

The Glucocorticosteroid, Budesonide, Partially Blocks Histamine Release from Human Lung Tissue *in Vitro*

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IgE-receptor dependent, but not A23187-induced, histamine release from passively sensitized chopped human lung tissue, or from a dispersed lung cell population obtained from the tissue after enzymatic digestion, is reduced after incubation overnight of cells/tissue with the glucocorticosteroid budesonide (10^{-7} M). Since the inhibitory effect of budesonide is reduced in the continuous presence of diluted reagin-rich serum used for passive sensitization, but not in the presence of heat-inactivated serum, it is suggested that the glucocorticosteroid acts by reducing the binding of IgE-antibody to the target cell(s).

Key words: glucocorticosteroids; histamine release; human lung tissue.

Accepted for publication 5 March 1986

The high therapeutic efficacy of glucocorticosteroids (GCS) in various allergic disorders is well known. Besides their anti-inflammatory activity, GCS also show anti-allergic activity, i.e. when appropriately administered they may reduce the immediate and even more effectively block the late bronchial or skin reaction in challenged subjects. As recently emphasized in a comprehensive review of GCS action (25), their clinical efficacy cannot be explained by a single mode of action. One main action is probably the blockage of the influx of leukocytes of all types into the tissue, which may be due to an action of the GCS on endothelial cells. Part of the anti-allergic action of GCS may be due to inhibition of the generation and release of inflammatory mediators from basophils/mast cells. Thus, GCS treatment of asthmatics is reported to reduce peak plasma levels of histamine and per cent increase in serum neutrophil chemotactic activity at allergen challenge (18). On the other hand, such treatment does not seem to reduce the respon-

siveness of the patients' basophils *in vitro* to immunologic stimuli; rather, it induces reduced levels of basophils in the circulation (7, 24). However, *in vitro* GCS reduce IgE-receptor dependent histamine release from human leukocytes (4, 26, 27) provided that the cells are incubated with the steroid a sufficient time before challenge.

Very little or no inhibition of IgE-mediated histamine or arachidonic acid metabolite release from chopped human lung tissue or dispersed lung mast cells was recorded after exposure of the tissue/cells to dexamethasone or betamethasone (10, 25, 28). The present experiments were performed to examine whether the highly potent glucocorticoid budesonide (32), widely used in treatment of asthma (6, 8, 12) and previously shown to be an effective inhibitor of mediator release (1, 4), also from human nasal biopsies (22), influences mediator release from human lung tissue.

MATERIAL AND METHODS

Lung specimens

Specimens of human lung tissue (kindly supplied by the Department of Thoracic Surgery, University Hospital, Lund) were obtained from various non-atopic patients suffering from carcinomas of the lung. Normal tissue specimens were immediately transferred into saline and processed as specified below, either within a few hours or after storage at 4°C overnight. No consistent difference was recorded in degree of release or in effect of GCS between freshly prepared tissue and tissue stored overnight. The tissue was rinsed free from gross blood contamination and, while being bathed in Tyrode buffer (lacking Ca^{2+} and Mg^{2+}) or saline buffered with phosphate (PBS), was cut into small fragments (approximately 1 mm³ in size) with a pair of scissors. The fragments were washed in Tyrode or PBS several times.

Sensitization procedure

For passive sensitization, the lung fragments were incubated for 2 h at 37°C in a reagin-rich serum (dilution 1:10 in medium RPMI 1640 (Flow Lab) of a high titre IgE serum from a birch pollen-sensitive individual). The fragments were then washed 3 times in RPMI and approximately 3–5 pieces of tissue (together weighing ca. 100 mg) were transferred to each test tube together with RPMI 1640 (pH 7.3) containing 25 mM N-2-hydroxyethylpiperazine-N'-2-ethane sulfonic acid (HEPES) (Sigma), 50 mg/l gentamycin, 4 mM glutamine, and 1 g/l human serum albumin (Continental Pharma Ltd., Canada). Moreover, diluted reagin-rich serum or the diluent or budesonide (at a final concentration as indicated below) / vehicle, was added. Incubation was performed in a total volume of 1 ml of 37°C overnight, the fragments were washed, and finally resuspended in HEPES-ACM buffer (21).

