

Inhibitory Effect of Glucocorticosteroids on Anti-IgE-Induced Histamine Release from Human Basophilic Leukocytes: Evidence for a Dual Mechanism of Action

HÅKAN BERGSTRAND, ANETTE BJÖRNSSON, BRITTA LUNDQUIST,
ANNIKA NILSSON and RALPH BRATTSAND

Dept. of Pharmacology, Research and Development Laboratories, AB Draco*, Lund, Sweden

Anti-IgE-induced histamine release from human leukocytes is inhibited when the cells before challenge are cultured overnight in the presence of glucocorticoids (GCSs). The present report suggests that the GCSs might exert their effect by at least a dual mechanism of action. Histamine release was induced by a suboptimum concentration of anti-IgE. When the release recorded in the presence of the steroid is plotted against the release recorded in its absence, the data points of several experiments fit a regression line characterized by two parameters: its slope and its intercept with the abscissa. Structure-activity examination with selected GCSs indicates that the orders of potency for affecting these two parameters are not identical. Furthermore, pulse experiments suggest that the cells require different times of contact with the steroid to express inhibition according to the two parameters. The removal of adherent cells or platelets did not markedly affect the degree of leukocyte histamine release or its inhibition by a given GCS, suggesting that the steroid interacts directly with the basophil. Finally, steroid-induced inhibition was not affected by the putative phospholipase A₂-inhibitor p-bromophenacylbromide (BPB) or the 5-lipoxygenase inhibitor nordihydroguaiaretic acid (NDGA).

Key words: basophil; budesonide; glucocorticoid; histamine release.

Accepted for publication 5 November 1983

CLINICAL ASPECTS

Glucocorticosteroids, notably budesonide, inhibit anti-IgE-induced histamine release from human leukocytes provided the cells are cultured overnight with the steroid before challenge. The degree of inhibition depends on the strength of immunological challenge and the glucocorticosteroid concentration.

Inhaled glucocorticosteroids (GCSs), such as budesonide, inhibit the immediate bronchoconstriction in asthmatic individuals after allergen provocation, but this effect requires longer pretreatment periods than blockage of the late response (12, 15, 33). GCS treatment reduces the peak plasma level of histamine and the per cent increase in serum neutrophil chemotactic activity at allergen challenge of asthmatic

patients sensitive to grass or ragweed pollen (32) but does not seem to reduce the responsiveness of basophils *in vitro* to immunologic stimuli; it leads to reduced levels of blood basophils (14, 40). *In vitro*, GCSs inhibit immunologically induced release of allergic mediators from human basophilic leukocytes (41, 42) provided that the cells before challenge are incubated overnight with the steroid. The rela-

* Subsidiary to AB Astra, Sweden.

tive inhibitory potency of a series of compounds in this system correlates with the relative anti-inflammatory activity *in vivo* of the compounds. However, incomplete inhibition is recorded with most GCSs. Furthermore, concentration-response curves do not seem to be uniformly parallel (42). Daëron et al. (11) described similar results for mouse peritoneal mast cells passively sensitized with monoclonal IgE antibody specific for DNP. Dexamethasone interacted directly with mast cells by binding to specific cytoplasmatic receptors.

In rats and guinea pigs treated *in vivo* with GCSs 18–24 h before test, inhibition of anaphylactic reactions is seen *in vitro* (antigen-induced release of histamine from chopped lung tissue or serosal mast cells) and also *in vivo* (antigen-induced bronchial anaphylaxis (1, 8, 31, 44). However, no inhibition of histamine release from dispersed human lung tissue mast cells was recorded with dexamethasone (43). Similarly, inhibition of allergen-induced histamine release from human chopped lung tissue was not observed even after overnight preincubation with 5–100 µg/ml betamethasone sodium phosphate (18); however, a high concentration of the GCS led to a significant reduction of SRS-A release. Thus, GCS can inhibit immunologically induced mediator release from some, but not all, mediator-secreting cells.

The mode of action of the GCSs has not been defined. The general hypothesis is that GCSs bind to cytoplasmatic receptors and induce synthesis of protein(s) which then mediate the effect. One such protein, lipomodulin/macrocortin, is proposed to inhibit triggering of the arachidonic acid cascade by blocking phospholipase A₂ (for ref. see 5, 19). Other inhibitors of phospholipase A₂ activity, such as p-bromophenacylbromide (BPB) and quinacrine (mepacrine), also inhibit histamine release from human basophils (27, 29, 35) and from human lung mast cells (Lichtenstein, II Int. Congr. Immunopharm. Washington, 1982). However, lipomodulin is not effective in the mouse mast cell (11).

In the present paper we utilized anti-IgE-induced basophil histamine release to further examine "anti-allergic" effects of GCSs.

EXPERIMENTAL PROCEDURE

Preparation and culture of leukocytes

Blood was drawn from healthy volunteers into sterile B-D Vacutainer tubes containing EDTA, and the cells were allowed to sediment in a mixture of sterile dextrane and glucose (30 mg glucose/ml 5% dextran T-500 solution made up in saline) according to previously described principles (4, 37, 41). After 45–60 min, the leukocyte-rich supernatant plasma was withdrawn and the cells were centrifuged at $250 \times g$ for 10 min and washed twice in sterile RPMI 1640 (Flow lab.); occasionally only one washing was performed. After centrifugation, the washed cells were resuspended in sterile prewarmed (37°C) 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).

