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The effect of prednisolone and ketotifen on the antigen-induced bronchoconstriction and mediator release in rat isolated lungs

Maria Ottenhof, Jan G.R. Ufkes[1] & Hans H. Van Rooij*

Department of Pharmacology, Academic Medical Centre, University of Amsterdam, and Department of Pharmacology & Pharmacotherapy*, Faculty of Pharmarcy, State University of Utrecht, The Netherlands

1 Using a new method for inducing IgE-mediated, systemic anaphylaxis in the rat both prednisolone and ketotifen had been shown previously to be effective in suppressing the bronchial anaphylaxis *in vivo*. In order to study the mode of action underlying their bronchoprotective effect, both agents were also tested on the antigen-induced bronchoconstriction in rat isolated lungs in relation to the mediator release in the lung-effluent.

2 The presence of histamine, 5-hydroxytryptamine (5-HT) and SRS-A could be detected biologically in the lung-effluent during bronchoconstriction. Histamine and 5-HT were determined quantitatively by means of h.p.l.c. with fluorimetric detection, whereas SRS-A was determined using the guinea-pig ileum in a cascade set-up.

3 Although both prednisolone and ketotifen inhibited the antigen-induced bronchoconstriction effectively, it appeared that only prednisolone suppressed the release of histamine, 5-HT and SRS-A in the lung-effluent significantly, whereas ketotifen had no effect.

4 On account of these data it is suggested that the bronchoprotective effect of prednisolone is mainly based on inhibition of the release of the mediators involved, whereas the effect of ketotifen may be based on receptor antagonism.

Introduction

Several studies indicate that 5-hydroxytryptamine (5-HT), possibly together with cyclo-oxygenase and lipoxygenase products, is a mediator of the bronchial anaphylaxis in actively sensitized rats (Church *et al.*, 1972; Stotland & Share, 1974; Brunet *et al.*, 1983; Ahlstedt *et al.*, 1983; Dahlbäck *et al.*, 1984). Using a recently developed method for inducing IgE-mediated, bronchial and cardiovascular anaphylaxis in Brown-Norway rats, five different types of anti-allergic agents were tested with regard to mortality, bronchoconstriction and cardiovascular events (Ufkes & Ottenhof, 1984). With the exception of the histamine H_1-receptor antagonist mepyramine (no activity at all), each anti-allergic agent tested showed a different and characteristic profile of anti-allergic activity. It was suggested that with regard to mortality and cardiovascular events lipoxygenase products are possibly important mediators whereas other mediators (including cyclo-oxygenase products) may be involved in the bronchoconstriction. Histamine was considered to be of minor importance compared to other mediators.

Although both prednisolone and ketotifen appeared to be effective in suppressing the bronchial anaphylaxis *in vivo*, they showed rather different profiles of anti-allergic activity (Ufkes & Ottenhof, 1984) which suggests a different mode of action. In the present paper we describe the identification and quantification of the putative mediators involved in the bronchial anaphylaxis in isolated lungs from sensitized Brown-Norway rats. To clarify the mode of action underlying their bronchoprotective effects the influence of prednisolone and ketotifen on the antigen-induced bronchoconstriction was established in relation to the mediator release in the lung-effluent.

[1]To whom all correspondence should be addressed: Department of Pharmacy, Plantage Muidergracht 24, 1018 TV Amsterdam, The Netherlands.

Methods

Sensitization procedure

Female Brown-Norway rats (K.U. Leuven, Belgium) weighing 160–190 g were used throughout. The animals were injected i.p. with a suspension of 2 ml trinitrophenyl haptenized ovalbumin (TNP-OVA) together with $AlPO_4$ as an adjuvant (see also Ufkes *et al.*, 1983). All rats were judged 12–21 days later to determine the extent of sensitization. The amounts of TNP-specific IgE in plasma were determined using a radioimmunoassay, as previously described by Aalberse (1978). If the titer was increased by up to 20% binding of radioactivity to TNP-sepharose, the rats were considered to be sensitized sufficiently.

Rat isolated lungs with vascular perfusion

Lungs were removed under pentobarbitone-Na (Nembutal, Abbott) anaesthesia (50 mg kg^{-1}), suspended in a plexiglass chamber and perfused through the pulmonary artery with Krebs-Ringer solution (37°C and gassed with 5% CO_2 in O_2) at a flow rate of 7.5 ml min^{-1}. The pulmonary arterial pressure (PAP) was recorded by means of an Ailtech Physiological Transducer (MEDIO-E). Lungs were rhythmically ventilated (25 min^{-1}) by an alternating negative pressure of 5.5 cmH_2O in the chamber using a Palmer respiratory pump. Using 5% CO_2 in O_2 a positive pressure of 5.5 cmH_2O was continuously maintained in the trachea. Through the tracheal cannula the tracheal pressure fluctuations (TP) were recorded by an Elema-Schönander pressure transducer (EMT 311), as a measure of the lung ventilation. TP and PAP were displayed on a Kipp BD9 recorder.

Bioassay

In order to identify the mediators released during the antigen-induced bronchoconstriction, samples (0.1–0.5 ml) of lung-effluent were added to a cascade of various isolated smooth muscle strips (see Table 1) superfused with Krebs-Ringer solution (37°C and gassed with 5% CO_2 in O_2) at a flow rate of 7.5 ml min^{-1}.

In some experiments the Krebs-Ringer solution contained indomethacin (2 μg ml^{-1}) to inhibit the endogenous synthesis of cyclo-oxygenase products. To increase the specificity of the tissues towards the putative mediators the following antagonists (alone or in various combinations) were added to the superfusion medium: atropine (200 ng ml^{-1}), mepyramine (500 ng ml^{-1}), methysergide (1 μg ml^{-1}) and FPL 55712 (1 μg ml^{-1}).

In experiments determining the SRS-A activity the total amount of lung-effluent(7.5 ml min^{-1}) was mixed with the Krebs-Ringer solution (1:1), containing atropine, mepyramine and methysergide, and subsequently this mixture superfused the cascade of tissues.

