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Glucocorticoid Inhibition of Antigen-evoked Histamine Release from Human Skin

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Summary. Prednisolone causes a dose-related inhibition of antigen-evoked histamine release from IgE-sensitized human skin *in vitro*. The effective concentrations are of the same order as are achieved in plasma therapeutically.

Analysis of prednisolone inhibition shows that it acts on the second histamine release stage, antigen–antibody combination being unaffected.

In contrast with the traditional view, our results show that, at least in human skin, glucocorticoids can inhibit antigen-evoked histamine release.

INTRODUCTION

The view that glucocorticoids are without effect on antigen-evoked histamine release in man is widely held and is based on early animal experiments, including the failure of cortisone acetate given *in vivo* and *in vitro* to inhibit both the Schultz–Dale anaphylactic contraction of the guinea-pig ileum *in vitro* (Gray, Pedrick and Winne, 1951) and antigen-evoked histamine release from rabbit blood cells (Carryer and Code, 1950). However, cortisone acetate is now known to be devoid of glucocorticoid activity *in vitro* (Briggs and Brotherton, 1970). New, more sensitive *in vitro* methods have been developed for study of inhibition of IgE-mediated hypersensitivity and it is now possible to carry out these studies on human tissues using new, more potent glucocorticoids. We have therefore investigated the effect of two glucocorticoids, hydrocortisone and prednisolone, on IgE-mediated hypersensitivity in human skin *in vitro*. Our results have revealed a previously unrecognized immunosuppressive action of prednisolone.

MATERIALS AND METHODS

Our method for preparation and sensitization of human skin slices has been described in detail elsewhere (Greaves, Yamamoto and Fairley, 1972). Healthy breast skin removed at mastectomy was sliced using a hand microtome into 500 μm thick slices. The histamine content of human skin and spontaneous histamine release from it has been described in a previous paper (Greaves *et al.*, 1972). Reaginic serum from three pollen sensitive donors (total serum IgE concentration = 930–950 ng/ml) was used for passive sensitization. Each slice was incubated at 37° for 2 hours in a separate glass tube with 2 ml of a 1:30 dilution of serum using Tyrode solution as a diluent. The sensitized slices were washed in Tyrode

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solution and then incubated in triplicate for 15 minutes at 37° in Tyrode solution containing a 1:250 dilution of the pollen antigen. The final reaction volume in all experiments was 4 ml. The reaction was terminated by cooling and histamine release measured by bioassay. The residual histamine in each slice was then determined by boiling in Tyrode solution for 5 minutes followed by bioassay of the histamine content of the cooled Tyrode solution. The evidence that the observed antigen-evoked histamine release is the result of combination of antigen with antibody of the IgE class is contained in a previous report (Greaves *et al.*, 1972). All experiments included negative controls in which slices were incubated in Tyrode solution for 2 hours followed by washing and then incubation with antigen for 15 minutes. Histamine bioassay was carried out using an isolated atropinized guinea-pig ileum preparation and an automatic bioassay apparatus. Results of histamine assays were expressed as histamine base. Histamine release from each sample was calculated as a percentage of the total histamine present in the sample. All antigen-evoked histamine release values were corrected by subtracting spontaneous release. The effect of glucocorticoids was studied by dissolving the antigen and the glucocorticoid in Tyrode solution and pre-warming to 37°. This mixture was then incubated with the sensitized slice. All determinations were carried out in triplicate and each experiment included reagent control samples in which slices were incubated in Tyrode solution for 2 hours followed by washing and then incubation for a further 15 minutes with the glucocorticoid and antigen. No evidence was obtained that either glucocorticoid interfered significantly with the response of the guinea-pig ileum to histamine during bioassay.

RESULTS

HYDROCORTISONE

The effect of 100 μg/ml of hydrocortisone on antigen-evoked histamine release was studied using skin from seven donors. The results are given in Table 1. Inhibition ranging

TABLE 1

HYDROCORTISONE ON ANTIGEN-EVOKED HISTAMINE RELEASE FROM HUMAN SKIN

Donor	Percentage histamine release		Percentage inhibition
	Positive control*	Hydrocortisone†	
1	18·1	12·3	32·0
2	17·0	17·9	−5·2
3	21·1	19·1	9·4
4	17·0	16·4	3·5
5	13·0	11·2	13·8
6	9·4	9·5	−1·1
7	10·6	9·5	10·0

Negative control 'spontaneous' histamine release from slices were zero in all experiments.

* Positive control = release evoked by antigen (no hydrocortisone).

† Hydrocortisone = antigen-evoked release in the presence of 100 μg/ml of hydrocortisone. Mean inhibition by hydrocortisone = 8·9 per cent ± 4·6 s.d.

from 3·5–32·0 per cent was obtained in five experiments. In the other two slight enhancement occurred (1·1–5·2 per cent). The mean inhibition for the group was 8·9 per cent ±4·6 s.d. and this value was not statistically significant.

PREDNISOLONE

Prednisolone in concentrations of 50–100 μg/ml produced dose-related inhibition of histamine release from human skin. Results using skin from eight donors are given in Table 2.

TABLE 2

PREDNISOLONE ON ANTIGEN-EVOKED HISTAMINE RELEASE FROM HUMAN SKIN

Donor	Percentage histamine release			Percentage prednisolone inhibition‡	
	Positive control*	Prednisolone† (μg/ml)			
		50	100	50 μg/ml	100 μg/ml
1	15·8	11·5	8·4	27·2	46·8
2	13·6	12·0	6·1	11·8	55·1
3	35·1	24·0	21·1	27·9	36·6
4	14·4	10·4	6·1	27·8	57·6
5	17·1		10·2	N.D.	43·6
6	17·0		15·4	N.D.	9·4
7	21·1		16·8	N.D.	20·4
8	17·0		15·1	N.D.	11·2

Negative control 'spontaneous' histamine release from slices was negative in all experiments except one (donor 3) which was 1·8 per cent.