In some experiments, part of the leukocyte suspension was cultured for 60–90 min at 37°C in plastic Petri dishes (Nunc, Denmark) to remove adherent cells. Non-adherent cells were collected after gentle rotation of the dishes, and were then resuspended in prewarmed (37°C) RPMI 1640-HEPES. The cells (non-adherent or the total leukocyte population) were distributed between tubes. The actual concentration of basophilic leukocytes in each tube was not determined and therefore may have varied in the various experiments. However, assuming a basophil histamine content of approximately $1\text{--}2 \mu\text{g}/10^6$ cells (see 24, 39), each tube contained a final amount of approximately $2\text{--}10 \times 10^4$ basophils obtained from 1–2 ml of blood, suspended in 0.9 ml of RPMI 1640 culture medium containing the above-mentioned constituents. 0.1 ml of an appropriate GCS dilution or vehicle was added as indicated below. In some experiments, the cells were incubated at 37°C for 30 min with cortaxolone or pyridoxin (Sigma) at the indicated concentrations or with the corresponding vehicles before addition of GCS. The GCSs were dissolved at 10^{-2} M in ethanol and were then diluted 1000-fold in culture medium RPMI 1640 containing the

above-mentioned constituents. Further dilutions were performed in 10-fold steps. Thus, the final concentration of EtOH at culture was 0.10% or less; this concentration of EtOH was found not to influence the histamine content of the cells at culture, spontaneous release or net histamine release after culture (not shown). After addition of GCS/vehicle, the cells were cultured for the indicated time (10 min, 1 h, 4 h or overnight (20–24 h)) at 37°C in an atmosphere of 5% CO₂ and air. At kinetic studies, the cells were spun down after incubation for the indicated time and were washed twice in culture RPMI medium (without HSA) to remove the GCS/vehicle. Fresh culture medium without GCS or vehicle was added and the cultures continued overnight. Then, again, the cells were spun down, washed in HEPES-ACM buffer (4) and resuspended in this buffer. Cells cultured overnight with GCS/vehicle were not washed with RPMI 1640 before being washed with HEPES-ACM after the end of culture. Thus, GCSs were not present during the release phase (see below); according to Schleimer et al. (41) GCS omission at this point does not influence the extent of histamine release inhibition.

Histamine release

Histamine release experiments were performed as described previously (4, 37). Challenge was performed with anti-IgE, at the indicated final dilution. The anti-IgE was prepared as previously described (37) and was kindly donated by Dr. B. Å. Petersson. There was no indication that the degree of release induced by a given concentration of anti-IgE varied with the total histamine content of the cell sample; i.e., basophil concentration (results not shown). Agents to be examined for inhibitory effect: i.e. p-bromophenacylbromide (BPB) (9, 27, 29); dimethyl-DL-2,3-distearoyloxypropyl-2-hydroxyethylammonium acetate (DDPH) (9); nordihydroguaiaretic acid (NDGA) (27); quinacrine (9, 27, 29); or vehicle(s) if present, were added 20 min before anti-IgE. BPB (locally synthesized and kindly provided by Dr. J. Trofast) was dissolved at 5×10^{-2} M in acetone and this

solution was diluted to 10^{-4} M or 10^{-5} M in HEPES-ACM before use. Control cells then received a similar dilution of acetone in HEPES-ACM. DDPH (Calbiochem) was dissolved in EtOH at 10^{-1} M concentration and further diluted in HEPES-ACM. Such dilutions of DDPH in HEPES-ACM were not soluble at 2.5×10^{-4} M; they were thoroughly sonicated before addition to leukocyte suspensions. NDGA (Sigma) was dissolved in dimethylsulphoxide at 0.3 M and further diluted in HEPES-ACM. Control cells received plain DMSO similarly diluted. Quinacrine (Sigma) was dissolved in HEPES-ACM at 3×10^{-3} M before further dilution.

The release reaction took place for 40 min. All samples were run in duplicates. Histamine secreted into the supernatant was assayed by spectrophotofluorimetry (4, 17). The amount of histamine released into the supernatant was expressed as per cent of total cellular histamine content after correction for spontaneous release. The total histamine content was determined after lysis of cells with perchloric acid (17). With quinacrine, interference with histamine determinations was encountered (see also 29). Therefore, the histamine in samples containing quinacrine and in corresponding controls was determined as the difference between histamine extracted from the pellets of cells incubated with vehicle and those incubated with anti-IgE. Histamine was extracted from cell pellets by adding HEPES-ACM (1.22 ml) and perchloric acid (56 µl of 35% perchloric acid).

GCSs were dissolved and diluted in glass tubes, otherwise laboratory plastics were utilized throughout; preliminary experiments indicated that no difference in GCS-effect could be recorded between experiments performed in glass ware and plastics.

Sources of steroids: Betamethasone 17 α -valerate and beclomethasone 17 α ,21-dipropionate (BDP) ((LARK S.p.A. Milan), budesonide (Draco (46)); this compound is an approximate 1:1 mixture of two epimers with slightly differing biological activity), cortexolone (EGA Chemie), deoxycorticosterone, dexamethasone, and hydrocortisone (Sigma). Fluocortin butyl-ester (FCB) was kindly donated by Schering AG.

