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IgG- and IgE-mediated Histamine Release from Superfused Guinea-pig Airway Tissues

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Abstract—Anaphylactic histamine release and the inhibition by the β-adrenoceptor agonist fenoterol has been investigated using lung and tracheal tissues from two groups of guinea-pigs, differently sensitized to respond with IgG or IgE antibodies, respectively. A superfusion method was introduced and compared with classical batch incubation. The difference between IgG- and IgE-mediated histamine release during superfusion of both tissues was much greater than the difference obtained during batch-incubations. Fenoterol inhibited IgG-mediated histamine release during superfusion at lower concentrations and to a larger extent than the release from IgE-sensitized tissues. The inhibition by fenoterol was less pronounced after batch-wise incubations, preincubation at 0°C abolished the quantitative difference of IgG- and IgE-mediated histamine release from lung slices as well as the difference in β-adrenergic inhibition. It is concluded that the new superfusion procedure for airway tissues enhances the sensitivity of antigen-induced histamine release for pharmacological modulation, compared with batch-wise incubation. In addition, the effects of 0°C pretreatment show that cooled transport and storage of airway tissue should be considered with care.

β-Adrenoceptor agonists are widely used for the treatment of allergic, as well as non-allergic, asthmatic disorders. These drugs reduce airway smooth muscle tone and inhibit the release of allergic mediators (Orange et al 1971; Marone et al 1979; Hughes et al 1983). Although both β_1- and β_2-adrenoceptors have been found to be involved in the relaxation of guinea-pig but not human airway smooth muscle (Zaagsma et al 1983), the inhibition of antigen-induced histamine release from lung mast cells is mediated by a homogeneous population of β_2-adrenoceptors in animal (Daly & Levy 1979; Undem & Buckner 1984; Zaagsma et al 1984) as well as human tissues (Butchers et al 1980). However, major differences have been observed between the concentrations of β-adrenoceptor agonists which inhibit contractile responses to antigen and the concentrations which decrease mast cell histamine release or relax airway smooth muscle. Fenoterol displayed these discrepancies most clearly (Hughes et al 1983; Zaagsma et al 1984). Whereas antigen-induced contractions were reduced at (sub)nanomolar concentrations, the inhibition of histamine release and the relaxation of airway smooth muscle showed EC50 values of more than 10 nM.

The apparent affinity of an agonist for its receptor is often determined by experimental conditions. For example, the relaxation of airway smooth muscle requires higher concentrations of a β-adrenoceptor agonist when the concentrations of an exogenously applied contractile agonist or endogenously released mediators increase (Van Amsterdam et al 1990). Allergic histamine release can be modulated by other mediators which also interfere with its β-adrenergic inhibition (Van Amsterdam et al, unpublished). The quantity and composition of released mediators depend on the method of sensitization (Andersson 1980; Regal 1984; Undem et al 1985a). Furthermore, tissue oxygen supply can change IgE-mediated mast cell degranulation (Nadziejko et al 1989) and rapid changes of temperature have been demonstrated to initiate the production of prostaglandins and leukotrienes in guinea-pig trachea (Souhrada et al 1983). This temperature effect is particularly relevant since many experimental protocols include cold storage of tissue.

In the present study we have compared histamine release from batch-wise incubated guinea-pig lung slices and tracheal rings with the release from superfused tissues. The latter method prevents the accumulation of released mediators. Furthermore, temperature and oxygen supply of the surrounding buffer are optimal during superfusion. Using the two methodologies, airway tissues from IgG- and IgE-sensitized guinea-pigs were compared for antigen-induced histamine release and its β-adrenergic inhibition.

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Materials and Methods

Sensitization procedure

Outbred guinea-pigs of either sex, 250–350 g, were actively sensitized to respond with mainly IgG or IgE class antibodies as described previously (Van Amsterdam et al 1991). In brief, IgG antibodies were induced using an emulsion of equal volumes of 0·9% NaCl (saline) with ovalbumin (100 μg mL^{-1}) and Freund's complete adjuvant (FCA). Of this mixture, 0·5 mL containing 50 μg ovalbumin was injected i.p. and 50 μL, containing 5 μg ovalbumin, was injected intradermally in the proximity of lymph nodes in paws, lumbar region, and neck. A shift to IgE class antibodies was obtained by injection of ovalbumin containing saline with 200 mg aluminium hydroxide powder per mL instead of FCA which had been gently rotated for 60 min to obtain a gel (alu-gel). The quantities of ovalbumin used and the injection sites were as for the IgG group. The passive cutaneous anaphylaxis test (Watanabe & Ovary 1977), was used to demonstrate that following sensitization, alu-gel-treated guinea-pigs produced

much less IgG and greater quantities of IgE antibodies than FCA-treated animals (Van Amsterdam et al unpublished).