Quantitative determination of histamine and 5-HT was performed by h.p.l.c. (see below). A quantitative estimation of SRS-A activity was obtained by expressing it as equivalents of leukotriene C_4 (LTC_4) or LTD_4, formed by comparing the SRS-A-induced contractions on the guinea-pig ileum with the standard dose-effect curves for LTC_4 and LTD_4 respectively. The effect of the anti-allergic (pre)treatment on SRS-A release was determined by measuring the amount of SRS-A in the effluent, which was then expressed as a percentage of the SRS-A activity in effluent from control (untreated) lungs (taken to be 100%).

Table 1 The tissue preparations used to identify the mediators released during antigen-induced bronchoconstriction

Tissue	*Bioassay*
Rat stomach strip (m)	5-Hydroxytryptamine, prostaglandins
Rabbit aorta strip (m)	Thromboxane A_2
Rat duodenum (t)	Bradykinin
Guinea-pig ileum (t)	Histamine, SRS-A

Bioassay tissues were prepared according to Henman *et al.* (1978) and suspended isometrically (m) or isotonically (t) in cascade set-up for the mediators of anaphylaxis to be detected.

H.p.l.c. assay for histamine and 5-hydroxytryptamine

For the determinations of histamine in lung-effluents reversed-phase liquid chromatography was used with Hypersil ODS 5 μM (Shandon) as the column packing and with a mobile phase consisting of a mixture of 2-propanol and water (2:8 v/v, pH = 6.2), containing 5 mM sodiumdodecylsulphate. On line post-column derivatization with o-phtalaldehyde and fluorimetric detection ($\lambda_{exc.}$ = 340 nm, $\lambda_{em.}$ = 455 nm) was performed, as described by Nondek *et al.* (1983). For the determination of 5-HT in lung-effluents reversed-phase liquid chromatography was used with Hypersil ODS 5 μM (Shandon) as the column packing and with a mobile phase consisting of a mixture of methanol and water (2:8 v/v, pH = 4.7) and 50 mM ammoniumacetate. Fluorimetric detection ($\lambda_{exc.}$ = 300 nm, $\lambda_{em.}$ = 340 nm) was used. Samples of lung-effluents (100 μl) were injected without any pretreatment. Calibration was performed using freshly prepared solutions of synthetic histamine and 5-HT in Krebs-Ringer solution.

Antigen challenge

Before starting the actual experiment, recordings were

made of TP and PAP for 15 min. The bronchoconstriction was induced by the administration of 0.2 ml trinitrophenyl haptenized bovine serum albumin (TNP-BSA, see Aalberse, 1978) to the perfusion fluid.

Drugs

Prednisolone (Di-Adreson-F aquosum, Organon) was administered in doses of 100–200 mg kg^{-1} body weight intraperitoneally 5 h before the operation procedure to remove the lungs was started. Ketotifen (Zaditen, Sandoz, kindly supplied by Wander Pharma, Holland) was added to the perfusion fluid at final concentrations of 100–500 ng ml^{-1}.

To standardize the activity of the smooth muscle strips in the cascade set-up the following synthetic mediators were used: histamine (B.D.H.), 5-hydroxytryptamine (Fluka A.G.), acetylcholine (O.P.G.), bradykinin (Bachem A.G.), prostaglandins (PGE_1, PGE_2 and $PGF_{2\alpha}$, kindly supplied by Unilever Research) and leukotrienes (LTC_4 and LTD_4, kindly supplied by Dr J. Rokach, Merck Frosst Laboratories). Mediator solutions were freshly prepared in appropriate concentrations and stored on ice during the experiments.

The following antagonists were used: atropine (O.P.G.), mepyramine (O.P.G.), methysergide (Sandoz), FPL 55712 (sodium 7-[3-(4-acetyl-3-hydroxy-2-propylphenoxy)-2-hydroxy propoxy]-4-oxo-8-propyl-4H-1-benzopyran-2-carboxylate) kindly supplied by P. Sheard, M.Sc., Fisons Pharmaceuticals), indomethacin (M.S.D.).

Analysis of data

The decrease of TP, as a measure of bronchoconstriction, was calculated as area under the curve and expressed as percentage of the basal TP. Bronchoprotective activity was determined by comparing the bronchoconstrictions (i.e. decrease of TP in %) in untreated, antigen challenged lungs (controls) with those in lungs (pre-) treated with the anti-allergic compound under investigation.

All data concerning the comparison between control experiments and those with anti-allergic treatment, were statistically analysed by means of Student's *t* test. Results given are expressed as mean ± s.e.mean (in both the text and Figures).

Results

Antigen-induced bronchoconstriction

After a 15 min period of measuring the PAP and the TP, the antigen (TNP-BSA) was administered to the fluid perfusing the lungs from rats previously sensitized with TNP-OVA. This resulted in a highly reproducible, immediate bronchoconstriction characterized by a slight but consistent increase in PAP and a considerable decrease in TP (see Figure 1). The duration of this bronchoconstriction corresponded largely with the 8–10 min period of antigen-induced bronchoconstriction *in vivo* (see Ufkes *et al.*, 1983). The mean decrease of TP, used here as a measure of the bronchoconstriction, was 23.1 ± 1.1% ($n = 58$), also shown in Figure 3. A second antigen challenge at the moment the PAP and TP were normalized, was considerably less effective, whereas a third challenge was without any effect at all. Administration of TNP-BSA to the perfusion fluid of lungs from non-sensitized rats did not cause bronchoconstriction.

