakage via histamine H_1 receptors (Chand and DeRoth, 1979; Saria et al., 1983). In some species, such as the rabbit, the activation of histamine H_2 receptors causes bronchodilatation (Chand and Eyre, 1975). It can also dilate human pulmonary vessels (Boe et al., 1980) or rabbit cerebral arteries (Ea-Kim and Oudart, 1988). Recently, a third type of histamine receptor (H_3 receptor) has been discovered in rat brain (Arrang et al., 1983), where it modulates histamine synthesis and release by a feedback mechanism on cerebral neurons. Such receptors have also been identified in the lung in binding studies (Arrang et al., 1987). They seem to be present on cholinergic nerves and capsaicin-sensitive fibres (C-fibres), since their stimulation inhibits both parasympathetic and non-adrenergic, non-cholinergic (NANC) neurotransmission in guinea pig and human airways (Ichinose and Barnes, 1989a,b). In addition, it was recently shown that stimulation of histamine H_3 receptors inhibits the synthesis of histamine in rat lung mast cells (Dimitriadou et al., 1994).

In a previous study on isolated perfused rabbit lungs, we have shown that acetylcholine can induce pulmonary oedema by increasing endothelial permeability (Delaunois et al., 1993a). The effect involves stimulation of C-fibres with subsequent release of substance P and calcitonin gene-related peptide (CGRP) (Delaunois et al., 1994a). The latter substances induce the release of 5-HT and histamine from mast cells and lead

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ultimately to oedema (Delaunois et al., 1993a). We have also demonstrated that several drugs, such as α_2-adrenoceptor agonists, morphine and neuropeptide Y (Delaunois et al., 1994b), can modulate the effects of acetylcholine by inhibiting the release of peptides from C-fibres.

Accordingly, the aim of the present study was: (1) to verify whether histamine H_3 receptors can modulate acetylcholine- and capsaicin-induced permeability changes in isolated rabbit lungs; (2) to determine if this modulatory effect is related to a feedback control on the C-fibres or to a postjunctional mechanism.

2. Materials and methods

2.1. General procedure

New Zealand White rabbits (male and female) weighing 2.5–3 kg were deeply anaesthetized with a single intramuscular injection of fentanyl (0.2 mg/kg) plus fluanisone (10 mg/kg) (Hypnorm; Janssen Pharmaceutica, Beerse, Belgium). The trachea of each animal was isolated and cannulated. Pancuronium (0.2 mg/kg) was administered into the marginal ear vein to prevent sudden reflex movements of the chest due to anoxia during midsternal incision. The animals were ventilated with a small animal respirator (Palmer; Analis, Namur, Belgium). The chest was opened by midsternal incision and heparin (2000 U/kg; Liquémine; Roche, Brussels, Belgium) was injected into the right ventricle before exsanguination. The heart-lung block was rapidly removed from the chest and weighed. Both ventricles were opened and glass cannulae (L: 15 mm; i.d.: 3 mm) were secured in the pulmonary artery and left atrium via the corresponding ventricles. The surrounding tissues were included in the ligature of the pulmonary artery and left atrium, so as to decrease the compliance of these structures (Wangensteen et al., 1977; Kern et al., 1984).

The isolated heart-lungs were suspended on one side of a beam balance. Lung weight was counterbalanced and further weight changes occurring during the experiments were measured with an isometric force transducer (Palmer; Analis, Namur, Belgium) attached to the lever of the balance. This system was calibrated before each experiment by suspending weights in place of the lung. The heart-lungs were connected to a recirculating perfusion circuit and perfused with a constant perfusate flow (20 ml/min/kg). The circuit included an open venous reservoir, an electronic roller pump (NY-7550-62 Masterflex, Bioblock, Illkirch, France) indicating the perfusate flow, a heat exchanger, and a bubble trap. Care was taken to avoid introducing air into the circuit. The lungs were first flushed until the remainder of blood in the vascular bed was completely removed, then perfused with 200 ml of artificial blood-free perfusate (Krebs-Ringer-bicarbonate buffer containing 2.5% bovine albumin (Sigma Chemicals Co., St. Louis, MO, USA)). The pH and temperature of the perfusate were continually monitored and maintained within physiological ranges (pH = 7.4--7.5; $t°$ = 37–38° C). The pH was controlled by adding 1 N $NaHCO_3$ or by aerating the liquid in the venous reservoir with 95% carbon dioxide and 5% oxygen. The pO_2 and pCO_2 of the perfusion liquid were checked at the beginning and at the end of each experiment. These parameters remained within physiological ranges in all experiments (pO_2 = 128–160.4 mm Hg; pCO_2 = 17.4–31.6 mm Hg). The lungs were ventilated with humidified room air at a frequency of 45 breaths per minute, a tidal volume of 5 ml/kg and an end-expiratory pressure (Pe) of 2 cm H_2O. They were periodically hyperinflated to avoid atelectasis and covered with a plastic bag to prevent evaporative fluid loss.