Anaphylactic histamine release from lung tissue

Four to eight weeks after sensitization, guinea-pigs were killed by a sharp blow on the head and exsanguinated. The lungs and trachea were rapidly removed and kept at 37°C in Krebs-Henseleit solution of the following composition (mM): NaCl, 117·5; KCl, 5·6; $MgSO_4$, 1·18; $CaCl_2$, 2·5; NaH_2PO_4, 1·28; $NaHCO_3$, 25·0; glucose, 5·5; pH 7·4 which was permanently gassed with 95% O_2–5% CO_2. Peripheral lung tissue was prepared free from major blood vessels and bronchi and chopped into 0·5 mm^3 fragments using a McIllwain tissue chopper. The trachea was cut into separate rings after careful removal of serosal and connective tissue.

Subsequently, the lung tissue was divided into 100 mg (wet weight) portions which were placed in up to sixteen 150 μL superfusion chambers. Also two to four tracheal rings, up to a total weight of approximately 200 mg, were placed in individual chambers. Tissues were then equilibrated at 37°C during 60 min at a superfusion rate of 400 $\mu L\ min^{-1}$ of gassed buffer. Subsequently, different concentrations of fenoterol or vehicle were added to the buffer superfusing separate chambers. Ovalbumin, 10 $\mu g\ mL^{-1}$, was added to the buffer 15 min later. During 90 s before and several periods of 90 s after antigen challenge, superfusate was sampled in separate polyethylene cups (Technicon). The histamine content, directly from these cups, was quantitated utilizing a Technicon automated continuous-flow fluorimetric assay system, essentially according to Siraganian (1974). After 6 min of antigen challenge, when no more measurable amounts of histamine were released, the superfusion was stopped and the Krebs-Henseleit replaced by 0·4 M perchloric acid solution (PCA). Residual histamine was extracted from the tissue during four brief superfusion periods of 15 s at 2 $mL\ min^{-1}$ with PCA, separated by 15 min intervals to ensure full penetration of the PCA in the tissue. This method was shown in separate experiments to extract the residual histamine as efficiently as by boiling for 10 min in PCA. Total histamine was calculated from the summation of released and extracted histamine.

For batch incubations, lung slices were prepared in the same way as for superfusion experiments. Part of the lung slices was equilibrated for 2 h in 10 mL of gassed Krebs-Henseleit at 37°C, which was continuously refreshed using a peristaltic pump at a rate of 3·9 $mL\ min^{-1}$ and the remaining part at 0°C for the same period. Subsequently, 30–50 mg (wet weight) portions of the 0°C- and 37°C-treated lung slices were incubated in 1·5 mL Krebs-Henseleit buffer for 20 min at 37°C in a Dubnoff metabolic shaker under an atmosphere of 95% O_2–5% CO_2 with the final 10 min in the absence or presence of different concentrations of fenoterol. The tissue was challenged with 10 $\mu g\ mL^{-1}$ ovalbumin and 10 min later histamine release was stopped by placing the vials on ice. Samples of 1 mL were taken for quantitation of histamine content, and the remaining volume was acidified with PCA to 0·4 M and boiled for 10 min in order to extract residual histamine.

Chemicals

Aluminium hydroxide powder was purchased from Janssen Pharmaceutica (Beerse, Belgium), Freund's complete H37 RA adjuvant from Difco Labs (Detroit, Michigan, USA), ovalbumin (twice crystallized) from Koch-Light (Haverhill, UK). Fenoterol hydrobromide (Boehringer-Ingelheim, Ingelheim, Germany) was kindly provided by the manufacturer. All other chemicals were of reagent grade.

Data evaluation

Anaphylactic histamine release was calculated as percentage of total histamine content of the tissue or as percentage of paired control release. The Wilcoxon rank correlation test and the Student's paired *t*-test were used to estimate statistical differences between groups.