RESULTS

General characteristics of the experimental system

The present experiments were all performed with leukocytes incubated at 37°C overnight. Practically no information is available regarding the responsiveness of leukocytes to antigen/anti-IgE, or responsiveness to inhibitors after such a culture in comparison with the responsiveness of fresh cells. Radermecker (38) previously showed that heparinized blood can be stored at 4°C for 1 or 2 days without the basophils losing responsiveness to allergen. We have ourselves had similar experience (unpublished). Ida et al. (20, 21) and Schleimer et al. (41, 42) cultured leukocytes in RPMI-medium supplemented with fresh autologous serum for 24 to 48 h without any stated problem; *purified* human basophils do not tolerate overnight culture very well (42).

We found 0.1% human serum albumin to be at least as good a medium supplement as autologous serum, if not better. We found no consistent difference in histamine content, spontaneous release, or degree of anti-IgE-induced release, when cells examined immediately after preparation and after culture overnight at 37°C were directly compared. Furthermore, cells from most individuals providing blood for the present experiments were previously examined in conventional "acute" tests at more than three occasions. Concentration-response curves for anti-IgE-induced release varied only marginally

from test to test (4). Cells from these individuals were found to display only a slightly lower degree of release after overnight incubation at 37°C than that previously recorded in direct tests. Thus, when for each individual the mean of the release recorded at anti-IgE 1:10000 from overnight cultured cells was plotted against the mean of release in experiments with fresh cells, variates could be fitted to a regression line through the origo and with a slope of 0.8.

Effect of an overnight incubation with budesonide 10^{-7} M on basophil histamine release induced by different anti-IgE concentrations

We first examined the effect of overnight incubation of cells with the newly developed GCS budesonide (10^{-7} M) or vehicle on histamine release induced by increasing concentrations of anti-IgE. The results from two experiments are shown in Fig. 1. The inhibitory effect of this GCS is apparently more pronounced on histamine release indicated by a low anti-IgE concentration than at a high one, irrespective of the actual degree of release obtained.

Fig. 2 gives the results from experiments with cells from six individuals as a plot of degree of release from cells cultured in the presence versus absence of inhibitor (again budesonide 10^{-7} M). Regression lines have been fitted to the data pairs at each concentration of anti-IgE by the least squares method. The degree of inhibition recorded at this specified concentration of

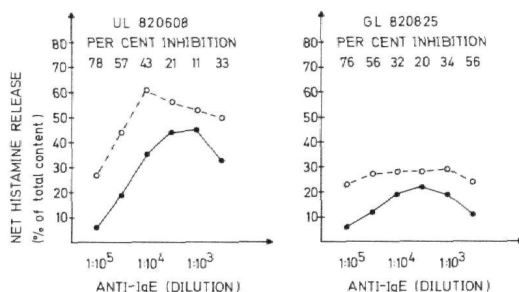


Fig. 1. Plot of net histamine release from cells cultured at 37°C overnight in the presence of budesonide at 10^{-7} M (●) or vehicle (○) versus concentration of anti-IgE at challenge. Data for cells from two individuals.

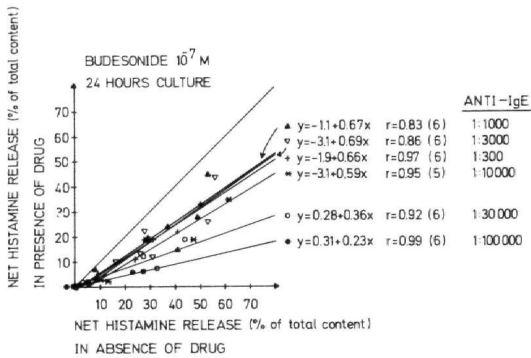


Fig. 2. Plot of net histamine release from cells cultured at 37°C overnight in the presence of 10^{-7} M budesonide versus that of cells from the same preparation cultured in its absence. Regression lines have been calculated for the data pairs recorded at each anti-IgE dilution.

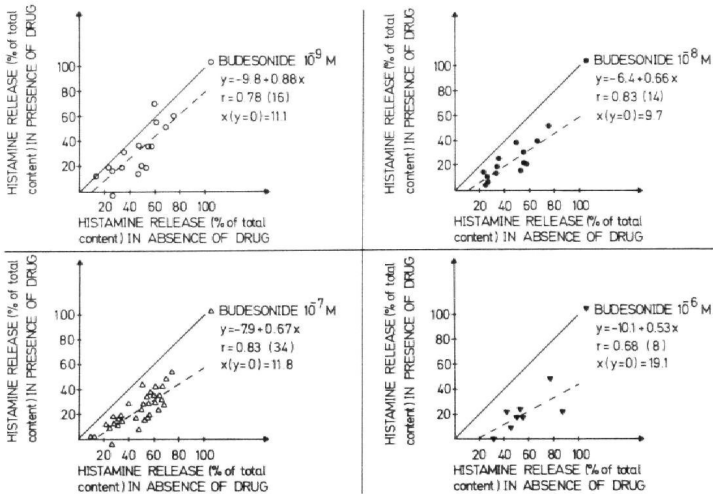


Fig. 3. Plot of net histamine release recorded at challenge with anti-IgE (1:10,000) of cells cultured at 37°C overnight with vehicle or budesonide 10^{-9} – 10^{-6} M. Calculated regression lines are also given for each budesonide concentration.

budesonide is not constant, but seems to depend more on the concentration of the secretagogue used than on the degree of release recorded in its absence.