Detection and quantification of the mediator release

Samples of 0.1–0.5 ml lung-effluent taken 2, 5 and 10 min after the antigen challenge were added to the cascade superfusion medium (flow rate 7.5 ml min^{-1}). Using selective receptor antagonists (i.e. atropine, methysergide or mepyramine), and in some experiments indomethacin to inhibit the endogenous synthesis of cyclo-oxygenase products, only 5-HT and histamine could be detected with the rat stomach strip and the guinea-pig ileum respectively (see Figure 1a). It appeared that both histamine and 5-HT reached maximum levels 2 min after the antigen challenge. The presence of other types of mediators could not be demonstrated in that manner. After mixing the total amount of lung-effluent with the superfusion medium (1:1) containing atropine, mepyramine and methysergide, a third component could be detected using the guinea-pig ileum. It manifested itself as a slow contracting substance and could only be antagonized by the specific SRS-A antagonist FPL 55712 (1 μg ml^{-1}, final concentration) (see Figure 1b). Therefore, this component is thought to be SRS-A, the leukotriene mixture of LTC_4 and LTD_4 (Lewis *et al.*, 1980). Unlike the release of histamine and 5-HT which was short-lasting, the SRS-A activity reached a maximum value circa 7 min after the antigen challenge after which time it slowly decreased during the following 60–90 min. A thromboxane A_2 (TXA_2)-like substance or other prostaglandins could not be detected in this manner. In order to make sure that any TXA_2 generated did not decay to the inactive TXB_2 before reaching the assay tissue, several experiments were conducted in which the lung-effluent directly (within seconds) superfused the rabbit aorta strip, this being the first assay tissue in the cascade, according to a method applied by Alabaster & Hawkeswood (1978). As a result no extra mediator activity was seen. The absence of TXA_2 or other prostaglandins was also confirmed in several experiments in which indomethacin (2 μg ml^{-1}) was added to the perfusion medium; no effect on either the antigen-induced bronchoconstriction or the mediator activity in the lung-effluent was observed.

The 5-HT and histamine concentrations in the lung-effluents were measured 2, 5 and 10 min after antigen challenge using h.p.l.c. separation and fluorimetric detection (see Figure 2a and b). The maximum concentration of 5-HT detected was 15.2 ± 2.3 ng ml^{-1} ($n = 22$) 2 min after antigen challenge, whereas

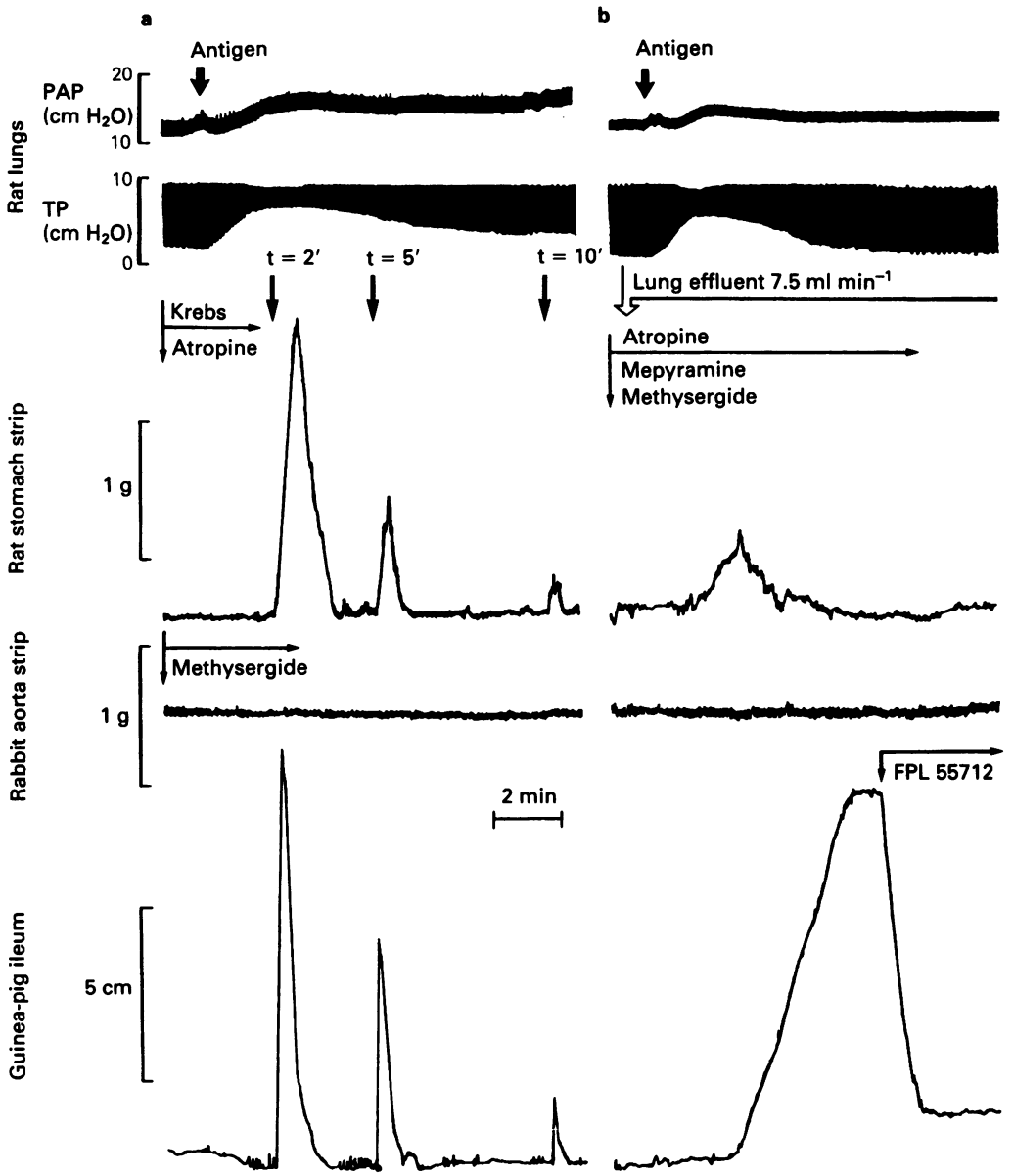

Figure 1 Detection of the mediators, released in the lung-effluent after antigen challenge, using a cascade of various assay tissues. The upper tracings show the pulmonary arterial pressure (PAP) and the tracheal pressure fluctuations (TP). (a) The left panel shows the responses of a rat stomach strip (blocked by atropine), a rabbit aorta strip and the guinea-pig ileum (both blocked by atropine and methysergide) to samples (0.2 ml) of lung-effluent taken 2, 5 and 10 min after antigen challenge. (b) The right panel shows the responses of these tissues (all blocked by atropine, mepyramine and methysergide) to the total amount of lung-effluent.

the maximum concentration of histamine was 189 ± 21 ng ml^{-1} ($n = 20$). These data are also incorporated in Figures 4 and 5.