Results

Antigen-induced histamine release

Spontaneous histamine release during superfusion (90 s samples), measured preceding antigen challenge, was below the detection level of the auto-analyser of 0·5–2 ng histamine. The total histamine content of the tissue samples in the superfusion chambers amounted to approximately 2 and 10 $\mu g\ g^{-1}$ wet weight for trachea and lung slices, respectively, which was similar for IgE- and IgG-sensitized tissue. Thus, less than 0·2% of total histamine was released spontaneously during a 90 s sampling period.

Antigen-induced histamine release (% total histamine) from superfused IgE-sensitized lung tissue amounted to 28·4 ± 2·5% (n = 24) which was much higher than from IgG-sensitized lung (13·2 ± 1·3%, n = 21, $P < 0.001$). A similar difference in the histamine release was observed between the tracheas from IgE- (21·9 ± 1·8%, n = 17) and IgG- sensitized animals (13·1 ± 2·1%, n = 13, $P < 0.001$). Fig. 1 illustrates these differences and also shows that the quantitative differences between IgE- and IgG-mediated histamine release from lung slices and tracheal rings obtained after batch-wise incubations were less pronounced. Furthermore, for lung slices under batch-wise conditions, it is shown that exposition to 0°C during the preincubation period virtually

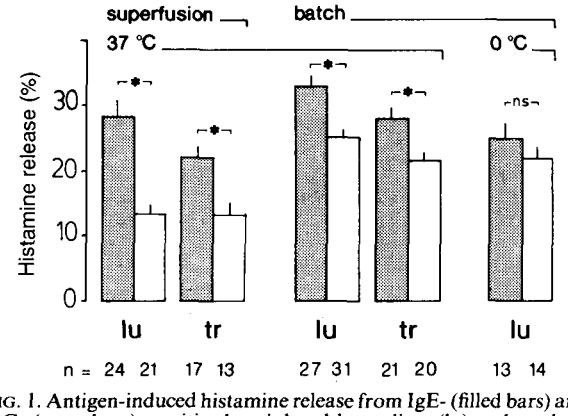

FIG. 1. Antigen-induced histamine release from IgE- (filled bars) and IgG- (open bars) sensitized peripheral lung slices (lu) and tracheal rings (tr). Data from superfusions are compared with data from batch incubations without and after cold treatment during preincubation as indicated. Data, expressed as % total histamine, represent means ± s.e.m. of n separate experiments. Statistical differences between IgE- and IgG-mediated histamine release are indicated by * $P < 0.001$ as determined in a Wilcoxon rank correlation test. ns = not significant.

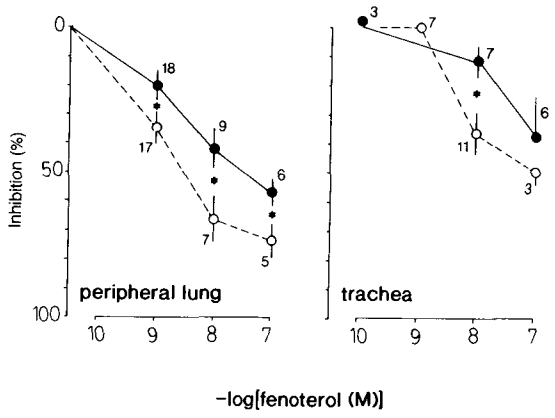

FIG. 2. Inhibition of antigen-induced histamine release from guinea-pig superfused lung slices (left panel) and trachea (right panel), taken from IgE- (●) and IgG- (○) sensitized guinea-pigs by different concentrations of fenoterol. Data, expressed as % inhibition of paired control release, represent means ± s.e.m. of separate experiments. Numbers of experiments are indicated along with the data points. Statistical differences between the inhibition of IgE- and IgG-mediated histamine release are indicated by *($P < 0.05$) as determined in a Wilcoxon rank correlation test.

abolished the quantitative difference between IgE- and IgG-mediated histamine release (Fig. 1, right panel).

Inhibition of histamine release by fenoterol

The inhibition by fenoterol of antigen-induced histamine release was investigated using the superfusion technique.

