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PROTECTIVE EFFECTS OF THE GLUCOCORTICOID, BUDESONIDE, ON LUNG ANAPHYLAXIS IN ACTIVELY SENSITIZED GUINEA-PIGS: INHIBITION OF IgE – BUT NOT OF IgG – MEDIATED ANAPHYLAXIS

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1 The effect of glucocorticoid pretreatment on antigen-induced bronchoconstriction was studied in guinea-pigs actively sensitized to two different ovalbumin regimens (one producing IgE- and IgG-like antibodies and the other exclusively IgG-like antibodies).

2 Budesonide (50 mg/kg) and hydrocortisone (50 mg/kg) given as one intraperitoneal injection 15–20 h before the anaphylactic test or as two consecutive intraperitoneal injections 5 and 6 days before, led to a decreased bronchial capacity. In this respect glucocorticoid pretreatment was effective only in guinea-pigs sensitized to produce both IgE-like and IgG-like antibodies.

3 Budesonide pretreatment also reduced the capacity of anaphylactically-challenged chopped lung tissue to release histamine in guinea-pigs sensitized to produce both IgE- and IgG-like antibodies.

4 Budesonide pretreatment did not change the levels of circulating IgG_{1a} and IgE-like homocytotropic antibodies as measured by passive cutaneous anaphylaxis; nor did it affect histamine or methacholine-induced bronchoconstriction *in vivo* or the capacity of histamine or methacholine to contract the guinea-pig isolated trachea preparation or the isoprenaline-induced relaxation of this preparation.

5 The selective inhibitory effects of budesonide and hydrocortisone on IgE-mediated but not IgG-mediated anaphylaxis and the relevance to human atopic disease are discussed.

Introduction

Glucocorticoids can effectively relieve the symptoms of atopic diseases such as asthma and rhinitis, but the mechanisms for this protection are still only partly understood. The well known anti-inflammatory effect of glucocorticoids is probably important, but inhibition of the immediate type I-reaction, leading to a reduced release of anaphylactic mediators, may also contribute (Pare & Hogg, 1980; Okuda & Mygind, 1980; Martin, Atkins, Dunsky & Zweiman, 1980).

Investigations in relevant animal models might throw further light on the protective mechanisms of glucocorticoids in atopic diseases. In sensitized rats, Church & Miller (1978) found that pretreatment with high glucocorticoid doses partly reduced the lung anaphylaxis induced by antigen challenge. However, the rat is very sensitive to the immunosuppressive action of glucocorticoids and direct cytotoxic effects, not relevant to man, have also be described (Claman, 1975). Studies are therefore also required in other species. Other workers have found it difficult to demonstrate a clearcut anti-anaphylactic action of glucocorticoid treatment against the bronchoconstriction obtained in guinea-pigs (Goadby & Smith, 1964; Hicks, 1970; Forsberg & Sörenby, 1981).

However, in these studies the provoked anaphylactic reaction was probably mediated mainly by IgG-like antibodies (because of the sensitization procedure) and this may be of less relevance for the situation in atopic human disease.

We recently described a sensitization procedure for guinea-pigs which leads to formation of IgE-like antibodies as well as IgG-like antibodies (Andersson, 1980; 1981). The aims of the present investigation were to compare the inhibitory effect of glucocorticoids against lung anaphylaxis provoked either mainly via IgG-like antibodies or by IgE-like antibodies, and to study the underlying mechanisms if any protection was obtained. Budesonide (Thalén & Brattsand, 1979), which has been shown to have clinical anti-asthmatic activity (Ellul-Micallef, Hansson & Johansson, 1980), and hydrocortisone were used as glucocorticoids.

Methods

Sensitization procedures for active sensitization

We used guinea-pigs of either sex (Dunkin-Hartley 250–300 g at sensitization), bred by Sahlins, Malmö, Sweden.

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Three sensitization procedures were used, referred to below as (A), (B) and (C).

(A) The animals were sensitized by one intraperitoneal injection of 0.5 ml of saline (0.9% w/v NaCl solution) containing 1 μg ovalbumin (OA) and 100 mg $Al(OH)_3$. The adjuvant was added to the antigen solution 1 h before injection. Anaphylactic challenge was performed on day 42 and the provocation dose was 5 μg/kg ovalbumin.

(B) The animals were injected intraperitoneally with 0.5 ml volumes containing OA 5 mg on day 0 and 10 mg on day 2. Anaphylactic challenge was performed on day 42 and the provocation dose was 120 μg/kg of ovalbumin.

(C) The procedure was exactly as for (A) except that the animals were injected with 30 mg/kg cyclophosphamide intraperitoneally two days before the primary sensitization with 1 μg OA + 100 mg Al $(OH)_3$. This procedure induces the same type of antibodies as in procedure A but serum levels are higher as required for performing the passive cutaneous anaphylaxis (PCA) tests.

Glucocorticoid pretreatment in vivo

Glucocorticoids were given as one intraperitoneal injection 15–20 h before testing or as two consecutive injections 5 and 6 days before testing. They were suspended in a vehicle of carboxymethylcellulose-sodium 0.75%, Tween 80 0.4% and NaCl 0.7% ad 100%.