Arterial (Pa) and venous (Pv) pressures were measured by means of two thin catheters connected to side ports of the cannulae secured in the pulmonary artery and left atrium, and to pressure transducers (P23 i.d. and P50; Gould, Brussels, Belgium). The pressures were zero-referenced at the level of the lung hilus. Equilibration, obtained after 10–15 min, was characterized by an isogravimetric state, i.e. one in which no gain or loss of weight was observed, and by stability of arterial and left atrium pressures in zone III conditions (Pa > Pv > Pe). All parameters were recorded on a Gould recorder (TA 2000; Brussels, Belgium).

2.2. Experimental measurements

Capillary pressure (Pc) was measured by the double occlusion method used by Dawson et al. (1982) in dogs and by Gustin et al. (1994) in pigs. Simultaneous occlusion of both inflow and outflow (for a few seconds) induces an increase in Pv and a decrease in Pa until Pa and Pv equilibrate at the same pressure, providing a good estimate of capillary pressure (Hakim, 1988).

The capillary filtration coefficient (Kf,c) was measured by increasing Pa and Pv, usually by 10–15 cm H_2O for 2–4 min. Pc was estimated before and at the end of this period in order to determine the hydrostatic pressure difference (ΔPc) inducing fluid filtration across the endothelium. The amount of fluid filtering across was calculated from the weight-gain plot. At first, due to congestion, the lungs gained weight rapidly for 30–60 s following the vascular pressure increases. Thereafter, lung weight increased more slowly due to subsequent fluid filtration out of the vessels and accumulation in the lung tissue. The slopes of the slow phase of the weight increase ($\Delta W/\Delta t$) were plotted on a semi-logarithmic scale as a function of time and

extrapolated to zero-time. The value obtained was divided by ΔPc and by lung weight to yield the Kf,c expressed in milliliters (the fluid filtered was assumed to have a density of 1.0) per minute per cm H_2O per 100 g wet lung weight. Details of this method have been published (Drake and Gabel, 1981; Sumita et al., 1989; Delaunois et al., 1992; Gustin et al., 1994).

2.3. Experimental design

In each group of rabbit lungs, the baseline values of all parameters were measured after the equilibration period of the lung, ranging from 10 to 15 min. All drug concentrations given in the text are final concentrations in the perfusate (200 ml).

The vasoconstricting effects of acetylcholine, substance P and 5-HT were prevented with a single dose of papaverine (3×10^{-4} M). This pretreatment enabled us to measure permeability changes without interfering with the vascular response (Delaunois et al., 1993a). In groups 1 ($n = 9$) and 2 ($n = 5$), graded concentrations of acetylcholine (10^{-8} to 10^{-4} M) or substance P (10^{-10} to 10^{-6} M) were added in the circuit and the Kf,c was determined after each concentration, at 20-min intervals. In two other groups, the effects of a single dose of capsaicin (10^{-4} M; $n = 6$) or 5-HT (10^{-4} M; $n = 5$) were investigated. Control values were calculated by measuring the Kf,c value at 20-min intervals for 2 h in lungs not receiving any drug.