Concentration-response relation for inhibitory effect of budesonide

Fig. 3 shows the results recorded at challenge with anti-IgE (1:10,000) of cells incubated over-

night with 10^{-9} , 10^{-8} , 10^{-7} , 10^{-6} M budesonide or vehicle. The slope of the regression line, fitted to the data recorded with budesonide at 10^{-9} M, does not differ from unity, but it does not intercept the abscissa in the origo. The slope of the regression line, fitted to the data for budesonide 10^{-7} M, clearly differs from unity, again its intercept with the abscissa differs from the origo.

Table 1

Effects on histamine release recorded with beclomethasone 17 α ,21-dipropionate (BDP), betamethasone 17 α -valerate, budesonide, dexamethasone, deoxycorticosterone, flucortin butylester (FCB) or hydrocortisone after culture at 37°C overnight. The table gives the slopes of regression lines fitting data pairs; their intercepts with the abscissa, correlation coefficients (*r*); and number of experiments (*n*)

Compound	Conc.	Slope	Intercept (<i>y</i> = 0)	<i>r</i>	<i>n</i>	% inhibition Mean $\pm$ SEM
BDP	10 ⁻⁹	1.04	7.7	0.99	9	19.4 $\pm$ 3.3
	10 ⁻⁸	0.79	8.3	0.84	7	39.6 $\pm$ 7.2
	10 ⁻⁷	0.48	-4.1	0.72	9	46.8 $\pm$ 6.0
	10 ⁻⁶	0.51	-0.6	0.81	6	49.5 $\pm$ 5.9
Betamethasone 17 α -valerate	10 ⁻⁹	0.78	1.3	0.97	9	24.4 $\pm$ 5.0
	10 ⁻⁸	0.71	9.9	0.88	10	46.3 $\pm$ 7.6
	10 ⁻⁷	0.47	-2.8	0.81	11	49.3 $\pm$ 7.0
	10 ⁻⁶	0.67	17	0.67	8	55.9 $\pm$ 10.6
Budesonide	10 ⁻¹⁰	0.95	8.3	0.90	6	21.5 $\pm$ 7.5
	10 ⁻⁹	0.88	11.1	0.78	16	33.9 $\pm$ 7.4
	10 ⁻⁸	0.66	9.7	0.83	14	50.6 $\pm$ 4.9
	10 ⁻⁷	0.65	11.9	0.83	34	53.6 $\pm$ 3.4
	10 ⁻⁶	0.53	19.1	0.68	8	67.5 $\pm$ 6.8
Dexamethasone	10 ⁻⁹	0.89	2.0	0.87	8	16.0 $\pm$ 8.4
	10 ⁻⁸	0.73	5.4	0.83	15	37.3 $\pm$ 6.1
	10 ⁻⁷	0.50	2.1	0.87	22	53.9 $\pm$ 3.1
	10 ⁻⁶	0.50	10.2	0.80	9	60.4 $\pm$ 5.8
Deoxycorticosterone	10 ⁻⁷	0.93	-1.4	0.99	7	3.4 $\pm$ 2.0
	10 ⁻⁶	0.91	-1.0	0.99	7	6.6 $\pm$ 2.9
FCB	10 ⁻⁸	0.85	-7.2	0.98	4	0.8 $\pm$ 3.8
	10 ⁻⁷	0.69	1.4	0.84	6	34.0 $\pm$ 10.6
	10 ⁻⁶	0.66	3.0	0.84	6	41.0 $\pm$ 9.5
Hydrocortisone	10 ⁻⁹	0.91	-10.4	0.88	8	-10.8 $\pm$ 7.4
	10 ⁻⁸	1.08	6.8	0.85	12	8.7 $\pm$ 8.1
	10 ⁻⁷	0.97	13.2	0.85	17	32.9 $\pm$ 6.4
	10 ⁻⁶	0.78	11.5	0.70	18	40.4 $\pm$ 5.5

Percent inhibition calculated as: (1 - degree of release in the presence of drug divided by degree of release in its absence) $\times$ 100.

Effects of other GCSs

Employing the same challenge concentration of anti-IgE (1:10,000), we then examined the effect of incubating cells with beclomethasone 17 α ,21-dipropionate, betamethasone 17 α -valerate, dexamethasone, flucortin butylester or hydrocortisone. The appropriate calculated figures for slopes and intercepts of regression lines fitting the data pairs of the examined

compounds are summarized in Table 1. For dexamethasone, regression lines for data pairs representing the effects at 10⁻⁹ to 10⁻⁷ M, despite displaying decreased regression coefficients with increasing concentration, all seem to go through the origo. A similar situation is encountered with FCB. For hydrocortisone 10⁻⁸ and especially 10⁻⁷ M, on the other hand, the regression lines display intercepts differing