Drug administration in vitro

The glucocorticoids were dissolved in ethanol.

Respiratory measurements

The respiratory measurements were performed as described earlier (Andersson & Bergstrand, 1981). Guinea-pigs actively sensitized according to procedures (A) and (B) were anaesthetized and challenged on day 42 with OA injected intravenously (doses indicated in the results section) through the left jugular vein. When a stable response was reached, 1000 μg/kg of OA was injected to produce maximum response. Blood pressure was recorded throughout the entire procedure by a catheter inserted in the right carotid artery. The variables used to measure pulmonary mechanics and bronchoconstriction were lung resistance (R_L) and dynamic lung compliance (C_{DYN}) as described previously. R_L and C_{DYN} have been defined and calculated as outlined by Amdur & Mead (1958).

Histamine release from anaphylactically-shocked chopped guinea-pig lung fragments

The effect of budesonide treatment on histamine release from anaphylactically-shocked chopped lung fragments was examined in lungs taken from sensitized guinea-pigs using a procedure described earlier (Andersson & Bergstrand, 1981). Guinea-pigs sensitized according to procedure (A) were treated with budesonide (50 mg/kg i.p.) 15–20 h or 5 and 6 days before challenge. Guinea-pigs sensitized according to procedure (B) were treated with budesonide (50 mg/kg i.p.) 15–20 h before challenge. The anaphylactic challenge was performed on day 42 after sensitization. The histamine was quantified by the spectrophotofluorometric method described by May, Lyman, Alberto & Cheng (1970), omitting the extraction procedure (separate experiments showed that extraction of histamine from the supernatant did not significantly change the results; this step was therefore omitted in the present investigation).

Guinea-pig isolated trachea

The trachea was dissected out, cut spirally and placed in an organ bath filled with Krebs solution (37°C), aerated with 95% O_2 and 5% CO_2. The trachea was connected to a force displacement transducer (FTO3) with an initial tension of 1.5 g and responses recorded on a Grass polygraph model 7.

The effect of acute treatment with budesonide and hydrocortisone (dissolved in ethanol) was measured in tracheas taken from unsensitized guinea-pigs weighing 150–250 g. They were first pretreated with 0.12 ml ethanol (final bath concentration = 0.25%) and after 20 min carbachol (2.5×10^{-6} mol/l) was added to induce contraction. Isoprenaline was then added in increasing concentrations to induce relaxation. The effect of each increase in dose was calculated as a percentage of the maximum effect obtained. The same tracheas were then treated with the addition of test corticoids dissolved in 0.12 ml ethanol and the procedures described above repeated. The effect of *in vivo* pretreatment with budesonide (50 mg/kg i.p., 15–20 h before testing) was examined in tracheas taken from unsensitized guinea-pigs and contracted with 1×10^{-6} mol/l carbachol or histamine 4.8×10^{-5} mol/l.

Passive cutaneous anaphylaxis

Passive cutaneous anaphylactic reactions were estimated according to the principles given by Watanabe & Ovary (1977). Blood samples obtained from guinea-pigs sensitized according to procedures (B) and (C) were stored over night at 4°C; 0.5 ml serum was taken from each animal, pooled and stored at

−80°C. Each serum pool was examined by passive cutaneous anaphylaxis both with and without heat treatment (56°C; 1 h). A volume (0.1 ml) of the test serum, appropriately diluted in saline, was injected into the shaved abdominal skin of normal animals.

After a latent period of 4 h or 7 days, depending on the predominant type of antibody in the serum, the animals were challenged by an intravenous injection of 2 mg OA together with 10 mg/kg Evans Blue. These latent periods are considered to reflect mainly the activities of IgG_{1a} – and IgE-like antibodies, respectively as outlined by Watanabe & Ovary (1977). The antibody titre was estimated by the end point dilution technique. Titres given are the highest dilution that showed blueing in four out of six animals.

The effect of budesonide pretreatment on the circulating antibody level was investigated in guinea-pigs sensitized according to procedures (B) (for assay of IgG-like antibodies) and (C) (for assay of IgE-like antibodies). Cyclophosphamide has been shown to induce high titres of IgE-like antibodies (Chiorazzi Fox & Kats, 1976; Andersson, 1981).

Drugs

These were obtained as follows: ovalbumin (OA, grade III), (−)-isoprenaline hydrochloride, Sigma; Mebumal, ACo, Sweden; $Al(OH)_3$, Reheis Chemical Company, obtained through AB Astra, Sweden; budesonide (16 α, 17 α-(22R, S)-propylmethylenedioxypregna-1, 4-diene 11 β, 21-diol-3, 20-dione) (micronized), Astra, Sweden; hydrocortisone acetate (micronized), histamine chloride, carbamylcholine chloride (carbachol), Apoteksbolaget, Sweden; O-phthalaldehyde, BDH Chemicals Ltd; mepyramine maleate, May & Baker, Ltd; Evans Blue Merck, West Germany; cyclophosphamide (Sendoxane, Pharmacia, Sweden). Other chemicals used were analytically pure standard chemicals. The Krebs solution contained (mM): NaCl 118, KCl 4.7, CaCl 2.5, $MgSO_4$ 1.16, $NaHCO_3$ 25, KH_2PO_4 1.18 and D-glucose 11.