In order to study the influence of histamine H_3 receptors on the response to these mediators, eight groups of lungs were pretreated with a single dose of carboperamide (5×10^{-6} M) or Imetit (5×10^{-8} M) before the administration of acetylcholine, substance P, capsaicin or 5-HT. A lower concentration of Imetit (5×10^{-9} M) was also tested in three lungs treated

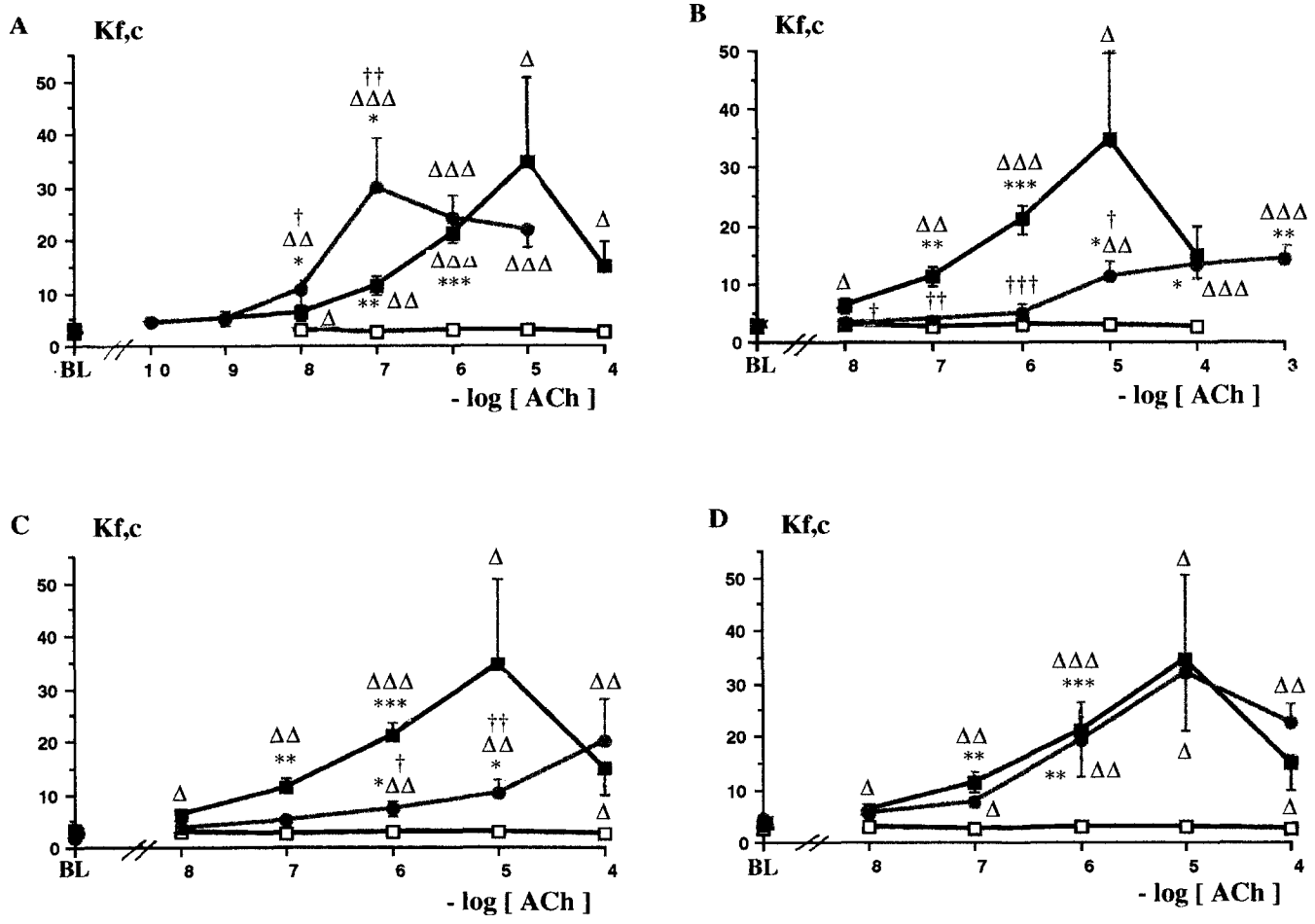

Fig. 1. Values for the capillary filtration coefficient (Kf,c), expressed as ml · min^{-1} · cm H_2O^{-1} · 100 g^{-1}, in control lungs (□; $n = 8$), in lungs treated with graded concentrations of acetylcholine without (■; $n = 9$) or with (●) pretreatment with carboperamide (5×10^{-6} M; $n = 5$) (panel A), Imetit (5×10^{-8} M; $n = 5$) (panel B), (*R*)-α-methylhistamine (3×10^{-7} M; $n = 3$) (panel C) or with Imetit (5×10^{-8} M) plus carboperamide (5×10^{-6} M) ($n = 3$) (panel D). Data are means and S.E.M. BL = baseline. Values significantly different from baseline in the same group: * $P < 0.05$, ** $P < 0.01$ or *** $P < 0.001$. Values significantly different from the value measured in the control group: $^{\Delta}$ $P < 0.05$, $^{\Delta\Delta}$ $P < 0.01$ or $^{\Delta\Delta\Delta}$ $P < 0.001$. Values significantly different from the value measured in the acetylcholine group: † $P < 0.05$ or †† $P < 0.01$.

with acetylcholine. In order to demonstrate the involvement of histamine H_3 receptors in these reactions, three lungs were also simultaneously pretreated with carboperamide and Imetit before the acetylcholine-concentration response curve was made. A second histamine H_3 receptor agonist, (*R*)-α-methylhistamine (3×10^{-7} M), was also used as pretreatment before acetylcholine administration.

It was verified that the histamine H_3 receptor agonists and antagonist had no direct effect on the parameters measured in this study.

2.4. Drugs

Acetylcholine chloride (Roche, Brussels, Belgium), substance P (Sigma Chemical Co., St. Louis, MO, USA), 5-hydroxytryptamine (Sigma Chemical Co., St. Louis, MO, USA) and (*R*)-α-methylhistamine (Bioprojet, Paris, France) were dissolved and diluted in NaCl 9‰. Imetit was provided by C.R. Ganellin (University College, London, UK) and dissolved in NaCl 9‰. Aliquots of substance