Challenge procedure

Challenge was performed by addition of birch pollen extract (final dilution 1:10000 or 1:1000

of a preparation obtained from Vitrum which was dialyzed against HEPES-ACM to remove phenol before use), anti-IgE (final dilution 1:10000 or 1:1000 of an antiserum, the preparation of which has been previously described (3)), ionophore A23187 (Lilly; final concentration 1 µg/ml) or vehicle (buffer control). The release process was stopped after 30 min by chilling the tubes on ice and the supernatants were removed. To extract remaining tissue histamine, 1.0 ml of medium HEPES-ACM was added to the fragments and the tubes were immersed in a boiling water bath for 8 min.

Determination of histamine

Pre- and postboiling supernatants were deproteinized by precipitation with perchloric acid (9). The histamine content of the supernatants was determined spectrophotofluorometrically after condensation with o-phthalaldehyde (9). All samples were run in duplicate. The amount of histamine released into the supernatant was expressed as per cent of total tissue histamine and corrected for spontaneous release.

Experiments with dispersed cells

Experiments with dispersed cells were performed as follows. The chopped tissue was treated with proteolytic enzymes according to principles described by Schulman et al. (29). To each gram of finely chopped lung tissue was added a few ml of RPMI 1640 made 1:10 with respect to the reagin-rich serum described above. After passive sensitization at room temperature for 2 h, pronase (Sigma protease P-5147; 2 mg/ml final concentration), chymopapain (Sigma C-9007; 0.5 mg/ml final concentration), and DNase (Sigma D-4763; 10 µg/ml final concentration) were added and the incubation continued for 30 min at room temperature. The released cells were freed from the tissue by filtration through a nylon net. Digestion of the tissue was repeated once as described above and then performed twice in RPMI 1640 - 10% reagin-rich serum made 1 mg/ml with respect to gelatin, 10 µg/ml with respect to DNase, 1 mg/ml with respect to collagenase

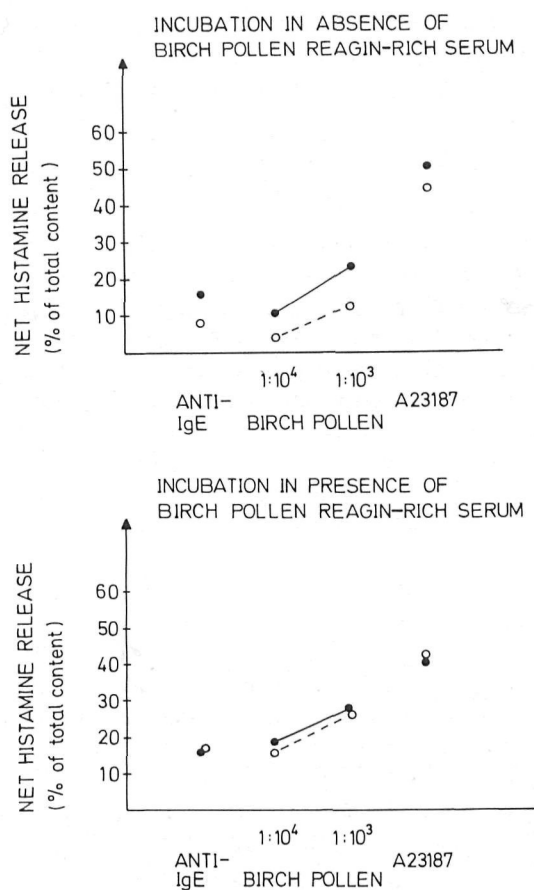


Fig. 1. Histamine release from chopped human lung tissue incubated overnight at 37°C without (top frame) or with (bottom frame) birch pollen reagin-rich serum (1:10) and either budesonide (O; 10^{-7} M) or vehicle (●). Challenge was performed with anti-IgE (final concentration 1:10000), birch pollen extract (1:1000 or 1:10000), or A23187 (1 μ g/ml). Figures given for secretagogue-induced release are corrected for vehicle-induced release (ranging from 4 to 8% of total histamine content).