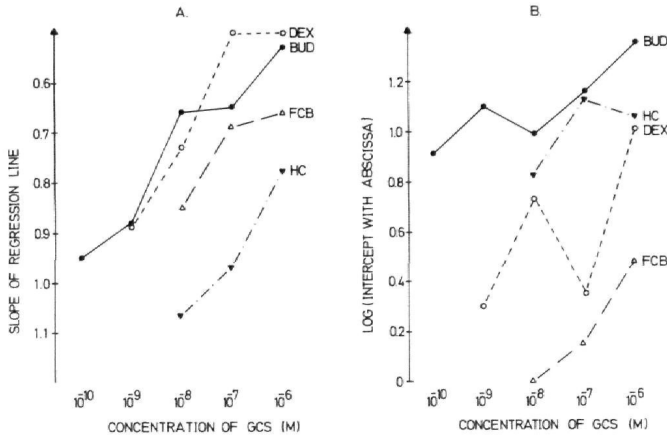


Fig. 4. Replots of slope of regression lines (A) and log (intercept of regression line with abscissa) (B) against concentration of budesonide (BUD), dexamethasone (DEX), flucortin butylester (FCB) and hydrocortisone (HC). A negative intercept for FCB 10^{-8} M has been given the value 0.

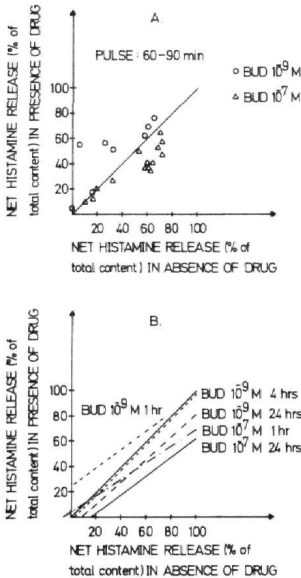


Fig. 5. A. Plot of histamine release recorded at challenge with anti-IgE (1:10,000) of cells cultured at 37°C for 60-90 min with budesonide at 10^{-9} M or 10^{-7} M. Following pulse with drug/vehicle, cells were washed and cultures were continued overnight before challenge.

B. Regression lines calculated for data pairs recorded for anti-IgE-induced basophil histamine release with cells pulsed with 10^{-7} or 10^{-9} M budesonide or vehicle for the indicated time period followed by culture overnight in plain medium.

from the origo, apparently without changing their slopes from unity.

Fig. 4 shows replots against concentration of slopes and log of intercepts for the regression lines for four of the examined steroids. The potency ratio of the GCS with respect to the two parameters is not identical.

For comparison, Table 1 also gives the values obtained for degree of inhibition calculated according to conventional methods (where per cent inhibition is calculated as $(1 - \text{net release in the presence of drug} / \text{net release in its absence}) \times 100$) for each concentration of the GCS examined. Concentration-response curves constructed with these figures will not be parallel (e.g., betamethasone 17 α -valerate vs dexamethasone) and therefore cannot be used for strict comparison of potencies.

Deoxycorticosterone, a mineral corticoid, did not affect histamine release even when the cells were cultured overnight with this drug at a concentration of 10^{-6} M (Table 1).

Incubation time with GCS required for effect

Schleimer et al. (41) and Daëron et al. (11) showed that the inhibition of release following incubation of leukocytes/mast cells with GCS for 4-8 h was lower than the inhibition observed

after incubation of cells with drug overnight. However, these data do not tell us how long time the drug (if added at the initiation of culture) has to be present to express its effect. Very short exposure times (down to no more than 5 min) were required for demonstration of some GCS-induced effects in other systems (see 7, 10, 13, 22). Therefore, leukocytes were incubated with 10^{-7} M or 10^{-9} M budesonide, or the corresponding vehicle, for 10, 60–90, or 240 min at 37°C . After incubation, the cells were spun down and washed twice in culture medium. Fresh culture medium was added, the cells were resuspended and cultured overnight without any further GCS addition. After culture, cells were collected by centrifugation, resuspended in HEPES-ACM and challenged with anti-IgE. The degree of release was determined for each aliquot of cells; it was compared to that of an aliquot of cells cultured for the full time (overnight) in the presence of GCS or vehicle. Fig. 5 gives the individual results for cells pulsed with budesonide for 1 to $1\frac{1}{2}$ h (Frame A), and the regression lines calculated from data for the 1 to $1\frac{1}{2}$ h and the 4 h pulses compared to 24 h exposures for the corresponding cells (Frame B). Incubation with the steroid at 10^{-7} M for 1 to $1\frac{1}{2}$ h apparently induces a certain degree of inhibition of release. The slope of the regression line is similar to that registered with cells cultured with the drug overnight; however, the intercept of the regression line with the abscissa does not seem to differ from the origin for cells incubated 1 to $1\frac{1}{2}$ h with 10^{-7} M budesonide. Cells incubated with budesonide 10^{-7} M for 10 min or for 4 h behaved as those incubated with the steroid for 1 h (not shown). Incubation of cells with 10^{-9} M budesonide for various time periods led to different results. Cells pulsed for 1 to $1\frac{1}{2}$ h showed a stimulation of release (Fig. 5A). Cells pulsed with 10^{-9} M budesonide for only 10 min also often showed a stimulation of release (not shown), whereas cells exposed for 4 h to the GCS displayed neither an enhanced nor a suppressed release.