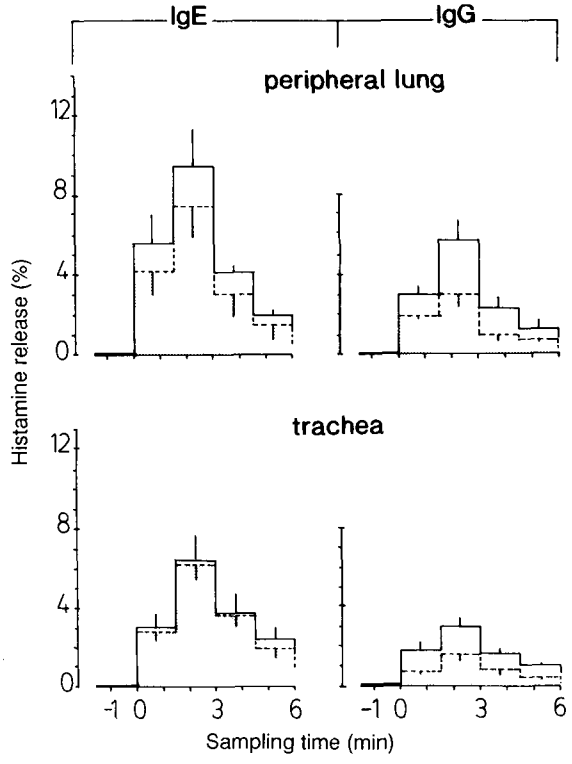

FIG. 3. Time courses of IgE-(left panels) and IgG-(right panels) mediated histamine release from guinea-pig superfused peripheral lung slices and tracheal rings in the absence (solid lines) and presence (broken lines) of 1 nM (lung, upper panels) or 10 nM (trachea, lower panels) of fenoterol. Data, expressed as % total histamine, represent means ± s.e.m. of 4 paired experiments.

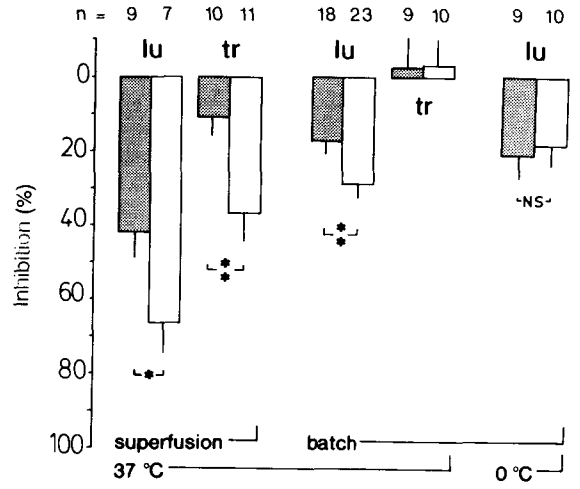

FIG. 4. Inhibition by 10 nM fenoterol of IgE- (filled bars) and IgG- (open bars) mediated histamine release from guinea-pig peripheral lung slices (lu) and tracheal rings (tr). Data obtained from superfusions and batch incubations with preincubations at 37 and 0°C are compared as indicated and are expressed as % inhibition of paired control release, and represent means ± s.e.m. of n separate experiments. Statistical differences between the inhibition of IgE- and IgG-mediated histamine release are indicated by *($P < 0.02$) and **$P < 0.01$ as determined in a Wilcoxon rank correlation test. ns = not significant.

Fig. 2 shows the effects of increasing concentrations of fenoterol for peripheral lung and trachea.

Histamine release from peripheral lung was inhibited by 1 nM fenoterol (IgE, 19·9 ± 5·2%, n = 18, $P < 0.01$; IgG, 34·7 ± 5·6%, n = 17, $P < 0.001$), whereas tracheal release required 10 nM (IgE, 11·2 ± 5·1%, n = 7, $0.05 < P < 0.1$; IgG, 36·7 ± 7·7%, n = 11, $P < 0.001$) or higher concentrations. At 1, 10, and 100 nM, fenoterol inhibited IgG-mediated histamine release from peripheral lung more strongly than IgE-mediated release ($P < 0.02$) and above 10 nM fenoterol on, a similar difference was found with tracheal tissue. The higher potency of fenoterol to inhibit IgG-mediated histamine release is also shown in Fig. 3 which illustrates the time courses of histamine release during superfusion of lung and trachea, respectively.

Fig. 4 compares the influence of superfusion and batch-wise incubation on the inhibition of histamine release from peripheral lung and trachea by 10 nM fenoterol. With both tissues, histamine release is more strongly inhibited by 10 nM fenoterol during superfusion than during batch incubations, irrespective of the sensitization procedure applied. Notably, tracheal histamine release during batch incubations was not reduced at all by 10 nM fenoterol. Previous cooling of lung tissue abolished the differences in inhibition by fenoterol between IgE- and IgG-mediated release.