Statistics

Significance of the differences between groups was estimated by Student's *t* test. The dose-response curves were compared by parallel line assay (Finney, 1952).

Results

Effect of budesonide and hydrocortisone on antigen-induced bronchial anaphylaxis in sensitized guinea-pigs

In guinea-pigs sensitized according to procedure (A) the prechallenge lung resistance (R_L) and dynamic lung compliance (C_{DYN}) were 25.4 ± 3.2 $cmH_2O\ l^{-1}\ s^{-1}$ and 2.47 ± 0.21 ml/cmH_2O respectively (mean ± s.e.mean, $n = 63$). The values for guinea-pigs sensitized according to procedure (B) were 21.4 ± 2.8 $cmH_2O\ l^{-1}\ s^{-1}$ and 2.12 ± 0.17 ml/cmH_2O respectively (mean ± s.e.mean, $n = 53$). A higher provocation dose (120 μg/kg of OA), was needed in animals sensitized according to procedure (B) to obtain an anaphylactic response comparable in intensity to that obtained with 5 μg/kg of OA in guinea-pigs sensitized according to procedure (A).

Figure 1A shows that pretreatment with budesonide and hydrocortisone (50 mg/kg, 15–20 h or 5 and 6 days respectively before testing) markedly reduced the subsequent *in vivo* bronchial anaphylactic reactivity in guinea-pigs sensitized according to procedure (A). However, the same glucocorticoid pretreatments did not significantly decrease the subsequent anaphylactic response in guinea-pigs sensitized according to procedure (B) (Figure 1B). Mepyramine reduced the antigen-induced bronchoconstriction in a dose-dependent way in anaesthetized guinea-pigs sensitized according to both procedures (A) and (B) (Figure 2, panels B_1, B_2 and C. Budesonide pretreatment (50 mg/kg, 15–20 h before testing) conferred an additive protection on the mepyramine-induced inhibition of the bronchial anaphylaxis produced by a low provocation dose (5 μg/kg) in guinea-pigs sensitized according to procedure (A) (Figure 2, panel B_1). If a high supramaximal, provocation dose of ovalbumin (40 μg/kg) was used, there was a slight, but not significant additive protection of budesonide on the mepyramine-induced inhibition of the bronchial anaphylaxis (Figure 2, panel B_2).

Effect of budesonide pretreatment on histamine release from anaphylactically shocked guinea-pig lung fragments

This was investigated with the chopped lung technique in guinea-pigs sensitized according to procedure (A) or (B). There was no difference in the total histamine content or in the spontaneous histamine release in lungs taken from vehicle-treated or budesonide-treated guinea-pigs. The lungs from guinea-pigs sensitized according to procedure (A), pretreated with budesonide (50 mg/kg) 15–20 h or 5 and 6 days respectively before testing and challenged with ovalbumin showed a markedly reduced histamine release compared with vehicle-treated animals (Figure 3). The dose-response relationships were also investigated by linear regression analysis. This showed that after pretreatment with budesonide 15–20 h or 5 and 6 days (before challenge) in the