P and Imetit were stored on ice. Papaverine chlorhydrate (Roche, Brussels, Belgium) was diluted in the perfusion liquid, to which was added HCl 1 N to facilitate dilution. Capsaicin (Sigma Chemical Co., St. Louis, MO, USA) was dissolved in ethanol (0.3 ml) and then diluted in the perfusion liquid. Carboperamide (1-(heptylcarbonyl)-4-(1*H*-imidazolyl-4)piperidine) (i.e. Arrang et al., 1992) was synthesized and provided by J.-C. Lancelot and M. Robba (Caen, France). It was dissolved in dimethyl sulphoxide and then diluted in NaCl 9‰. Aliquots were stored on ice. Reagents and solvents used to prepare the drugs had no effect on any measurement made.

2.5. Statistics and calculations

The data are expressed as means ± standard error of the mean (S.E.M.). Data were subjected to two-way analysis of variance (ANOVA 2). When the ANOVA was significant, a paired or unpaired Student's *t*-test was used for comparison of two means. When necessary, the *P* values for significance were corrected by Bonferroni's method to allow multiple comparisons (Wallenstein et al., 1980). Differences were considered significant when $P < 0.05$.

In response to acetylcholine and substance P, each lung exhibited a concentration-dependent increase in the Kf,c, followed by a decrease (see Results). This decrease was due to an artifact, explained by the overflow of the lung. Accumulation of fluid in the interstitial tissue can induce an increase in the hydrostatic tissue pressure, which opposes new fluid filtration across the endothelium. For each lung, the Kf,c value obtained just before the decrease was taken as the maximum effect (E_{max}). E_{max} is the highest value the Kf,c can reach under such experimental conditions. The EC_{50}, i.e. the molar concentration of agonist producing a Kf,c increase equivalent to 50% of the E_{max}, was calculated for each lung from the linear regression equation obtained with the values ranging from 10% to 90% of E_{max} and with the logarithm of the concentration. At least three values were considered. A mean EC_{50} was then calculated in each group. The pD_2 value was defined as the negative logarithm of the EC_{50}. This parameter allows comparison of the potency of several agonists to produce a similar effect.