Pulsing was also performed for 1 h with dexamethasone 10^{-7} M or hydrocortisone 10^{-6} M and the effects obtained were compared to those recorded after overnight culture in the

presence of the drug(s). No inhibition was observed in cultures pulsed with either drug for 1 h, despite good effects after overnight exposure to the drugs in parallel cultures (results not shown).

Effect of cortisolone or pyridoxine on the inhibitory effect of budesonide, dexamethasone or hydrocortisone

Cortisolone displays anti-glucocorticoid activity *in vitro*. By increasing intracellular pyridoxal phosphate levels, pyridoxin is reported to modulate cellular glucocorticoid-induced responses depending on nuclear function, but apparently not responses that do not seem to involve an interaction between the activated hormone-receptor complex and chromatin (see 22).

Leukocytes were incubated with cortisolone (10^{-6} M) or vehicle for 30 min before addition of budesonide (final concentration 10^{-8} M), dexamethasone (10^{-8} M), hydrocortisone (10^{-7} M) or vehicle. The cells were then cultured overnight before challenge. Treatment with cortisolone did not in itself affect release. The inhibitory effect of budesonide on release was not markedly affected by the presence of cortisolone. For dexamethasone and hydrocorti-

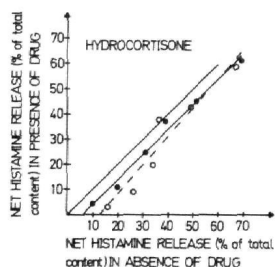


Fig. 6. Effect of cortisolone (10^{-6} M) added 30 min before hydrocortisone on inhibition of basophil histamine release by hydrocortisone (10^{-7} M) after overnight culture. Filled symbols represent experiments in the presence of cortisolone (regression line: $y = -6.1 + 1.0 x$; $r = 0.96$ (6)), open symbols represent experiments in the absence of cortisolone ($y = -15.0 + 1.14 x$; $r = 0.99$ (6)).

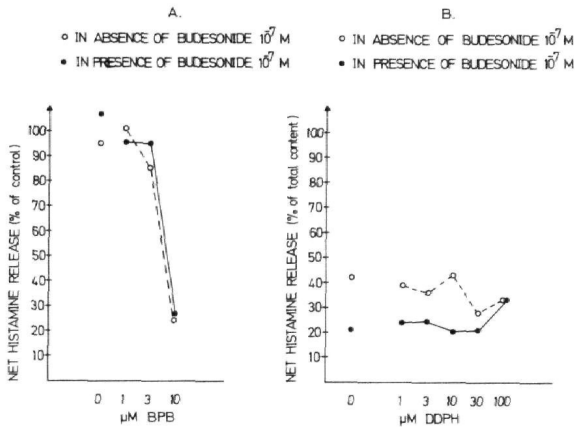


Fig. 7. Anti-IgE-induced histamine release from leukocytes cultured overnight at 37°C together with budesonide 10^{-7} M (filled symbols) or vehicle (open symbols).

A. Release experiments were performed with each of the two aliquots of cells in the presence of BPB (1, 3 or 10 μM) or vehicle_{BPB}. Histamine release in the absence of budesonide was 66%; in the presence of budesonide 10^{-7} M, 34%. Data given are means of two experiments.

B. Release experiments were performed with each of the two cell aliquots in the presence of DDPH or vehicle. Data given are means of two experiments.

sone, cortexolone seems partly to block inhibition. Data recorded with hydrocortisone are shown in Fig. 6.

Leukocytes were also incubated for 30 min at 37°C with 5 mM pyridoxine or vehicle before addition of budesonide (final concentration 10^{-7} M), dexamethasone (10^{-7} M), hydrocortisone (10^{-6} M) or vehicle. The cells were cultured overnight before challenge. The results of six experiments with each GCS (not shown) do not indicate that pyridoxine should affect the inhibitory effect(s) of the GCSs.

Effect on responsiveness to anti-IgE and to GCS of removal of adherent cells

Factors derived from monocytes (or from lymphocytes in monocyte-dependent reactions) influence basophil histamine release. Such factors could either enhance IgE-mediated release (2, 20, 21) or induce release independently of IgE-mechanisms (45, 47, 48). Mahmood et al. also recorded a monocyte-dependent suppression of basophil histamine release (28). Monocyte-derived factors, e.g., IL-1, can also block

the inhibitory effect of GCSs on lymphoid cells (notably helper but not suppressor cells) (6, 34). The possibility exists, that a similar modulation by monocyte product(s) of GCS-mediated inhibition of basophil histamine release might exist.

For that reason experiments were performed where half the leukocyte suspension was cultured at 37°C for 1 h in plastic Petri dishes to remove the majority of adherent cells before culture overnight of nonadherent cells with GCS or vehicle. The other half of the cell suspension was cultured with GCS/vehicle directly. Occasionally, anti-IgE-induced basophil histamine release was markedly enhanced in nonadherent cell populations as compared to total leukocyte populations after overnight culture, but this was in no way a consistent observation. There was no clear indication that the inhibitory efficiency of budesonide (10^{-7} M), dexamethasone (10^{-7} M), or hydrocortisone (10^{-6} M) varied between nonadherent or total leukocyte populations (results not shown).