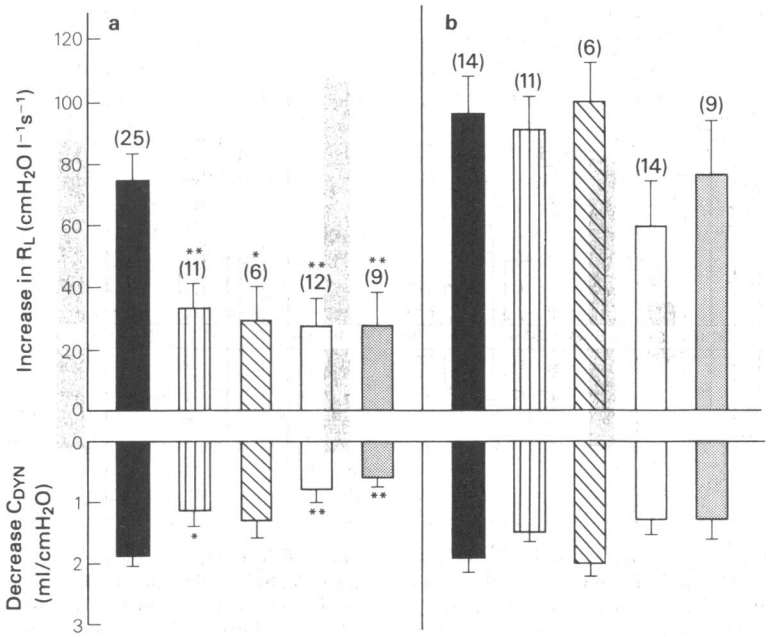

Figure 1 Effect of budesonide and hydrocortisone pretreatment on antigen-induced bronchial anaphylaxis in sensitized guinea-pigs. Bronchoconstriction is expressed in terms of lung resistance (R_L) and dynamic lung compliance (C_{DYN}); (a) Guinea-pig sensitized by procedure (A); (b) guinea-pigs sensitized by procedure (B). Solid column: control response to ovalbumin provocation (5 μg/kg in (a) and 120 μg/kg in (b); striped columns: budesonide (50 mg/kg, i.p.) given 15–20 h before testing; hatched columns: hydrocortisone (50 mg/kg, i.p.) given 15–20 h before testing; open columns: budesonide (50 mg/kg, i.p.) given 5 and 6 days before testing; stippled columns: hydrocortisone (50 mg/kg, i.p.) given 5 and 6 days before testing. Columns represent mean results and bars show s.e.mean; figures in parentheses indicate the number of experiments. Statistical significance of differences are shown with respect to control anaphylaxis: $*P<0.05$; $**P<0.01$.

procedure (A), 5–8 times ($P<0.01$) higher antigen doses were necessary to induce the same amount of histamine release as in vehicle-pretreated animals (Figure 3). However, pretreatment with budesonide (50 mg/kg, 15–20 h before testing) did not decrease the histamine release in lungs taken from guinea-pigs sensitized according to procedure (B).

Effect of budesonide pretreatment on the in vivo *bronchial sensitivity to methacholine and histamine*

Injections of histamine (2–8 μg/kg) or methacholine (2–16 μg/kg) produced dose-related increases in R_L and decrease in C_{DYN}. Pretreatment with budesonide (50 mg/kg i.p., 15–20 h before test) did not affect these responses.

Effect of budesonide on isoprenaline-induced relaxation of guinea-pig isolated trachea

Two consecutive tests with 0.12 ml of ethanol, the budesonide solvent, added to the same trachea, did not change the sensitivity to isoprenaline. (ED_{50} 137 nmol/l before, 140 nmol/l after). The tracheas were contracted with carbachol, 1×10^{-6} mol/l. The acute effect of budesonide was tested at a final concentration of 6.5×10^{-5} mol/l as this was the solubility limit for the compound in buffer. Budesonide had only very slight acute effects on isoprenaline-induced relaxation of the carbachol-contracted tracheas, reducing the ED_{50} from 120 nmol/l to 96 nmol/l (difference not significant).

In vivo pretreatment with budesonide (50 mg/kg i.p., 15–20 h before testing) had no significant effect on the *in vitro* relaxant activity of isoprenaline on histamine- or carbachol-contracted tracheas. The ED_{50} values for isoprenaline in control and budesonide-pretreated tissues were 3.1 and 2.5 nmol/l (histamine as agonist, 4.8×10^{-6} mol/l) and 26.3 and 31.3 nmol/l (carbachol as agonist, 1×10^{-6} mol/l).

Effect of in vivo *pretreatment with budesonide on the serum levels of homocytotropic antibodies*

Pretreatment with budesonide (50 mg/kg i.p. 15–20 h before testing) did not change the levels of circulating IgG_{1a}- or IgE-antibodies as determined