Discussion

Guinea-pigs were actively sensitized to respond to IgG or IgE antibodies. The latter group is considered to reflect the human situation more. A superfusion method for the study of antigen-induced histamine release was introduced to obtain optimal stability of temperature and oxygen supply

for the tissue and was compared with a batch-wise incubation procedure.

It was found that IgE-sensitized airway tissue released much more of its histamine following antigen challenge than IgG-sensitized tissue, and that the β-adrenoceptor agonist fenoterol was less potent against IgE- than against IgG-mediated release. As we found in a previous study, using batch-incubations (Van Amsterdam et al unpublished), there was no relation between the inhibition of histamine release by fenoterol and the quantity of histamine released in the absence of the β-adrenoceptor agonist in the presently applied paired superfusion procedure. Other investigators obtained data in favour of such a relation, but they challenged the tissues with increasing amounts of antigen (Tung & Lichtenstein 1981; Undem & Buckner 1984; Church & Hiroi 1987). The quantitative difference between IgE- and IgG-mediated histamine release was more pronounced after superfusion of the tissue than after batch-wise incubations. This difference between IgE and IgG is at variance with the findings of Undem et al (1985a), obtained with guinea-pig tissues passively sensitized with IgG_1 or IgE antibodies, but is in agreement with the studies of Andersson & Bergstrand (1981) who applied a similar sensitization procedure to that in the present study.

Fenoterol has been reported to inhibit histamine release from passively sensitized human lung fragments significantly at concentrations from 10 nM upwards (Peters et al 1982; Hughes et al 1983), and from guinea-pig lung slices with an EC50 value of ~ 10 nM (Zaagsma et al 1984). However, the present study demonstrates that under superfusion conditions EC50 values close to 1 nM, for IgG-sensitized lung, can be obtained. Differences in the effective concentrations, as well as in the maximal inhibition, have also been found with other β-adrenoceptor agonists (Assem & Schild 1971; Butchers et al 1980; Undem & Buckner 1984; Undem et al 1985b; Church & Hiroi 1987). Both variations in the sensitization procedures and the sub-optimal batch-wise incubation conditions may have contributed to these differences. A source of variation may be the accumulation of mediators during batch incubations which modulate histamine release. Modulatory roles of histamine H_1- (Orehek et al 1973; Tauber et al 1973; Berti et al 1979; Undem et al 1987). H_2- (Holroyde & Eyre 1977) and, recently, H_3-receptor (Arrang et al 1987)-mediated responses have been proposed. In addition, lipoxygenase products have been claimed to enhance IgE-mediated histamine release and to modulate control mechanisms that are linked to adenylate cyclase (Marone et al 1979). A role of cyclo-oxygenase and of lipoxygenase products has been related to the presence of cartilage (Raeburn et al 1987) or temperature fluctuations (Souhrada et al 1983). Finally, different populations of mast cells have been suggested to be involved, originating from central and peripheral airway tissues (Undem et al 1987).

In the present study, after cooling, followed by a preincubation period preceding batch-wise antigen challenge during which the temperature increased from 0 to 37°C, the quantitative differences between IgE- and IgG-mediated histamine release and in the sensitivity towards β-adrenergic inhibition were abolished. From the studies of Souhrada et al (1983) it might be inferred that the modulation of histamine release by leukotrienes and prostaglandins, produced in response to the changes in temperature, may interfere with the response to antigen.

Histamine release from trachea was much less sensitive to β-adrenergic inhibition than the release from peripheral lung, irrespective of whether the release was IgE- or IgG-mediated. On superfusion of the trachea the inhibition by 10 nM fenoterol was clearly reduced, and with batch-wise incubations tracheal histamine release was found to have become completely insensitive to this concentration of fenoterol. A possible explanation for the reduced β-adrenergic inhibition may be that mediators which modulate β-adrenoceptor function are released from the cartilage. Tracheal cartilage has been reported to be a rich source of prostaglandins, including PgE_2, $PgF_{2\alpha}$, and prostacyclin (Raeburn et al 1987).

In the present study, a superfusion procedure was introduced for the measurement of antigen-induced histamine release from airway tissues. Both the β-adrenergic inhibition and the differential modulatory role of IgE and IgG were found to be greatly enhanced compared with the conventional batch-wise incubations. In addition, previous cooling of the tissue to 0°C and subsequent rewarming to 37°C severely diminished the sensitivity to pharmacological modulation. The findings are relevant for the interpretation of mast cell β-adrenoceptor function and for the transport and storage of (human) tissues, which are often carried out at 4 or 0°C.

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