The SRS-A activity in the lung-effluents was quantified by comparing the SRS-A-induced contractions on the guinea-pig ileum with the standard dose-effect curves for LTC_4 and LTD_4, determined in several experiments. It appeared that the SRS-A activity in effluents from the control (untreated) lungs might be considered equivalent to contractions evoked by a (single) dose of 10–13 ng LTC_4 or 4–6 ng LTD_4.

The effect of prednisolone and ketotifen

Prednisolone 100 mg kg^{-1}, administered i.p. 5 h before the experiment was performed, appeared to be the optimum dose for inhibiting the antigen-induced bronchoconstriction. Addition of prednisolone to the perfusion fluid in final concentrations up to 1 mg ml^{-1} did not cause any significant change with regard to the bronchoconstriction. The antigen-induced bronchoconstriction after the prednisolone pretreatment appeared to be $11.2 \pm 1.1\%$ ($n = 30$), indicating a

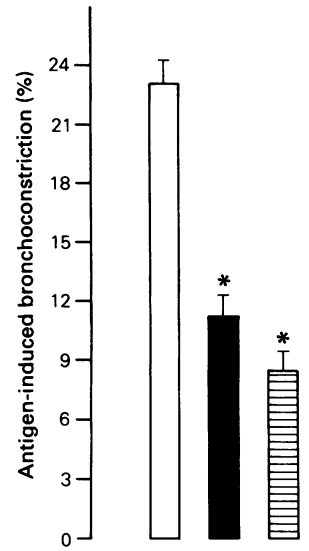

Figure 3 The antigen-induced bronchoconstriction in untreated lungs (controls, open column; $n = 58$), in lungs from rats pretreated with prednisolone (solid column; $n = 30$) and in lungs perfused with ketotifen (hatched column; $n = 28$). $^*P < 0.005$.

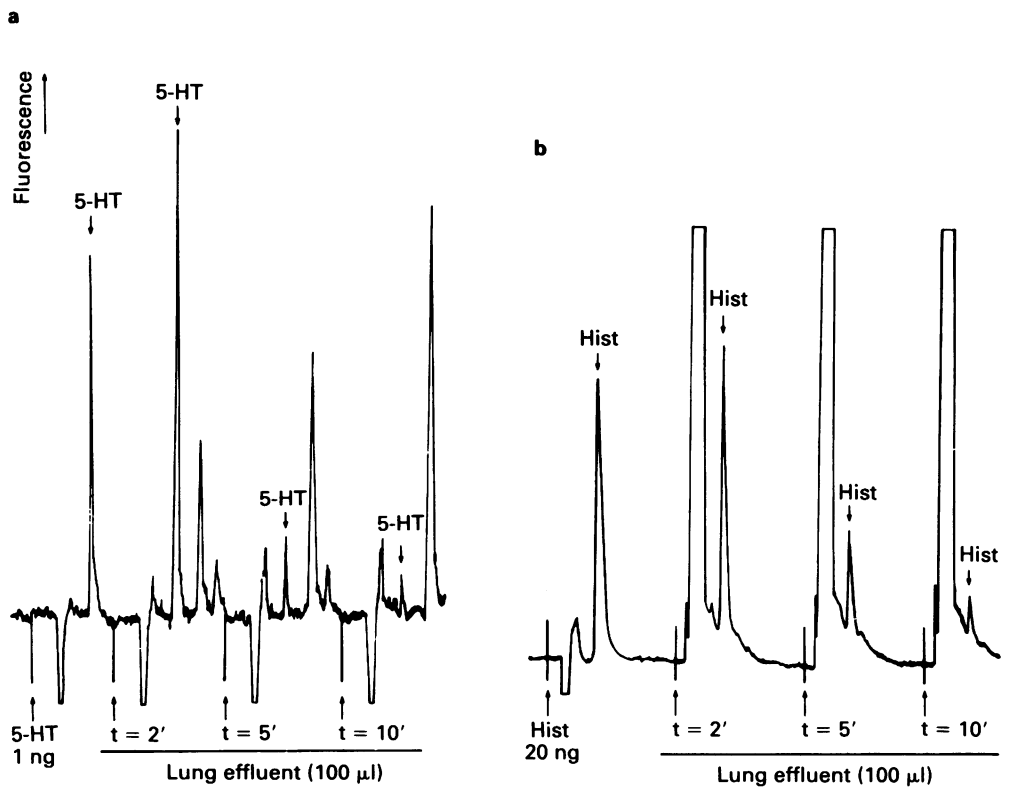

Figure 2 H.p.l.c. separation and fluorimetric detection of 5-hydroxytryptamine (5-HT) (a) and histamine (Hist)(b) in lung-effluents taken 2, 5 and 10 min after the antigen challenge.

52% reduction as compared to the control values (see Figure 3).

The data concerning the effect of prednisolone on antigen-induced 5-HT, histamine and SRS-A release are summarized in Figures 4, 5 and 6, respectively. To avoid confusion concerning the expression of the SRS-A activity in LTC_4 or LTD_4 equivalents, the effect of prednisolone on the SRS-A activity detected was expressed as a percentage of the SRS-A activity in effluents from the control lungs (taken to be 100%).

Ketotifen, added to the perfusion fluid in final concentrations of 100–500 ng ml^{-1}, caused a concentration-dependent suppression of the antigen-induced bronchoconstriction. Intraperitoneal administration several hours before the experiment was performed did not cause any significant change in the bronchoconstriction. It was established that 500 ng ml^{-1} ketotifen, this being the maximum usable concentration, decreased the antigen-induced bronchoconstriction to 8.5 ± 0.9% ($n = 28$), which means a 63% reduction as compared to the control values (see Figure 3). Ketotifen in higher concentrations caused effects of its own on the PAP and TP, which prevented a correct interpretation of its influence on the antigen-induced bronchoconstriction.