3. Results

3.1. Effects of carboperamide on the permeability changes induced by acetylcholine, capsaicin, substance P and 5-HT

Fig. 1A illustrates the effects of acetylcholine on the Kf,c. Acetylcholine induced a concentration-dependent increase in this parameter. The mean pD_2 was 6.59 ±

Table 1
Values of pD_2 and E_{max} (ml · cm H_2O^{-1} · min^{-1} · 100 g^{-1}) obtained in the different groups of lungs treated with acetylcholine or substance P and the histamine H_3 receptor agonist or antagonist

Group	pD_2	E_{max}	*n*
Acetylcholine	6.59 ± 0.32	34.61 ± 15.88	9
Acetylcholine + carboperamide	7.99 ± 0.12 [a]	29.37 ± 9.35	5
Acetylcholine + Imetit	Not calculated [b]	14.25 ± 1.15 [c]	5
Acetylcholine + (*R*)-α-methylhistamine	Not calculated [b]	19.86 ± 8.16 [c]	4
Acetylcholine + carboperamide + Imetit	6.41 ± 0.23	32.14 ± 10.88	3
Substance P	8.59 ± 0.08	23.39 ± 6.07	5
Substance P + carboperamide	8.97 ± 0.37	20.67 ± 5.37	5
Substance P + Imetit	Not calculated [b]	8.43 ± 2.66 [c]	6

n = number of animals. Values are means ± S.E.M. [a] Value significantly different from that obtained in the acetylcholine group ($P < 0.001$). [b] pD_2 was not calculated because E_{max} had not recovered. [c] This value is the Kf,c obtained with the highest concentration of acetylcholine or substance P. It does not represent E_{max}.

Table 2
Values for capillary filtration coefficient (Kf,c), expressed as percentage of baseline value obtained in the different groups of lungs treated with capsaicin (10^{-4} M) or 5-HT (10^{-4} M) and the histamine H_3 receptor agonist or antagonist

Group	Kf,c	n
Control	100.4 ± 15.5	5
Capsaicin	271.3 ± 48.6 [a,d]	6
Capsaicin + carboperamide	907.4 ± 267.5 [a,d,e]	5
Capsaicin + Imetit	125.8 ± 29.4 [f]	4
5-HT	742.2 ± 312.2 [a]	5
5-HT + carboperamide	505.7 ± 112.8 [b,c]	3
5-HT + Imetit	607.6 ± 146.5 [b,c]	3

n = number of animals. Values are means ± S.E.M. [a] Value significantly different from the baseline in the same group (100%) ($P < 0.05$). [b] Value significantly different from the baseline in the same group ($P < 0.01$). [c] Value significantly different from the value measured in the control group ($P < 0.05$). [d] Value significantly different from the value measured in the control group ($P < 0.01$). [e] Value significantly different from the value measured in the group treated with capsaicin or 5-HT alone ($P < 0.05$). [f] Value significantly different from the value measured in the group treated with capsaicin or 5-HT alone ($P < 0.01$).

0.32. The effect was maximal (34.61 ± 15.88 ml · min^{-1} · cm H_2O^{-1} · 100 g^{-1}) at 10^{-5} M, lower values being recorded at higher concentrations. Lung volume increased considerably and pulmonary oedema was observed.

Carboperamide (5×10^{-6} M) had no direct effect on the Kf,c. The value recorded 10 min after administration of this drug (2.51 ± 0.36 ml · min^{-1} · cm H_2O^{-1} · 100 g^{-1}) did not differ from the baseline (2.67 ± 0.82 ml · min^{-1} · cm H_2O^{-1} · 100 g^{-1}). This histamine H_3 receptor antagonist induced a shift to the left of the concentration-response curve to acetylcholine (Fig. 1A), as shown by the significant increase in the pD_2 from 6.59 ± 0.32 to 7.99 ± 0.12 ($P < 0.001$) (Table 1).

Like acetylcholine, capsaicin (10^{-4} M) caused the Kf,c to increase to 271 ± 48% of baseline (Table 2). Pretreatment with carboperamide enhanced the effect of capsaicin ($P < 0.01$) (Table 2).

As shown in Fig. 2A, substance P increased the Kf,c in a concentration-dependent manner, with a pD_2 of 8.65 ± 0.08 and E_{max} of 23.62 ± 6.04 ml · min^{-1} · cm H_2O^{-1} · 100 g^{-1}. Carboperamide did not significantly affect this substance P-evoked response. The pD_2 value obtained with the histamine H_3 receptor antagonist (8.97 ± 0.37) did not differ from that observed without pretreatment (Table 1).