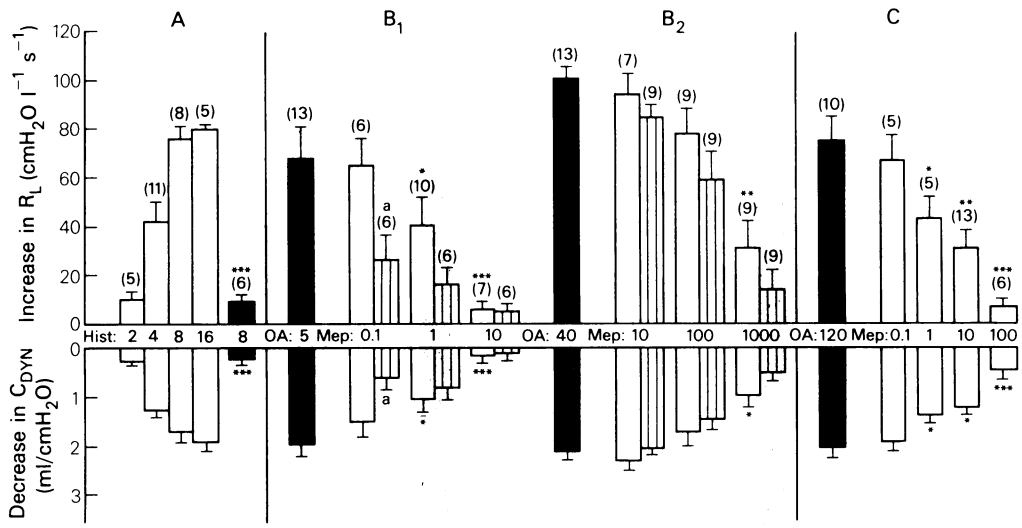

Figure 2 Effects of mepyramine (Mep) on histamine (Hist)- or antigen-induced bronchoconstriction in anaesthetized guinea-pigs. (A) Open columns: dose-related bronchoconstriction to histamine in unsensitized guinea-pigs; solid columns: inhibition by 10 μg/kg mepyramine of bronchoconstriction induced by 8 μg/kg histamine. (B) Effect of mepyramine or budesonide pretreatment on ovalbumin (OA) anaphylaxis, procedure (A). Solid columns: control response to ovalbumin (5 μg/kg in panel B_1 and 40 μg/kg in panel B_2; open columns: dose-response for mepyramine (given 10 min before anaphylactic shock); striped columns: dose-response for mepyramine (as above) in animals pretreated with budesonide (50 mg/kg given 15–20 h before testing). (C) Effect of mepyramine pretreatment on ovalbumin anaphylaxis in guinea-pigs sensitized by procedure (B). Solid columns: control response to anaphylaxis induced by 120 μg/kg ovalbumin; open columns: dose-response for mepyramine (given 10 min before anaphylactic shock). Mepyramine doses are given as μg/kg. Columns represent mean results and bars show s.e.mean; figures in parentheses indicate the number of experiments. Statistical significance of differences are shown with respect to control anaphylaxis: $*P<0.05$; $**P<0.01$; $***P<0.001$; (a) indicates difference at $P<0.05$ with respect to mepyramine treatment alone.

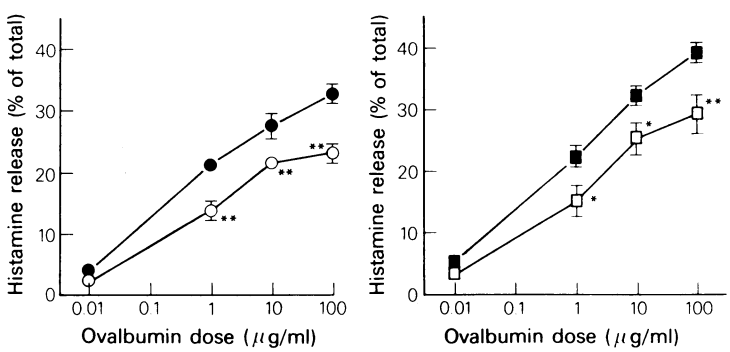

Figure 3 Effect of budesonide pretreatment on *in vitro* antigen-induced histamine release in sensitized guinea-pigs. Guinea-pigs were sensitized by procedure (A) and the lung fragments shocked with varying doses of ovalbumin.(●, ■) Control responses, no pretreatment ($n = 10$ or 14). (○, □) Animals pretreated with budesonide (50 mg/kg, i.p.) given 15–20 h (○, $n = 8$) or 5–6 days (□, $n = 9$) before testing. Points show mean values and bars represent s.e.mean. Differences with respect to control: $*P<0.05$ and $**P<0.01$.

by tests of passive cutaneous anaphylaxis. In guinea-pigs sensitized according to procedure (B), the serum titre of IgG_{1a}-antibodies was 512 in control of animals and 512 in budesonide-treated animals. The serum level of IgE-antibodies was estimated in guinea-pigs sensitized according to procedure (C). The titre was found to be 64 in control animals and 32 in budesonide-treated animals.

Discussion

The aim of the present study was to investigate the anti-anaphylactic effects and mechanisms of action of glucocorticoids in sensitized guinea-pigs. The two selected glucocorticoids (budesonide and hydrocortisone) were administered in a high dose by intraperitoneal injection because of the low sensitivity of guinea-pigs to glucocorticoids (Claman, 1975) and the difficulty of administering efficiently such compounds by aerosol to guinea-pigs *in vivo*. They were given as pretreatments (5 and 6 days or 15–20 h before anaphylactic challenge) because of the latency time of steroid hormone action.

Release of anaphylactic compounds via IgE-mediated reactions is considered to play a major role in the pathogenesis of atopic human asthma (Johansson & Foucard, 1978). However, mast cells and basophils from different species also possess Fc-receptors for homocytotropic antibodies of the IgG-class (Spiegelberg, 1974) and there are reports indicating different receptors for IgG and IgE on mast cells (Daëron, Pronovost-Danon & Voison, 1980; Moodley & Mongar, 1981). Although there are reports of anaphylactic antibodies of the IgG-type in man (Bryant, Burns & Lazarus 1973; 1975), the pathophysiological importance of IgG-mediated reactions for human asthma symptoms is not clear. Thus in an animal model it would be desirable to obtain a type I anaphylactic response mediated by IgE-like antibodies.

Andersson (1980; 1981) showed that in the guinea-pig it is possible to vary the types of anaphylactic antibodies elicited by changing the sensitization procedure. Animals sensitized with small amounts of ovalbumin together with $Al(OH)_3$ as adjuvant (sensitization procedure (A)) produce both IgE- and IgG-like antibodies, whereas those sensitized with large amounts of OA without adjuvant (sensitization procedure (B)) produce only IgG-like antibodies. Higher provocation doses are needed to induce an anaphylactic bronchoconstriction and much less histamine is liberated from the lungs upon antigen challenge in guinea-pigs sensitized according to procedure (B), compared with procedure (A) (Andersson, 1980; Andersson & Bergstrand, 1981). This is in accordance with the findings that IgG-mediated liberation of histamine from leucocytes and mast cells is much less pronounced than the IgE-mediated release (Ishizaka, Ishizaka, Johansson & Bennich, 1969; Lichtenstein, Levy & Ishizaka, 1970; Barnet & Justus, 1975).