(Sigma C-0130), and 10 U/ml with to respect elastase (Sigma E-1250). Recovered cells were combined and washed, erythrocytes were lysed by treatment with H_2O for 1 min and cells were resuspended in Tyrode solution lacking Ca^{2+} and Mg^{2+} made 10% with respect to reagin-rich serum and 10 μ g/ml DNase. The cells were counted and stained with alcian blue to examine mast cell/basophil count. The cells

were resuspended in RPMI 1640 made 50 mg/l in gentamycin, 4 mM in glutamine, 1 g/l in human serum albumin, and 10% in naive or heat-inactivated (56°C 4 h) reagin-rich serum. Budesonide (final concentration 10^{-7} M) or the corresponding amount of vehicle (ethanol) was added. The cells were cultured overnight and release experiments were performed as described for leukocytes (4). Challenge was performed with the agents employed with the chopped lung tissue as described above.

RESULTS

Effects of GCS on histamine release from chopped human lung tissue

We first examined effects of GCS on histamine release from chopped human lung tissue *in vitro* induced by either antigen, anti-IgE, or calcium ionophore A23187. Following passive sensitization for a 2 h period, the chopped tissue was incubated overnight in either the presence or the absence of a further sample of the diluted (1:10) reagin-rich serum and GCS (or vehicle). Then, the tissue was washed and challenged with the above-mentioned secretagogues. Fig. 1 shows the results of one such experiment indicating that inhibition of release initiated by IgE-receptor dependent secretagogues, but not that induced by A23187, is observed for fragments cultured with budesonide. The inhibitory effect of the GCS is reduced if the overnight incubation is performed in the presence of diluted reagin-rich serum. Fig. 2 shows plots of histamine release from passively sensitized fragments incubated overnight with budesonide versus that of fragments incubated with vehicle. These experiments were performed in the absence of any further addition after sensitization of birch pollen reagin-rich serum. The figure shows that culture of lung fragments with budesonide 10^{-7} M as compared to culture with vehicle leads to reduced histamine release at challenge with secretagogues acting via IgE-receptors, but not at challenge with A23187. The degree of inhibition is not clearly correlated to the strength of the stimulation as was previously (3) shown to be the case for leukocytes

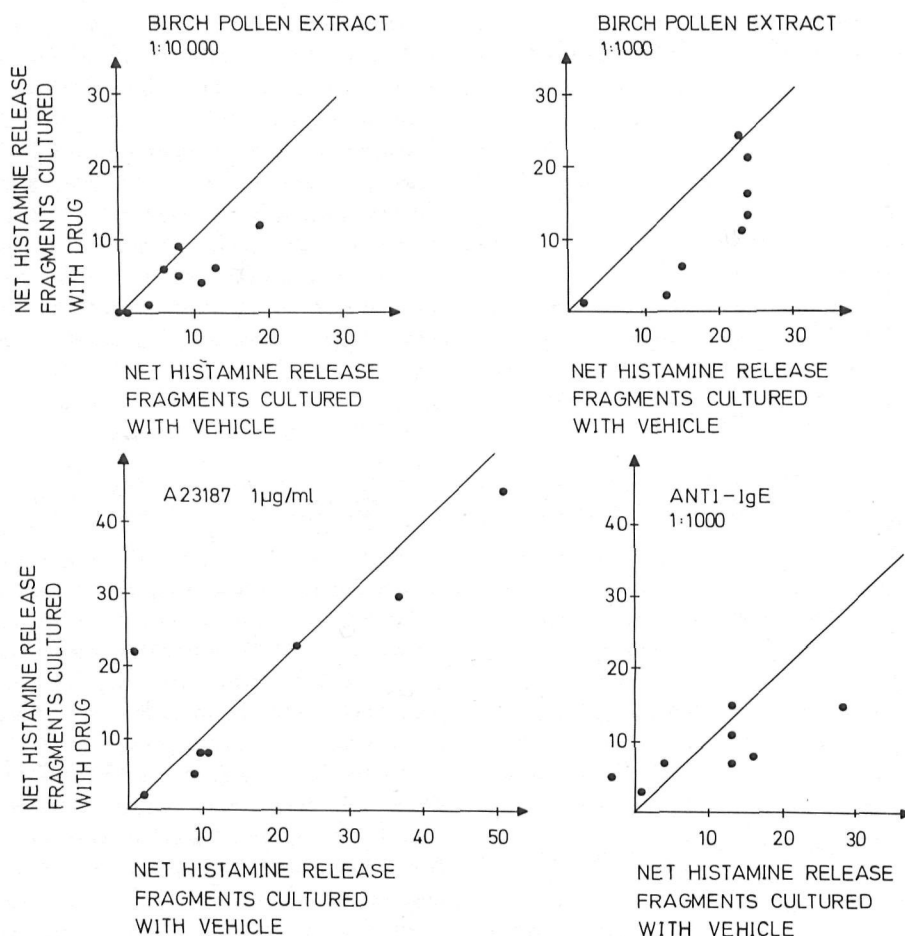


Fig. 2. Plot of net histamine release from fragments cultured overnight at 37°C in the presence of 10^{-7} M budesonide versus that of fragments of the same lung specimen cultured in the presence of vehicle. All cultures were performed in the absence of birch pollen reagin-rich serum. Within each frame, points represent data recorded with separate lung specimens.