Carboperamide, likewise, did not significantly change the 5-HT-induced Kf,c increase (742.16 ± 312.16% of baseline without carboperamide ($n = 5$) vs. 505.67 ± 112.85% with carboperamide ($n = 4$)) (Table 2).

3.2. Effects of Imetit and (R)-α-methylhistamine on the permeability changes induced by acetylcholine, capsaicin, substance P and 5-HT

Imetit (5×10^{-8} M) had no direct effect on the Kf,c (3.32 ± 0.21 after Imetit vs. 2.87 ± 0.69 ml · min^{-1} · cm H_2O^{-1} · 100 g^{-1} for baseline). Pretreatment with Imetit (5×10^{-8} M) strongly inhibited the response of the Kf,c to acetylcholine (Fig. 1B). The pD_2 value was not calculated in this group because the E_{max} value had not recovered, even at 10^{-3} M of acetylcholine. Pretreatment with a lower concentration of Imetit (5×10^{-9} M) induced a rightward shift of the concentration-response curve to acetylcholine. The pD_2 value

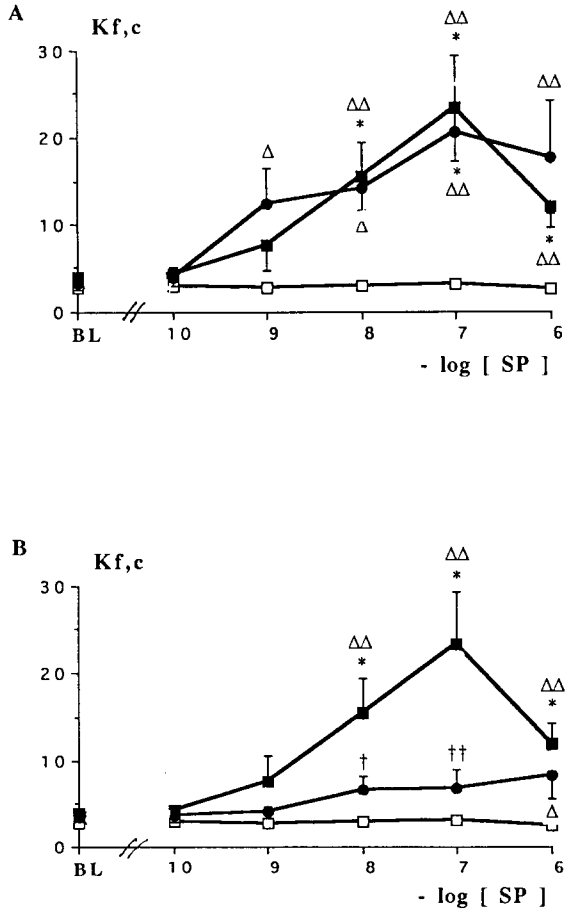

Fig. 2. Values for the capillary filtration coefficient (Kf,c), expressed as ml · min^{-1} · cm H_2O^{-1} · 100 g^{-1}, in control lungs (□; $n = 8$), in lungs treated with graded concentrations of substance P without (■; $n = 5$) or with (●) pretreatment with carboperamide (5×10^{-6} M; $n = 5$) (panel A) or Imetit (5×10^{-8} M; $n = 6$) (panel B). Data are means and S.E.M. BL = baseline. Values significantly different from baseline in the same group: * $P < 0.05$, ** $P < 0.01$ or *** $P < 0.001$. Values significantly different from the value measured in the control group: Δ $P < 0.05$, ΔΔ $P < 0.01$ or ΔΔΔ $P < 0.001$. Values significantly different from the value measured in the substance P group: † $P < 0.05$ or †† $P < 0.01$.

was reduced to 5.65 (mean for $n = 3$). Imetit also completely inhibited the effects of capsaicin on the Kf,c ($P < 0.01$) (Table 2). The substance P-induced response was strongly inhibited by Imetit (Fig. 2b). As with acetylcholine, the E_{max} value did not recover, even at high doses of substance P. In contrast, the histamine H_3 receptor agonist did not prevent the 5-HT-induced permeability changes (Table 2).