We examined whether GCS could induce release from leukocytes of principle(s) similar

to those exhibiting anti-inflammatory activity (lipomodulin?), which are released in the peritoneal cavity of the rat 1 h after GCS administration (5). Leukocytes were cultured for 1 h, or overnight, in the presence of GCS or vehicle. The supernatants were harvested and examined for effects on anti-IgE-induced histamine release from fresh leukocytes. Confirming previous work (45, 47), medium from leukocyte cultures was found to enhance histamine release induced by anti-IgE; even medium harvested after 1 h was occasionally effective. However, the presence of dexamethasone 10^{-7} M during the culture did not affect the enhancing effect of the medium, whether it was harvested 1 h or 24 h after culture initiation.

A recent report by Maclouf et al. (26) showed that leukocyte 5-lipoxygenase activity is stimulated by products of the 12-lipoxygenase of platelets. Furthermore, Knauer et al. (23) observed an enhancement of IgE-mediated histamine release in the presence of platelets. We examined therefore, in preliminary experiments, the effect on responsiveness to anti-IgE and to GCS of removing the majority of platelets by repeated centrifugation of leukocytes at $140 \times g$ and culturing overnight the platelet-depleted leukocytes with GCS or vehicle. The responses of these platelet-depleted leukocyte suspensions were compared to those of leukocyte suspensions restituted with autologous platelets. Basophil histamine release and inhibitory effects of budesonide 10^{-7} M did not differ between leukocyte cultures poor or rich in platelets.

Interaction of GCS with inhibitors of arachidonic acid metabolism

Our inability to obtain "complete" inhibition with any of the GCSs examined, their proposed mode of action as (indirect) phospholipase A_2 inhibitors, and the reported efficiency at basophil histamine release of other known phospholipase A_2 inhibitors (27, 29, 35) prompted a study of the interaction of a GCS (budesonide), putative phospholipase A_2 inhibitors (BPB, DDPH, quinacrine), and the lipoxygenase inhibitor NDGA. Leukocytes were cultured over-

night in the presence of budesonide 10^{-7} M or vehicle, washed, and then challenged with anti-IgE in the presence of increasing concentrations of BPB, DDPH, quinacrine, or NDGA. The acute inhibitory effects of BPB, quinacrine or NDGA did not differ in leukocytes cultured with budesonide or with vehicle. This is exemplified in Fig. 7A where the results for BPB are shown. DDPH at 10^{-4} M was found to enhance slightly histamine release for cells cultured with budesonide but to block slightly release in cells cultured with vehicle (Fig. 7B).

DISCUSSION

The present work examines in some detail the effect on human leukocyte responsiveness to anti-IgE when the cells have been precultured with GCS. Our results support the conclusions earlier drawn by Schleimer et al. (41, 42) and by Daéron et al. (11) (with mouse mast cells) that a substantial degree of inhibition of histamine release can be registered provided culture of cells with the glucocorticoid is performed for more than a few hours. However, the salient result of the present work is that GCSs inhibit anti-IgE-induced basophil histamine release seemingly by at least a dual mechanism of action. This opinion is based on the following findings:

- 1) A regression line fitted to data pairs representing release induced by anti-IgE with cells cultured for 24 h in the presence of 10^{-9} M budesonide or vehicle crossed the abscissa outside the origo but its slope did not differ from the unity (Fig. 3A). When examining the effect of 10^{-8} – 10^{-6} M budesonide, similar intercepts of the regression lines with the abscissa were recorded as for 10^{-9} M budesonide, but now the slopes registered for the regression lines seemed to decrease concentration-dependently (Fig. 3A). These data conform to the general characteristics of inhibition of release described previously in this and other systems (4, 11, 16, 37, 42, 49, 50). They caution against an uncritical use of the parameter "per cent inhibition" to characterize the effect of a drug; the effects on intercept and slope of the regression line should rather be dealt with separately.

2) Concentration-response curves recorded for some other GCSs revealed drugs with seemingly opposing effects; dexamethasone (and also fluocortin butylester) decreased concentration-dependently the slope of the regression line without markedly affecting its intercept with the abscissa, whereas hydrocortisone affected dose-dependently the intercept of the regression line, apparently without primarily affecting its slope (Fig. 4 and Table 1). Contrary to our results, Daéron et al. (11) clearly showed in the mouse mast cell system that the degree of inhibition exerted by dexamethasone at 10^{-6} M varied with the actual percentage of histamine release in control tubes.

3) Pulse experiments suggest that the slope and the intercept of the regression line are influenced differentially. Thus, cells incubated with 10^{-7} M budesonide for 4 h or less, and then cultured in its absence overnight, were partly inhibited in their ability to release histamine at challenge with anti-IgE. There was no indication that the regression line for these data should not pass through the origo, its slope corresponded to that recorded with cells cultured with GCS overnight. Leukocytes cultured with 10^{-9} M budesonide displayed three forms of response. When the cells had been cultured with 10^{-9} M budesonide for 1 h, washed and then cultured overnight, the stimulation of histamine release was registered. A 4-h pulse with budesonide under similar conditions did not affect leukocyte responsiveness. However, overnight culture of cells with 10^{-9} M budesonide led to a change in the intercept with the abscissa of the regression line but not to a change in its slope. At the moment, it cannot be clearly settled whether the effects recorded at pulsing with budesonide are due to the short exposure of the cells to the indicated concentration of the steroid, or to a prolonged exposure to a lower concentration remaining after the washing procedure. Rough calculations show that the budesonide concentration in the medium surrounding the cells, initially exposed to 10^{-7} M, after the washing procedure probably would equal 10^{-11} M. Separate experiments with (^{3} H)-labelled budesonide indicated that some steroid could be released from the

cells or the culture tubes during the overnight culture. However, with reference to Fig. 3A and 5B, a 1-h pulse exposure to budesonide 10^{-7} M, followed by culture overnight in its "absence", apparently leads to a qualitatively different kind of inhibition (no change of slope) than overnight culture does at either 10^{-7} or 10^{-9} M budesonide (changed slope), which argues against trivial explanations for the effects.