(compare results obtained with the two concentrations of birch pollen extract).

Table 1 gives the values for percent inhibition defined as:

In these calculations, experiments where net release for vehicle-treated fragments is less than 10% have been excluded. This table also lists results obtained in parallel experiments where

10% birch pollen reagin-rich serum was present during the GCS treatment. These results show that incubation of fragments overnight with budesonide leads to a reduced histamine release at challenge with birch pollen and possibly also at challenge with anti-IgE, whereas very little inhibition is seen at challenge with the calcium ionophore A23187. The inhibitory capacity of the GCS seems to be reduced by the simultaneous presence during overnight culture of reagin-rich serum when birch pollen allergen is employed as secretagogue. For cultures treated

with budesonide there is a significant reduction ($P < 0.05$) in degree of inhibition at both concentrations of birch pollen (Table 1).

Effects of GCS on histamine release from dispersed human lung tissue cells

Pieces of human lung tissue, passively sensitized with reagin-rich serum, were enzymatically disintegrated according to the procedure described by Schulman et al. (29), slightly modified as detailed in Experimental Procedure. Dispersed, washed cells containing from a few up to approximately 10 % alcian blue staining cells were cultured overnight in the presence of naive or heat-inactivated (56°C 4 h) reagin-rich

serum and in the presence of budesonide (10^{-7}M) or vehicle. The cells were washed and resuspended in HEPES-ACM; challenge was performed with the specified concentration of the secretagogues as described for leukocyte histamine release (4). Table 2 gives the results (means from three experiments). If the culture was performed in the presence of heat-inactivated reagin-rich serum (1:10), cells cultured in the presence of budesonide released less histamine than those cultured with vehicle at allergen and anti-IgE challenge, but not at challenge with the calcium ionophore. When the cells are incubated in the presence of reagin-rich serum diluted 1:10, budesonide inclusion during the overnight culture did not affect the

Table 1

Effects on histamine release from passively sensitized chopped human lung tissue of culture overnight with budesonide (10^{-7}M) either in the presence or absence of birch pollen reagin-rich serum diluted 1:10. Following culture, challenge was performed with the indicated concentrations of secretagogues. Only experiments where net release in vehicle-treated cultures were $> 10\%$ have been included in the calculations. n indicates number of experiments, each of which was performed in duplicate with a different specimen of lung tissue

Compound	Culture condition	% Inhibition of release (mean $\pm$ SEM)			
		Birch pollen extract		Anti-IgE 1:1000	A23187 1 $\mu\text{g/ml}$
		1:10000	1:1000		
Budesonide	presence of reagin-rich serum	18.1 $\pm$ 7.9* (8)	13.7 $\pm$ 3.1* (12)	-9.8 $\pm$ 30.4 (4)	13.4 $\pm$ 7.5 (8)
Budesonide	absence of reagin-rich serum	51.7 $\pm$ 7.0* (3)	40.5 $\pm$ 11.3* (7)	28.8 $\pm$ 12.7 (5)	6.5 $\pm$ 9.8 (6)

* Degree of inhibition recorded in presence of reagin-rich serum varies significantly from that recorded in absence of reagin-rich serum ($P < 0.05$).