With regard to the effect of ketotifen on the antigen-

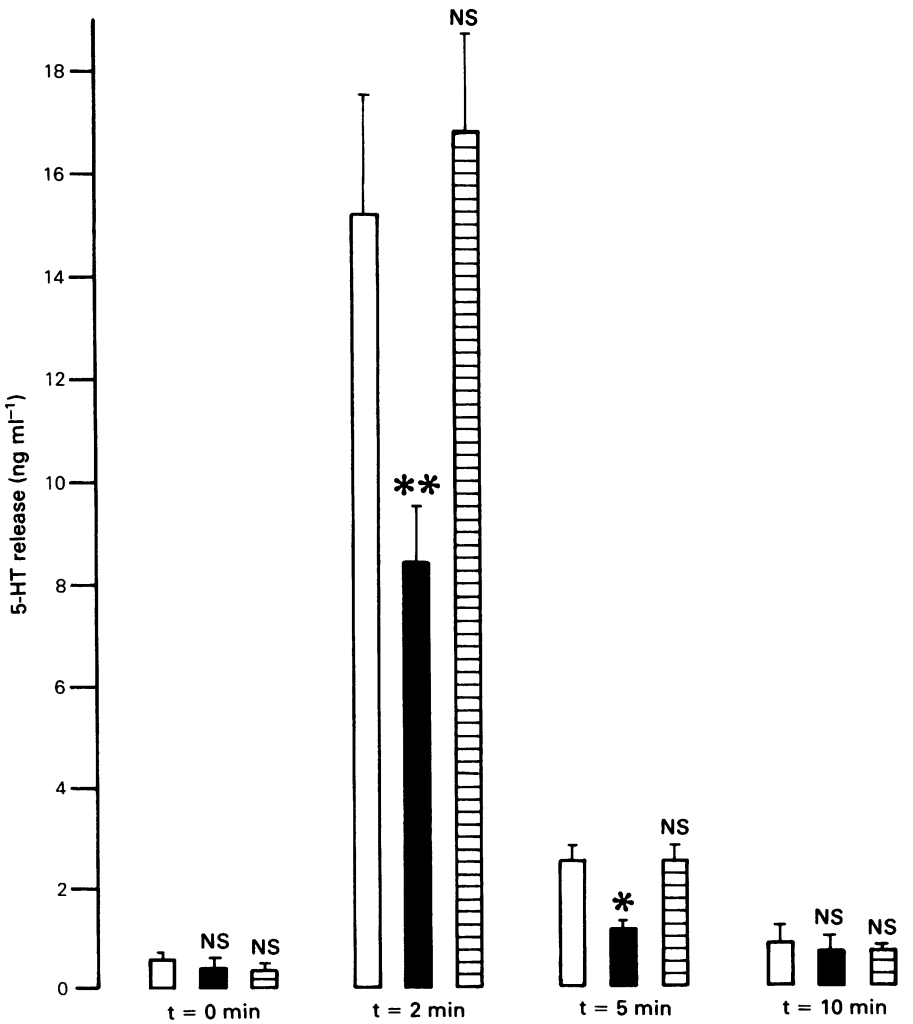

Figure 4 The release of 5-hydroxytryptamine (5-HT) in effluents from untreated lungs (controls; open columns; $n = 22$), lungs from rats pretreated with prednisolone (solid columns; $n = 15$) and lungs perfused with ketotifen (hatched columns; $n = 13$) before (t = 0) and 2, 5 and 10 min after the antigen challenge. $^*P < 0.05$, $^{**}P < 0.02$, NS = not significant.

induced release of 5-HT, histamine and SRS-A, it was clearly established that ketotifen had no effect at all as can be seen in Figures 4, 5 and 6.

Table 2 summarizes the data of Figures 3, 4, 5 and 6 expressing the relative scores of prednisolone and ketotifen tested with regard to the antigen-induced bronchoconstriction and the release of 5-HT, histamine and SRS-A.