Using these two sensitization procedures in guinea-pigs it has been possible to investigate whether glucocorticoids (i.e. hydrocortisone and budesonide) inhibit lung anaphylaxis mediated mainly by IgE-like antibodies or mainly by IgG-like antibodies. Sensitization procedure (A) certainly also induces formation of IgG-like antibodies, but the provocation dose used on challenge in such animals (5 μg/kg) was so low that it would not induce bronchoconstriction due to agents released by IgG-mediated reactions, assuming the sensitivities of the antibodies to be similar, as in procedure (B) (Andersson, 1980).

The results in this paper show that pretreatment with budesonide or hydrocortisone inhibited anaphylactic reaction in animals sensitized according to procedure (A) by more than 50%, as measured in terms of lung resistance and dynamic lung compliance (Figure 1). As this type of anaphylaxis is provoked mainly by IgE-like antibodies, the inhibition may be of clinical interest and is also in accordance with recent clinical findings. Thus Martin *et al.* (1980) found that 40 mg prednisolone given daily for 1 week markedly reduced antigen-induced bronchoconstriction. Inhalation of budesonide (1 mg) daily for one week counteracted by more than 40% the antigen-induced immediate drop of peak expiratory flow rate in allergic patients (Dahl & Johansson, 1981).

On the other hand these results show that no significant protection was afforded by glucocorticoid pretreatment on the IgG-induced (sensitization procedure (B)) type of anaphylaxis. This absence of clear protection is in conformity with earlier studies on anti-anaphylactic effects of glucocorticoids in guinea-pigs. All such studies, as far as we know, have been performed with sensitization methods similar to the present procedure (B). Goadby & Smith (1964) and Forsberg & Sörenby (1981) found no protection from glucocorticoids alone and Hicks (1970) reported a weak inhibition after pretreatment with potent glucocorticoids.

The budesonide pretreatment did not affect the level of circulating IgE- or IgG-like antibodies, which is in accordance with most of the earlier studies on the effect of glucocorticoids on antibody production (Baxter & Forsham, 1972). However, it was not possible in the present investigation to study the antibody levels directly in lung tissue.

Complementary investigations were performed to study whether the demonstrated protection against the mainly IgE-induced anaphylaxis depended on inhibition of release of anaphylactic mediators or on inhibition of the pharmacological actions of such mediators. In either case the inhibition must be specific as it was demonstrated in only one of the sensitization procedures. Budesonide had no clear bronchorelaxant action on contractions provoked by histamine or methacholine (see above) or by acetylcholine or prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) (results not shown). Nor did budesonide potentiate β-receptor-mediated bronchorelaxation. However, the demon-

strated antianaphylactic effects of budesonide in animals sensitized by procedure (A) may instead be related to changes in anaphylactic histamine release. A greatly reduced anaphylactic histamine release was found in fragments from lungs of animals sensitized by procedure (A) (Figure 3) while no budesonide-induced protection could be demonstrated in lungs from guinea-pigs sensitized according to procedure (B). Hicks (1970) and Forsdberg & Sörenby (1981) found no or only a small decrease of antigen-induced histamine liberation after glucocorticoid pretreatment of guinea-pigs sensitized according to procedures similar to procedure (B). However, in these other studies glucocorticoid pretreatment plus mepyramine, an H_1-antagonist, decreased the anaphylactic response *in vivo* more than mepyramine alone did. This was explained by glucocorticoid-induced inhibition of SRS-A formation (Goadby & Smith, 1964; Forsberg & Sörenby, 1981). Similar, but very weak, tendencies for an additive protection by mepyramine and budesonide were seen in the present IgE-mediated anaphylaxis (Figure 2, panels B_1 and B_2). The lack of clear additive or synergistic behaviour of the combination of budesonide and mepyramine may be because SRS-A has less importance than histamine for the bronchoconstriction in this type of IgE-mediated anaphylaxis. Moreover, cyclo-oxygenase products of arachidonate metabolism (e.g. thromboxane A_2) do not seem to be major mediators because indomethacin does not inhibit this type of anaphylaxis (P. Andersson, unpublished experiments).

Taken together the information about different Fc-receptors for IgE- and IgG-antibodies, and the demonstrated different pharmacological protection against anaphylaxis involving these antibodies, suggests that budesonide and hydrocortisone inhibit anaphylaxis by specific interactions with early events in the triggering of the IgE-receptor. Such a hypothesis is in accordance with some recent findings described in the literature. Thus glucocorticoid treatment has been reported to reduce the number of monocytes with Fc-receptors for IgE in patients with severe atopic diseases (Melewicz, Zeiger, Mellon, O'Connor & Spiegelberg, 1981). Glucocorticoids also prevent the IgE-induced expression of Fc_ε-receptors and change the nature of the IgE-binding factors formed (Yodoi, Hirashima & Ishizaka, 1981). As glucocorticoids inhibit phospholipase A_2-activity (Flower & Blackwell, 1979), it is of great interest that phospholipase A_2 has been proposed as a participant in the regulation of IgE-binding factors in T-lymphocytes (Yodoi, Hirashima, Hirata, De Blas & Ishizaka, 1981), as well as in the formation of SRS-A. It remains to be established whether glucocorticoids also affect Fc_ε-receptors in guinea-pigs sensitized according to the present procedure (A), and if so, what kind of anaphylactic lung cells are affected.