Table 2

Effects on passively sensitized human lung tissue dispersed cell histamine release induced by indicated secretagogues. Culture of the cells was performed overnight with budesonide (10^{-7}M) or vehicle in the presence of naive or heat-inactivated (56°C 4 h) reagin-rich serum diluted 1:10. Figures given are the net degree of release expressed as % of total cellular histamine content (means of three experiments). Figures in parentheses denote % inhibition of release in the presence of budesonide

	Net histamine release (% of total content)									
	Vehicle					Budesonide				
	Anti-IgE		Birch pollen		A23187	Anti-IgE		Birch pollen		A23187
	1:10 ⁴	1:10 ³	1:10 ⁴	1:10 ³		1:10 ⁴	1:10 ³	1:10 ⁴	1:10 ³	
Reagin-rich serum (1:10)	8	18	25	34	58	10 (-25)	17 (5)	27 (-8)	35 (-3)	58 (0)
Reagin-rich serum (1:10)										
heat-inactivated	8	21	16	22	74	5 (37)	17 (19)	11 (33)	13 (40)	69 (7)

degree of release induced by any of the secretagogues at the ensuing challenge. These results are similar to those recorded in the experiments with chopped tissue, except that the inhibitory effect of budesonide is now completely reduced in the presence of diluted reagin-rich serum.

DISCUSSION

Glucocorticosteroid treatment *in vitro* partially reduces IgE-mediated histamine release from human basophils (4, 26, 27) and from mouse and rat peritoneal mast cells (5, 11) provided that the treatment is performed for a sufficient time period (usually 18–24 h). Moreover, *in vivo* treatment with GCS reduces rat mast cell degranulation and mediator release *in vitro* as well as *in vivo*, especially for the so-called mucosal mast cell type (15, 17). Similar effects have been recorded at guinea pig chopped lung tissue histamine release (1). However, IgE-receptor dependent mast cell mediator release from human lung tissue or dispersed lung mast cells is reported not to be affected by analogous treatment with GCS (including dexamethasone) (10, 28).

In the present experiments we recorded a partial but consistent reduction of IgE-dependent but not of calcium ionophore-induced histamine release from passively sensitized chopped human lung tissue, or dispersed mast cell containing lung cell populations, by treatment overnight with the glucocorticosteroid, budesonide. Although not shown, similar results were also recorded with dexamethasone.

The cell(s) being the target of the GCS were not identified. The human lung tissue mast cell is mainly thought of as a so-called connective tissue mast cell type due to its heparin content. However, bronchoalveolar lavage fluid from human beings contains cells with basophil-like characteristics (20) and/or cells characterized as being of mucosal type of mast cells (35). Moreover, the lung tissue employed contains variable amounts of blood, and despite extensive washing procedures blood cells may remain in the tissue. Therefore, the possibility that such cells contribute to the histamine release cannot be excluded. However, our previous experience

with leukocytes indicates a higher degree of inhibition by budesonide of basophil histamine release than observed with the lung tissue cells.

The mechanism of the action of the GCS remains to be established. The effect may be indirect or direct. Mononuclear cell-derived factors are known to enhance or even directly induce basophil and to a lesser degree lung mast cell histamine release (2, 13, 14, 16, 30, 31, 33, 34). Since GCS treatment often affects synthesis of lymphokines/cytokines, any endogenous production of such factors may be reduced by the GCS treatment, which would be expected to lead to reduced release at challenge with the IgE-dependent secretagogues. However, the observation that the GCS-inhibitory effect is blocked in the presence of diluted reagin-rich serum, but not in the presence of heat-inactivated reagin-rich serum, suggested that the GCS affects the mediator-releasing cell(s) directly and that its mechanism of action is to reduce the degree of sensitization of the cells. The observation that A23187-induced histamine release is not affected by the GCS treatment suggested an action of the drug(s) early in the stimulus-response sequence. Previous experiments have shown that GCS treatment of atopyics reduces the number of the "low" affinity Fc_ϵ -receptors on monocytes from such patients (19). It seems to be a plausible working hypothesis for further experiments with purified lung mast cells that GCS treatment increases the dissociation of IgE from the mast cell IgE-receptors and/or reduces the density of Fc_ϵ -receptors expressed on the cell membrane, thereby reducing IgE-receptor dependent mediator release.

Note added in submitted manuscript: Robin et al. (23) recently reported that culture with dexamethasone reduced mouse bone marrow-derived mast cells mediator release and Fc_ϵ -receptor expression.

ACKNOWLEDGEMENTS

The authors are grateful to the staff and personnel at the Department of Thoracic Surgery, University Hospital, Lund, for providing specimens of lung tissue, to Prof. S. G. O. Johansson, Karolinska Institute, for providing serum

with high anti-birch pollen IgE titre, and to Mrs. Ulla Larsson for skilful secretarial assistance. Mr. Robert Dew-snap kindly revised the text.

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