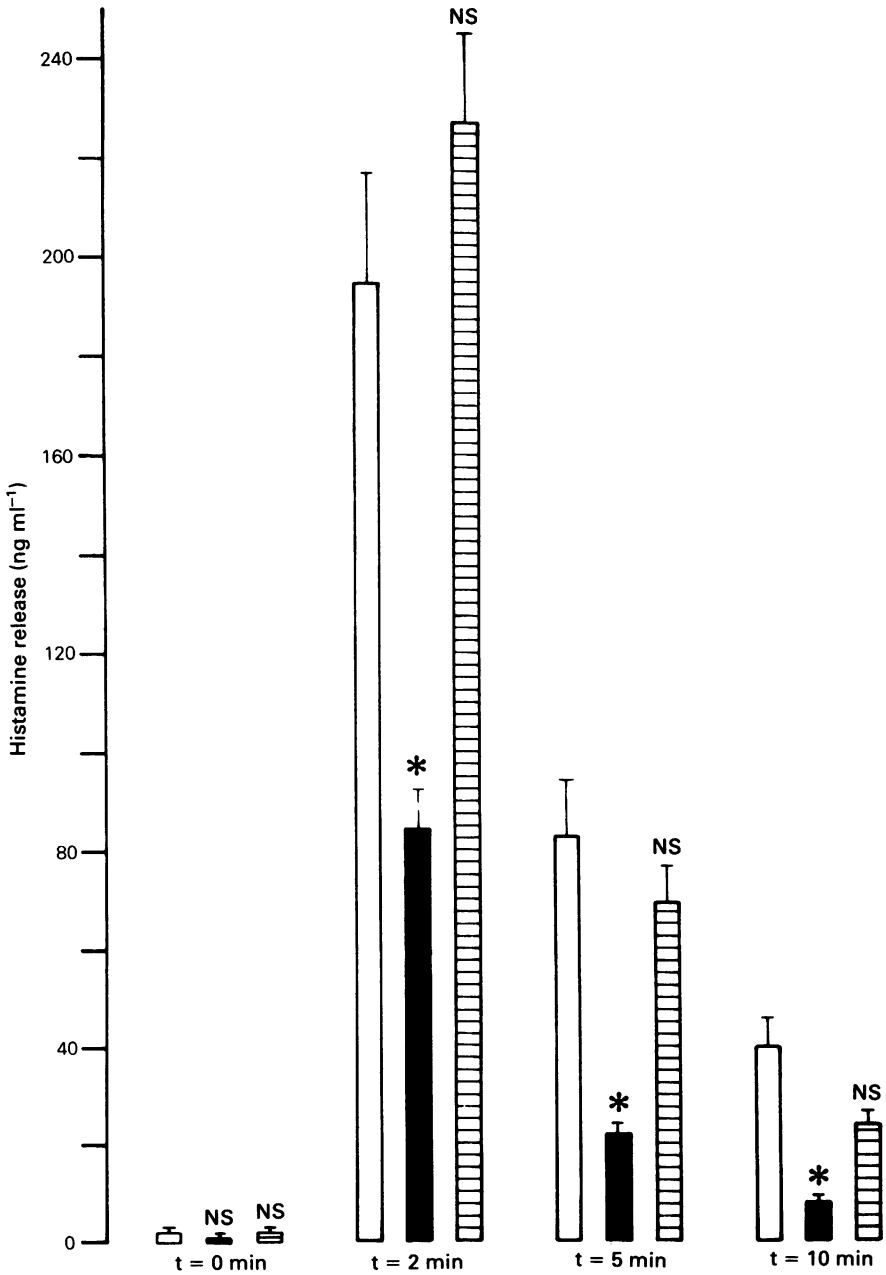

Figure 5 The release of histamine in effluents from untreated lungs (controls; open columns; $n = 20$), lungs from rats pretreated with prednisolone (solid columns; $n = 15$) and lungs perfused with ketotifen (hatched columns; $n = 13$) before (t = 0) and 2, 5 and 10 min after the antigen challenge. $^*P < 0.005$, NS = not significant.

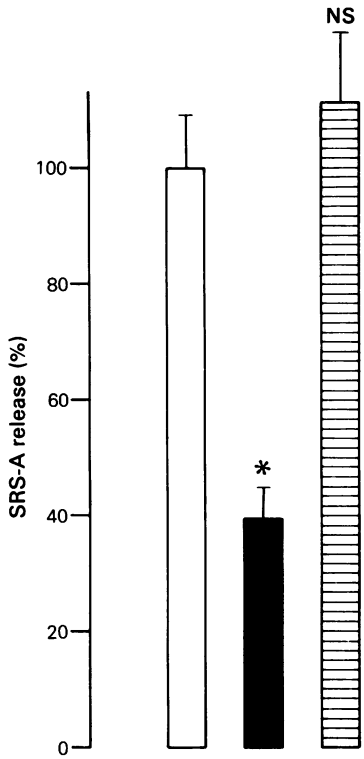

Figure 6 The release of SRS-A in effluents from untreated lungs (controls; open column; $n = 35$) taken to be 100%, as compared to the SRS-A release in effluents of lungs from rats pretreated with prednisolone (solid column; $n = 17$) and lungs perfused with ketotifen (hatched column; $n = 15$). $P < 0.005$, NS = not significant.

Additionally several experiments were performed to examine the effect of prednisolone and ketotifen on the bronchoconstriction induced by various, exogenously administered bronchoconstrictors. Acetylcholine (25 and 50 μg), bradykinin (50 and 100 μg) and 5-HT (50 and 100 μg) induced bronchoconstriction of a magnitude similar to that after antigen challenge.

Table 2 Relative effects of prednisolone and ketotifen on the antigen-induced bronchoconstriction and the release of 5-hydroxytryptamine (5-HT), histamine and SRS-A in the lung effluent

	Bronchoconstriction	*5-HT*	*Histamine*	*SRS-A*
Prednisolone	+ +	+ +	+ + +	+ + +
Ketotifen	+ + +	0	0	0

+ + + = high effectiveness; + + = medium effectiveness; 0 = no effect.

Histamine in doses up to 500 μg did not cause any demonstrable bronchoconstriction. With the exception of the 5-HT-induced bronchoconstriction which was blocked by ketotifen to a considerable extent, it appeared that neither prednisolone nor ketotifen, tested in their optimal effective doses, exhibited any significant effect on the bronchoconstrictions induced by the other mediators.

Discussion

In this study we have attempted to identify and quantify the putative mediators involved in the antigen-induced bronchoconstriction in rat isolated lungs and to establish the effect of prednisolone and ketotifen. To this end we used a newly developed immunization procedure for inducing IgE-related, systemic anaphylaxis in Brown-Norway rats.

Our results show that large amounts of histamine and smaller but still considerable amounts of 5-HT were released in the lung effluent during the first minutes after antigen challenge. In addition, using the guinea-pig ileum we were able to detect a slow contracting substance which appeared in the effluent somewhat later after the antigen challenge. This substance was identified as SRS-A, the leukotriene mixture consisting of LTC_4 and LTD_4 (Lewis *et al.*, 1980). Surprisingly no other products of arachidonic acid such as thromboxane-like substances, could be detected by means of the cascade superfusion technique. In view of the short half-life of TXA_2 (about 30 s at 37°C) special attention was given to the point that the lung effluent directly (without any delay) superfused the rabbit aorta strip, considered to be very sensitive to TXA_2 (Alabaster & Hawkeswood, 1978; Zijlstra & Vincent, 1981). This finding is in contrast to the results of Dahlbäck *et al.* (1984) who concluded that besides 5-HT some not yet identified products of cyclo-oxygenase/thromboxane synthetase are main mediators of allergen-induced bronchial anaphylaxis in rats and that products of the lipoxygenase pathway play a minor role but may modulate the response. On the other hand Brunet *et al.* (1983) presented results from *in vitro* studies suggesting that the respiratory response of rats to antigen challenge is mediated by 5-HT in the trachea and by 5-HT and leukotrienes in the lung parenchyma. These *in vitro* effects were also found *in vivo*. Iacopina *et al.* (1984) showed that LTC_4 produced dose-dependent increases of the pulmonary perfusion pressure in rat isolated lungs accompanied by a dose-dependent increase in effluent levels of cyclo-oxygenase products. These results differed from those of Al-Ubaidi & Bakhle (1980) who did not observe a release of cyclo-oxygenase products in rats after SRS-A administration. Our experiments using indomethacin to block the cyclo-oxygenase pathway

of arachidonic acid metabolism, demonstrate no significant effect on the antigen-induced bronchoconstriction and mediator release. This also implies no direct role for cyclo-oxygenase products in this *in vitro* model. However, it does not exclude a role for these products in the bronchial anaphylaxis *in vivo*, since in that case various other cell types are involved such as platelets, an important source of thromboxanes. Although histamine was released in large amounts its role in antigen-induced bronchoconstriction still remains uncertain since it has been established that antihistamines are ineffective in modifying the bronchoconstriction (Church, 1975; Farmer *et al.*, 1975; Menassé *et al.*, 1979; Ufkes & Ottenhof, 1984), and exogenously administered histamine does not cause bronchoconstriction (this paper; Church, 1975). The release of considerable amounts of 5-HT corresponds with the findings reported by others (Church *et al.*, 1972; Stotland & Share, 1974; Brunet *et al.*, 1983; Ahlstedt *et al.*, 1983; Dahlbäck *et al.*, 1984).