One objection to the above hypothesis is that no protection was obtained from the IgG-mediated anaphylaxis. Crabtree, Gillis, Smith & Munch (1979) and Suzuki, Sadasivan, Saito-Taki, Stechschulte, Balentine & Helmkamp (1980) found that glucocorticoids decrease the number of Fc_γ-receptors on granulocytes and β-lymphocytes, respectively. However, the molecular induction of IgG-mediated anaphylaxis may be less clear than that of IgE-provoked reactions as there are suggestions that IgG-anaphylaxis can proceed over deposition of immune complexes that might also trigger complement receptors (for references see Theofilopoulos & Dixon, 1979). Further studies are required to discover the mechanisms underlying the anaphylactic responses obtained in guinea-pigs using the two sensitization procedures and the effects of glucocorticoid on them.

In conclusion, the present investigation shows that treatment with budesonide or hydrocortisone reduces anaphylactic bronchoconstriction *in vivo* and histamine release *in vitro* on antigen challenge in guinea-pigs sensitized to produce mainly IgE-antibodies. The anti-anaphylactic activity of budesonide is not correlated with decreased antibody production, decreased total histamine content of the lung or to changed broncho-reactivity to β-sympathomimetics or contracting agents like histamine or carbachol. It is concluded that guinea-pigs sensitized with small amounts of ovalbumin may be a suitable animal model for evaluating the effects and mechanisms of glucocorticoids on IgE-mediated anaphylaxis. Continuing investigations also demonstrate that is is possible to refine the model for testing the anti-anaphylactic effects of glucocorticoids after local delivery to the lungs.

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References

AMDUR, M.O. & MEAD, J. (1958). Mechanics of respiration in unanaesthetized guinea-pigs. *Am. J. Physiol.*, **192,** 364–368.

ANDERSSON, P. (1980). Antigen-induced bronchial anaphylaxis in actively sensitized uinea-pigs. Pattern of response in relation to immunization regimen. *Allergy*, **35,** 65–71.

ANDERSSON, P. (1981). Antigen-induced bronchial anaphylaxis in actively sensitized guinea-pigs. The effect of booster injection and cyclophosphamide treatment. *Int. Archs Allergy appl. Immunol.*, **64,** 249–258.

ANDERSSON, P. & BERGSTRAND, H. (1981). Antigen-induced bronchial anaphylaxis in actively sensitized guinea-pigs: effect of long-term treatment with sodium cromoglycate and aminophylline. *Br. J. Pharmac.*, **74,** 601–609.

BARNETT, J.B. & JUSTUS, D.E. (1975). Differential effects of homocytotropic antibodies on the mast cell and anaphylactic responses in actively and passively sensitized mice. *Infection and immunity*, **11,** 1342–1351.

BAXTER, J.D. & FORSHAM, P.H. (1972). Tissue effects of glucocorticoids. *Am. J. Med.*, **53,** 573–589.

BRYANT, D.H., BURNS, M.W. & LAZARUS, L. (1973). New type of allergic asthma due to IgG 'reaginic' antibody. *Br. med. J.*, **4,** 589–592.

BRYANT, D.H., BURNS, M.W. & LAZARUS, L. (1975). Identification of IgG antibody as carrier of reaginic activity in asthmatic patients. *J. Allergy clin. Immunol.*, **56,** 417–428.

CHIORAZZI, N., FOX, D.A. & KATZ, D.H. (1976). Hapten-specific IgE antibody responses in mice. VI. Selective enhancement of IgE antibody production by low doses of X-irradiation and cyclophosphamide. *J. Immunol.*, **117,** 1629–1637.

CHURCH, M.K. & MILLER, P. (1978). Time courses of the anti-anaphylactic and anti-inflammatory effects of dexamethasone in the rat and mouse. *Br. J. Pharmac.*, **62,** 481–486.

CLAMAN, H.N. (1975). How corticosteroids work. *J. Allergy clin. Immunol.*, **55,** 145–151.

CRABTREE, G.R., GILLIS, S., SMITH, K.A. & MUNCK, A. (1979). Glucocorticoids and immune responses. *Arthritis and Rheumatism*, **22,** 1246–1256.

DAËRON, M., PRONOVOST-DANON, A. & VOISON, G.A. (1980). Mast cell membrane antigens and Fc receptors in anaphylaxis II. Functionally distinct receptors for IgG and IgE on mouse mast cells. *Cell. Immunol.*, **49,** 178–189.

DAHL, R. & JOHANSSON, S.Å. (1981). The effects of inhaled budesonide on dual asthmatic reactions. XIV *Scandinavian Congress in Allergology*, Gothenburg, 10–13th June, 1981. Abstract.