(*R*)-α-Methylhistamine (3×10^{-7} M) also strongly inhibited the effects of acetylcholine on the Kf,c (Fig. 1C).

The inhibitory effect of Imetit (5×10^{-8} M) on the acetylcholine-induced response was completely prevented by carboperamide (5×10^{-6} M) (Fig. 1D). The E_{max} and pD_2 values obtained with carboperamide plus Imetit were similar to that observed in the lungs treated with only acetylcholine (Table 1).

4. Discussion

This study demonstrated that histamine H_3 receptor stimulation by Imetit prevents the acetylcholine- and capsaicin-induced pulmonary oedema, while carboperamide enhanced these effects. The permeability changes induced by exogenous substance P were also inhibited by Imetit, but not modified by carboperamide.

The role of the NANC nervous system in controlling vascular and airway smooth muscle or glandular secretions is now well recognized in many species, including the human (Lundberg and Saria, 1987). Afferent nerve endings, also called C-fibres, are located in the upper airway as well as in the lower respiratory tract (Joos et al., 1988). These fibres contain neuropeptides such as substance P, calcitonin gene-related peptide and neurokinins A and B which act as neurotransmitters. These substances can be released locally with various stimuli such as cigarette smoke or administration of capsaicin (Lundberg et al., 1983). In a previous study, we showed that acetylcholine induces pulmonary oedema in the rabbit by increasing endothelial permeability (Delaunois et al., 1993a). The mechanisms involved in this reaction have been investigated. Acetylcholine activates muscarinic receptors located on C-fibres, which subsequently release neuropeptides such as substance P and CGRP (Delaunois et al., 1994a). These neuropeptides trigger a cascade of events involving 5-HT, most likely released from mast cells, and arachidonic acid derivatives. Capsaicin, by activating the same cascade of events, also induces pulmonary oedema (Delaunois et al., 1993b). The vasoconstrictive effects of acetylcholine in our preparation, which have previously been shown to be related to a direct action on vascular smooth muscle (Delaunois et al., 1994b), are not considered here.

Various drugs can interfere with C-fibres at a prejunctional level. α_2-Adrenoceptor agonists (Grundström and Andersson, 1985; Matran et al., 1989), opiate agonists (Frossard and Barnes, 1987) and γ-aminobutyric acid (Belvisi et al., 1989) can prevent C-fibre activation, thus inhibiting the release of neuropeptides. Such substances probably act on their respective receptors, located on the nerve fibres. We have recently shown that clonidine, morphine, and also neuropeptide Y, can prevent acetylcholine- and capsaicin-induced oedema by a negative control on these fibres (Delaunois et al., 1994b). The aim of the present study was to verify whether histamine H_3 receptor stimulation can protect the lung against capsaicin- and acetylcholine-induced permeability changes via a prejunctional mechanism.

Histamine H_3 receptors, which appear pharmacologically distinct from the histamine H_1 and H_2 receptor subtypes, were first detected in rat brain (Arrang et al., 1983). Their stimulation inhibits the synthesis and release of histamine from cerebral neurons via a feedback mechanism. There is now increasing evidence of prejunctional histamine H_3 receptors in various peripheral tissues. For instance, binding studies have shown that histamine H_3 receptors are also present in guinea pig lung (Arrang et al., 1987). The role of these receptors in the airways was investigated by using (*R*)-α-methylhistamine and thioperamide as selective agonist and antagonist, respectively. In vitro studies on human (Ichinose and Barnes, 1989b) and guinea pig (Ichinose et al., 1989) airways demonstrated that histamine H_3 receptors can modulate cholinergic neurotransmission by inhibiting acetylcholine release from parasympathetic nerves. The stimulation of histamine H_3 receptors also appears to inhibit the NANC-evoked bronchoconstriction and airway microvascular leakage induced by electrical stimulation of the vagus nerve in atropine-pretreated guinea pigs (Ichinose and Barnes, 1989a; Ichinose et al., 1990), probably by reducing the release of neuropeptides from the C-fibres. These inhibitory presynaptic histamine H_3 receptors also modulate histamine synthesis in mast cells from rat lung (Dimitriadou et al., 1994). In our preparation, Imetit, a novel histamine H_3 receptor agonist with marked selectivity and higher potency than (*R*)-α-methylhistamine (Garbarg et al., 1992), strongly inhibited the effects of capsaicin and acetylcholine on endothelial permeability, while carboperamide, a new potent histamine H_3 receptor antagonist showed opposite effects. The concentration of Imetit used in the present work was similar to that necessary to develop a maximal response in the rat brain slice model (Garbarg et al., 1992). However, we also used a lower concentration of Imetit in order to confirm that the inhibitory role of the agonist not only exists at maximal concentrations. Moreover, the second histamine H_3 receptor agonist,