In an attempt to clarify the mode of action underlying their broncho-protective effects, prednisolone and ketotifen were tested on the antigen-induced bronchoconstriction in relation to the mediator release. Although their effectiveness in suppressing the bronchial anaphylaxis *in vivo* has been established (Ufkes & Ottenhof, 1984), they showed rather different profiles of anti-allergic activity which suggests a different mode of action. This could be clearly confirmed by our present results. Although both prednisolone and ketotifen were able to inhibit the antigen-induced bronchoconstriction effectively, only prednisolone suppressed the release of histamine, 5-HT and SRS-A considerably, whereas ketotifen had no effect at all. As distinct from ketotifen, prednisolone was only effective if the rats from which the lungs were removed, were pretreated 5 h before the antigen challenge. This time lag may be explained by the slow appearance of the protein synthesis-dependent intermediary macrocortin, which is considered to be the 'second messenger' in the anti-inflammatory action of glucocorticosteroids in perfused lung and peritoneal leukocytes (Flower & Blackwell, 1979; Carnuccio *et al.*, 1981). This polypeptide has been shown to inhibit the action of phospholipase A_2 leading to suppression of the synthesis of arachidonic acid products including SRS-A. The manner in which glucocorticosteroids inhibit the histamine and 5-HT release is not completely clarified. Several data point to a role for the lipoxygenase products 5-HPETE and 5-HETE, generated by phospholipase A_2 as well, in enhancing the histamine release in human basophils (Peters *et al.*, 1981) and rat mast cells (Stenson *et al.*, 1980). As a consequence the prednisolone-induced, macrocortin-mediated inhibition of phospholipase A_2 not only decreases the SRS-A release but might decrease the release of 5-HPETE and 5-HETE as well, leading to a decreased histamine and (in rodents) 5-HT release from mast cells.

With regard to the preventive effect of ketotifen in bronchial asthma, several mechanisms have been suggested (see also Craps & Ney, 1984): inhibition of the antigen-induced release of histamine (Martin & Römer, 1978; Radermecker *et al.*, 1980) and SRS-A (Ross *et al.*, 1979; Greenwood, 1982); SRS-A (Ney *et al.*, 1980) but not histamine H_1-(Martin & Römer, 1978; Martin & Baggiolini, 1981) receptor-antagonism; calcium antagonism (Lowe & Richardson, 1980) and prevention or reversal of decreased β-adrenoceptor sensitivity (Bretz *et al.*, 1983). In contrast to the findings of Greenwood (1982) and Ross *et al.* (1979), who used sensitized guinea-pig and human chopped lung preparations to study the effect of ketotifen on antigen-induced histamine and SRS-A release, we failed to find any inhibition of the antigen-induced mediator release by ketotifen. Apart from differences in immunization procedures, species used and experimental conditions, much higher ketotifen concentrations (10^{-4} mol l^{-1}) were used compared to the more appropriate concentrations (500 ng ml^{-1} = 1.18×10^{-6} mol l^{-1}) in our experiments, which may explain this discrepancy. Since exogenously administered bradykinin and acetylcholine but not 5-HT were still able to cause bronchoconstriction during the lung perfusion in the presence of ketotifen a mechanism based on calcium antagonism (Lower & Richardson, 1980) may be excluded as well. Although reversal and prevention of β-adrenoceptor-mediated tachyphylaxis (Bretz *et al.*, 1983) might be a very relevant, additional factor for the anti-asthmatic activity of ketotifen in the clinical situation, it does not play a role in our present experiments. Taking into account the fact that ketotifen is a strong histamine H_1-receptor antagonist (Martin & Römer, 1978) and also exhibits functional SRS-A antagonism *in vivo* (Ross *et al.*, 1979), and the finding in our experiments that ketotifen effectively inhibited the 5-HT induced bronchoconstriction without affecting the mediator release, it can be assumed that the bronchoprotective effect of ketotifen is mainly based on (multiple) receptor antagonism.

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References

AALBERSE, R.C. (1978). An indirect antibody assay using haptenated antigen and ^{125}I-labelled anti-hapten antibody. *J. Immunol. Methods*, **19**, 87–93.

AHLSTEDT, S., SMEDEGÅRD, G., NYGREN, H. & BJÖRKSTÉN, B. (1983). Immune responses in rats sensitized with aerosolized antigen. Antibody formation, lymphoblastic responses and mast cell and mucous cell development related to bronchial reactivity. *Int. Arch. Allergy appl. Immunol.*, **72**, 71–78.

ALABASTER, V.A. & HAWKESWOOD, E. (1978). Detection and bioassay of pharmacologically active substances released by arachidonic acid from guinea-pig perfused lungs. *J. Pharmac. Meth.*, **1**, 341–350.

AL-UBAIDI, F. & BAKHLE, Y.S. (1980). Differences in biological activation of arachidonic acid in perfused lungs from guinea-pig, rat and man. *Eur. J. Pharmac.*, **62**, 89–96.

BRETZ, U., MARTIN, U., MAZZONI, L. & NEY, U.M. (1983). β-adrenergic tachyphylaxis in the rat and its reversal and prevention by ketotifen. *Eur. J. Pharmac.*, **86**, 321–328.

BRUNET, G., PIECHUTA, H., HAMEL, R., HOLME, G. & FORD-HUTCHINSON, A.W. (1983). Respiratory responses to leukotrienes and biogenic amines in normal and hyperreactive rats. *J. Immunol.*, **131**, 434–438.