ELLUL-MICALLEF, R., HANSSON, E. & JOHANSSON, S.Å. (1980). Budesonide: a new corticosteroid in bronchial asthma. *Eur. J. respir. Dis.*, **61,** 167–173.

FINNEY, D.J. (1952). *Statistical Methods in Biological Assay*. London: Charles Griffin & Co. Ltd.

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

FORSBERG, K. & SÖRENBY, L. (1981). The influence of a new corticosteroid, budesonide, on anaphylactic bronchoconstriction and SRS-A release in the guinea-pig. *Agents and actions*, **11,** 391–395.

GOADBY, P. & SMITH, W.G. (1964). Observations on the anti-anaphylactic activity of hydrocortisone and related steroids. *J. Pharm. Pharmac.*, **16,** 108–114.

HICKS, R. (1970). The influence of corticosteroid pretreatment on anaphylactic bronchoconstriction in the guinea-pig. *J. Pharm. Pharmac.*, **22,** 170–179.

ISHIZAKA, T., ISHIZAKA, K., JOHANSSON, S.G.O. & BENNICH, H. (1969). Histamine release from human leukocytes by anti-αE antibodies. *J. Immunol.*, **102,** 884–892.

JOHANSSON, S.G.O. & FOUCARD, T. (1978). IgE in immunity and disease. In: *Allergy: Principles and Practice.* ed. Middleton Jr., E., Read, C.E. & Ellis, E.F. pp. 551–562. Saint Louis, U.S.A.: C.V. Mosby Company.

LICHTENSTEIN, L.M., LEVY, D.A. & ISHIZAKA, K. (1970). In vitro reversed anaphylaxis: Characteristics of anti-IgE mediated histamine release. *Immunology*, **19,** 831–842.

MARTIN, G.L., ATKINS, P.C., DUNSKY, E.H. & ZWEIMAN, B. (1980). Effects of theophylline, terbutaline and prednisone on antigen-induced bronchospasm and mediator release. *J. Allergy clin. Immunol.*, **66,** 204–212.

MAY, C.D., LYMAN, M., ALBERTO, R. & CHENG, J. (1970). Procedures for immunochemical study of histamine release from leucocytes with small volume of blood. *J. Allergy*, **46,** 12–20.

MELEWICZ, F.M., ZEIGER, R.S., MELLON, M.H., O'CONNOR, R.D. & SPIEGELBERG, H.L. (1981). Increased peripheral blood monocytes with Fc receptors for IgE in patients with severe allergic disorders. *J. Immunol.*, **126,** 1592–1595.

MOODLEY, I. & MONGAR, J.L. (1981). IgG receptors on the mast cell. *Agents and actions*, **11,** 77–83.

OKUDA, M. & MYGIND, N. (1980). Pathophysiological basis for topical steroid treatment in the nose. In: *Topical Steroid Treatment for Asthma and Rhinitis.* ed. Mygind, N. & Clark, T.J.H. pp. 22–33. London: Baillière Tindall.

PARE, P.D. & HOGG, J.C. (1980). Pathophysiological basis for inhaled steroid treatment in the bronchi. In: *Topical Steroid Treatment for Asthma and Rhinitis.* ed. Mygind, N. & Clark, T.J.H. pp. 12–21. London: Baillière Tindall.

SPIEGELBERG, H.L. (1974). Biological activities of immunoglobulins of different classes and subclasses. *Adv. Immunol.*, **19,** 259–294.

SUZUKI, T., SADASIVAN, R., SAITO-TAKI, T., STECHSCHULTE, D.J., BALENTINE, L. & HELMKAMP, G.M. (1980). Studies of Fc_γ receptors of human B lymphocytes: Phospholipase A_2 activity of Fc_γ receptors. *Biochemistry*, **19,** 6037–6044.

THALÉN, A. & BRATTSAND, R. (1979). Synthesis and anti-inflammatory properties of budesonide, a new nonhalogenated glucocorticoid with high local activity. *Arzneim.-Forsch./Drug Res.*, **29,** 1687–1690.

THEOFILOPOULOS, A.N. & DIXON, F.J. (1979). The biolo-

gy and detection of immune complexes. *Adv. Immunol.*, **28**, 89–220.

WATANABE, N. & OVARY, Z. (1977). Antigen and antibody detection by *in vivo* methods: a reevaluation of passive cutaneous anaphylactic reactions. *J. immunol. Methods*, **14**, 581–590.

YODOI, J., HIRASHIMA, M. & ISHIZAKA, K. (1981). Lymphocytes bearing Fc receptors for IgE. VI. Suppressive effect of glucocorticoids on the expression of Fc_ε receptors and glycosylation of IgE-binding factors. *J. Immunol.*, **127**, 471–476.

YODOI, J., HIRASHIMA, M., HIRATA, F., DE BLAS, A.L. & ISHIZAKA, K. (1981). Lymphocytes bearing Fc receptors for IgE. VII. Possible participation of phospholipase A_2 in the glycosylation of IgE-binding factors. *J. Immunol.*, **127**, 476–482.

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