(*R*)-α-methylhistamine, at a maximal concentration, had the same inhibitory effect. The fact that the modulatory role of Imetit was blocked by carboperamide also confirms that this effect is due to histamine H_3 receptor activation. Moreover, the enhancement of the response to capsaicin and acetylcholine by carboperamide alone suggests that these histamine H_3 receptors are under tonic control by endogenous histamine.

Since carboperamide had no effect on the response induced by exogenous substance P and 5-HT, it could be speculated that histamine H_3 receptors were located on the C-fibres where they could inhibit the release of neuropeptides. However, the fact that Imetit also strongly protects the lung against the effects of exogenous substance P, but not against 5-HT, suggests that histamine H_3 receptors, different from those located on C-fibres, could also be present at a postjunctional site. Postsynaptic histamine H_3 receptors could likely be located on the mast cells, as previously speculated by Ichinose and Barnes (1990) in the guinea pig lung. However, another localization could also be proposed based on our previous data (Delaunois et al., 1993b,1995) showing that substance P induces permeability changes by acting on mast cells but also partly by stimulating parasympathetic fibres, with subsequent release of acetylcholine responsible for C-fibre activation. The presence of histamine H_3 receptors on cholinergic nerves could also explain the protective effect of Imetit against exogenous substance P. However, the fact that this inhibitory effect against substance P was very marked, while blockade of parasympathetic effects by hemicholinium-3 or atropine provided only a partial inhibition of exogenous substance P effects (Delaunois et al., 1995), argues instead in favour of the existence of histamine receptors on mast cells. Thus, we could suggest the presence of two subpopulations of histamine H_3 receptors in the rabbit lung. One, stimulated by endogenous histamine, could be located on the C-fibres and could be sensitive to carboperamide, i.e. explaining the enhancing effect of this antagonist against acetylcholine and capsaicin. The other population, probably not occupied by endogenous histamine under experimental conditions, is insensitive to carboperamide and could be present on mast cells or on cholinergic nerves.

In the light of all these results, the following mechanism could be proposed. In a non-stimulated lung, substance P is released from C-fibres at a basal level. This is suggested by the fact that thiorphan (10^{-5} M), an endopeptidase inhibitor, induced a spontaneous increase in endothelial permeability in our preparation (non-published data). Basal release of substance P has also been demonstrated in guinea pig trachea (Bloomquist and Kream, 1990). Spontaneous or substance P-induced release of basal levels of histamine (and 5-HT) could also be speculated upon. By a feedback control, histamine could activate histamine H_3 receptors located on C-fibres, then modulating the release of substance P by these fibres. However, the fact that blockade of histamine H_3 receptors by carboperamide did not spontaneously increase endothelial permeability shows that the basal release of endogenous histamine does not seem important. A negative feedback of histamine on its own release from mast cells cannot be precluded.

When the C-fibres are stimulated by acetylcholine or capsaicin, higher amounts of substance P are released. Stimulation of histamine H_3 receptors located on both C-fibres and mast cells or cholinergic nerves by specific agonists enhanced the inhibitory effect of the endogenous histamine, thus protecting the lung against acetylcholine-, capsaicin- and substance P-induced oedema. In contrast, the use of the histamine H_3 receptor antagonist displaced endogenous histamine and enhanced the effects of acetylcholine and capsaicin.

Thus, we conclude that histamine H_3 receptors could protect the rabbit lung against acetylcholine- and capsaicin-induced permeability changes via a prejunctional modulatory effect on the C-fibres. The presence of other subpopulations of histamine H_3 receptors, located on mast cells or cholinergic nerves, could also be suggested to explain the protective effect against exogenous substance P obtained after histamine H_3 receptor stimulation.

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