CARNUCCIO, R., DiROSA, M., FLOWER, R.J. & PINTO, A. (1981). The inhibition by hydrocortisone of prostaglandin biosynthesis in rat peritoneal leucocytes is correlated with intracellular macrocortin levels. *Br. J. Pharmac.*, **74**, 322–324.

CHURCH, M.K. (1975). Responses of rat lung to humoral mediators of anaphylaxis and its modification by drugs and sensitization. *Br. J. Pharmac.*, **55**, 423–430.

CHURCH, M.K., COLLIER, H.O.J. & JAMES, G.W.L. (1972). The inhibition by dexamethasone and disodium cromoglycate of anaphylactic bronchoconstriction in the rat. *Br. J. Pharmac.*, **46**, 56–65.

CRAPS, L.P. & NEY, U.M. (1984). Ketotifen: current views on its mechanism of action and their therapeutic implications. *Respiration*, **45**, (4), 411–421.

DAHLBÄCK, M., BERGSTRAND, H. & SÖRENBY, L. (1984). Bronchial anaphylaxis in actively sensitized Sprague Dawley rats: studies on mediators involved. *Acta pharmac. tox.*, **55**, 6–17.

FARMER, J.B., RICHARDS, I.M., SHEARD, P. & WOODS, A.M. (1975). Mediators of passive lung anaphylaxis in the rat. *Br. J. Pharmac.*, **55**, 57–64.

FLOWER, R.J. & BLACKWELL, G.J. (1979). Anti-inflammatory steroids induced biosynthesis of a phospholipase A_2 inhibitor which prevents prostaglandin generation. *Nature*, **278**, 456–459.

GREENWOOD, C. (1982). The pharmacology of ketotifen. *Chest*, **82S**, 45S–48S.

HENMAN, M.C., NAYLOR, I.L. & LEACH, G.D.H. (1978). A critical evaluation of the use of a cascade superfusion technique for the detection and estimation of biological activity. *J. Pharmac. Meth.*, **1**, 13–26.

IACOPINO, V.J., COMPTON, S., FITZPATRICK, T., RAMWELL, P., ROSE, J. & KOT, P. (1984). Responses to leukotriene C_4 in the perfused rat lung. *J. Pharmac. exp. Ther.*, **229**, 654–657.

LEWIS, R.A., AUSTIN, K.F., DRAZON, J.M., CLARK, D.A., MARFAT, A. & COREY, E.J. (1980). Slow reacting substances of anaphylaxis: identification of leukotrienes C-1 and D from human and rat sources. *Proc. natn. Acad. Sci. U.S.A.*, **77**, 3710–3714.

LOWE, D.A. & RICHARDSON, B.P. (1980). The effects of cyproheptadine, ketotifen and sodium nitroprusside on mechanical activity and calcium intake in guinea-pig taenia coli *in vitro*. *Prog. resp. Res.*, **14**, 134–140.

MARTIN, U. & BAGGIOLINI, M. (1981). Dissociation between the anti-anaphylactic and the anti-histaminic actions of ketotifen. *Arch. Pharmac.*, **316**, 186–189.

MARTIN, U. & RÖMER, D. (1978). The pharmacological properties of a new, orally active anti-anaphylactic compound: ketotifen, a benzocycloheptathiophene. *Arzneim. Forsch.*, **28**, 770–782.

MENASSÉ, R., FERRINE, P.G., NIEDERHAUSER, U. & SZESZAK, J. (1979). Influence of various anti-allergic agents on IgE-mediated anaphylactic alterations of pulmonary function in rats. *Monogr. Allergy*, **14**, 126–129.

NEY, U., BRETZ, U., GRADWOHL, P. & MARTIN, U. (1980). Further characterization of the antianaphylactic action of ketotifen. *Allerg. Immunopath.*, **8**, 386–391.

NONDEK, L., FREI, R.W. & BRINKMAN, U.A.Th. (1983). Heterogeneous catalytic post-column reaction detectors for high-performance liquid chromatography application to N-methylcarbamates. *J. Chromatog.*, **282**, 141–150.

PETERS, S.P., SIEGEL, M.I., KAGEY-SOBOTKA, A. & LICHTENSTEIN, L.M. (1981). Lipoxygenase products modulate histamine release in human basophils. *Nature*, **292**, 455–457.

RADERMECKER, M., GUSTIN, M. & SAINT-REMY, P. (1980). Inhibition of anaphylactic histamine release from human lung by ketotifen. *Allerg. Immunopath. (MADR.)*, **8**, 384–385.

ROSS, W.J., HARRISON, R.G., JOLLEY, M.R.J., NEVILLE, M.C., TODD, A., VERGE, J.P., DAWSON, W. & SWEATMAN, W.J.F. (1979). Antianaphylactic agents, 1,2-(acylamino)oxazoles. *J. med. Chem.*, **22**, 412–417.

STENSON, W.F., PARKER, C.W. & SULLIVAN, T.J. (1980). Augmentation of IgE-mediated release of histamine by 5-hydroxyeicosatetraenoic acid and 12-hydroxyeicosatetraenoic acid. *Biochem. biophys. Res. Commun.*, **96**, 1045–1052.

STOTLAND, L.M. & SHARE, N.N. (1974). Active bronchial anaphylaxis in the rat. *Can. J. Physiol. Pharmac.*, **52**, 1114–1118.

UFKES, J.G.R. & OTTENHOF, M. (1984). Characterization of various anti-allergic agents using a new method for inducing systemic anaphylaxis in the rat. *J. Pharmac. Meth.*, **11**, 219–226.

UFKES, J.G.R., OTTENHOF, M. & AALBERSE, R.C. (1983). A new method for inducing fatal, IgE-mediated, bronchial and cardiovascular anaphylaxis in the rat. *J. Pharmac. Meth.*, **9**, 175–181.

ZIJLSTRA, F.J. & VINCENT, J.E. (1981). Contractile characteristics of the rat stomach strip and the rabbit aorta and mesenteric artery. *J. Pharmac. Meth.*, **6**, 1–4.

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