The reason for doing these experiments is that some recent reports suggest that certain GCS-induced responses on the cellular level might be independent of a nuclear association of the steroid-receptor complex (7, 13, 22). These responses, in contrast to those requiring interaction between activated hormone-receptor complex and chromatin, do not seem to be influenced by increased intracellular levels of pyridoxal phosphate or by the GCS receptor antagonist cortexolone. Furthermore, biochemical studies on nuclear GCS-receptor interactions revealed heterogeneity, among other things, in the kinetics of association and dissociation and in structure-activity relation (7). There are also recent reports that in the neural retina of chick embryo GCS-free cytosol contains two GCS-receptors with possibly two different functions (3). Therefore, we also examined the influence exerted by cortexolone and pyridoxin on GCS-agonist activity. There was no clear indication that pyridoxin treatment affected the inhibition induced by budesonide, dexamethasone, or hydrocortisone. On the other hand, cortexolone seems to partially counteract the blockage of release induced by certain glucocorticosteroids. Evidently, cortexolone affects more easily the intercept of the regression line with the ordinate than its slope.

Taken together, these data suggest that the capacity of budesonide to change the slope of the regression line is a process which does not require prolonged presence of the GCS; whether it requires nuclear association of activated GCS receptor complexes and induction of protein synthesis is not yet known. In this context, the failure of a 1-h pulse with 10^{-7} M dexamethasone to affect release is hard to explain since dexamethasone affects the slope of the regression line but not its intercept. On the

other hand, the capacity of a steroid to change the intercept of the regression line from the origo seems to require a prolonged exposure time and it may be partially blocked by cortisone. Thus, this process might be suggested to depend on nuclear association of activated steroid-receptor complexes.

What could be the biological basis for a drug affecting basophil histamine release in two principally different ways, i.e., in the present context, to change independently the slope and the intercept of the regression line? Previous experience of β -agonist inhibition of histamine release from chopped guinea pig lung tissue (50) clearly has a bearing on this issue. These experiments showed that the inhibition of release by β -agonists is due to two independent mechanisms: one is a shift to the right of the dose-response curve for the allergen and the other a reduction of the maximum obtainable histamine release. It is not clear in what way the latter parameter is a function of mast cell reaginic antibody density and/or the function of a parameter which for basophils has been defined as releasability. In the guinea pig lung system, two different β -receptor populations were postulated to mediate the two effects. It is conceivable that, analogously, GCS might influence differentially two independent mechanisms for histamine release inhibition in the leukocyte system. A shift to the right in the dose-response curve for the stimulus would then correspond to a shift in the intercept of the regression line with the abscissa, a reduction of maximal histamine release would correspond to a reduction of the slope of the regression line. Possibly, one of these drug effects may be indirect; i.e., the drug may interact with a cell which in some way modulates the release of histamine from the mast cell in the guinea pig lung tissue or the basophil in the leukocyte suspension. However, from the data of the present experiments, there is no indication that the GCSs should exert part of their inhibitory effect indirectly by interference with mononuclear cells or platelets. Our failure to differentiate basophil histamine release in leukocyte preparations poor or rich in platelets is consistent with the data of Peters et al. (36) who found that 5-HPETE and 5-HETE,

but not the platelet product 12-HPETE, enhanced antigen-induced basophil histamine release. With anti-IgE as a stimulus not even 5-HPETE was consistently enhancing histamine release (36).

Finally, there is no indication that GCS pretreatment influences responsiveness of leukocytes to other putative phospholipase A_2 -inhibitors. Thus, these experiments cannot provide suggestive evidence for an indirect (lipomodulin-mediated) general phospholipase A_2 inhibition as a mechanism of action of the glucocorticoids. Again, these data agree with the interpretations of other workers (31, 42) but much more experimental data are needed to settle this point.

In conclusion, GCSs, notably budesonide, inhibit immunologically induced human basophil histamine release *in vitro*. Our results suggest that the effects of the GCSs may be due to at least a dual mode of action. Anti-IgE-induced release of histamine from basophils has long been regarded as equivalent to allergen-induced release. However, differences between these two secretagogues were recently recorded with respect to the shape of dose-response curves, the kinetics or release enhancement exerted by lipoxygenase products (30, 36). The fact that allergen is predominantly a multimeric and anti-IgE only a dimeric stimulus may explain these differences (cf. 25). This should be kept in mind when attempting to apply the present findings to the clinical situation.

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Address:

Håkan Bergstrand

Research and Development Laboratories

AB Draco, Box 1707

S-221 01 Lund

Sweden

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