* Positive control = release evoked by antigen (no prednisolone).

† Prednisolone = antigen-evoked release in the presence of 50–100 μg/ml of prednisolone.

‡ Mean inhibition by prednisolone = 23·7 per cent ± 7·9 s.d. (50 μg/ml) and 35·0 per cent ± 6·8 s.d. (100 μg/ml).

EFFECT OF PREDNISOLONE ON DIFFERENT STEPS OF HISTAMINE RELEASE

The inhibitory effect of prednisolone on different steps of antigen-evoked histamine release was then studied. The reaction can be separated experimentally into two stages depending on calcium requirements (Yamamoto and Greaves, 1973). In the first stage, which is independent of calcium, antigen combines with cell-bound reaginic antibody, leading to activation of the mast cell. In the second stage, which is calcium-dependent, the mast cell releases histamine. The effect of prednisolone on these separate stages was therefore studied using skin from six donors. The results are summarized in Table 3 and Fig. 1. In six experiments sensitized skin slices were incubated with antigen and 100 μg/ml of prednisolone in the absence of calcium. No histamine release occurred. After 15 minutes the slices were washed three times in calcium-free Tyrode solution to eliminate all excess uncombined antigen, and then recalcified by addition of normal Tyrode solution (calcium = 1·8 mM), followed by measurement of histamine release. Histamine release from these slices did not differ significantly from release from control slices which had been treated in an identical way except for omission of prednisolone. Thus prednisolone did not cause inhibition of the first stage of antigen-evoked histamine release from human skin. In six experiments (Table 3 and Fig. 1) the effect of prednisolone on the second histamine release stage was studied. In these experiments sensitized slices were incubated with antigen in the absence of calcium followed by washing in calcium-free Tyrode solution. As in the previous experiments no release occurred in the first stage. The sensitized antigen-

treated slices were then recalcified by addition of normal Tyrode containing 100 μg/ml of prednisolone, followed by measurement of histamine release. Release from these slices was reduced by 42–67 per cent compared with releases in positive control experiments

TABLE 3

PREDNISOLONE ON FIRST AND SECOND STAGES OF ANTIGEN-EVOKED HISTAMINE RELEASE FROM HUMAN SKIN

Donor	Percentage histamine release		
	Positive control*	Prednisolone†	
		Stage 1‡	Stage 2§
1	13·7	12·2	7·9
2	8·1	9·7	2·7
3	11·7	10·9	5·8
4	6·2	6·1	2·2
5	5·1	5·3	2·5
6	15·3	11·0	7·2

All values for releases are given after subtraction of negative control (spontaneous) histamine release (range 0–4·5 per cent).

* Positive control = release evoked by antigen in the absence of prednisolone.

† Prednisolone = antigen-evoked release in the presence of 100 μg/ml of prednisolone.

‡ Stage 1 = prednisolone present during incubation of antigen with skin slices (no calcium).

§ Stage 2 = prednisolone present during incubation of sensitized, antigen-treated, washed slices in the presence of calcium.

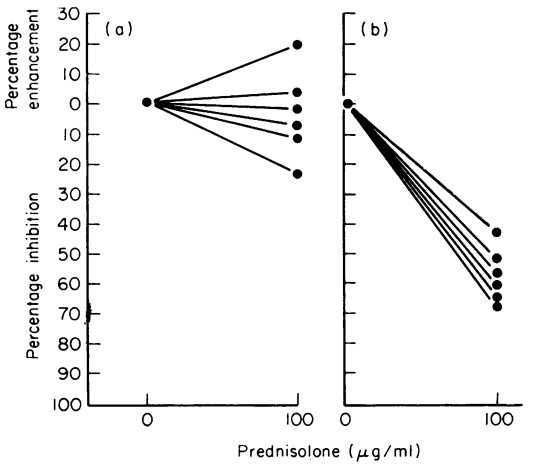

FIG. 1. Effect of 100 μg/ml of prednisolone on two stages of antigen-evoked histamine release from human skin. All values are expressed as percentage inhibition (or enhancement) of histamine release by prednisolone. (Positive control = zero.) (a) Stage 1 = calcium-independent antigen–antibody combination and skin mast cell activation. (b) Stage 2 = histamine release from washed, antigen-treated skin following addition of calcium.

where sensitized slices were treated in an identical way except for omission of prednisolone in the second stage. Thus we have demonstrated that prednisolone inhibits the second histamine release stage of antigen-evoked histamine release from human skin.

DISCUSSION

The present results demonstrate for the first time a dose-related inhibition of antigen-evoked histamine release from human tissues by a glucocorticoid. The inhibitory effect of prednisolone is exerted on the second histamine release stage, there being no inhibition of the initial stage of antigen–antibody combination. Hydrocortisone, which possesses approximately one quarter of the glucocorticoid activity of prednisolone (Nelson, 1962) had little or no inhibitory effect on release. The concentrations used do not differ greatly from those achieved in the blood following administration of therapeutic doses (Turcotte, Carpenter and Bacon, 1972). Our results contrast with results of animal experiments (Gray *et al.*, 1951; Carryer and Code, 1950) in which cortisone acetate failed to inhibit the Schultz–Dale reaction and anaphylactic histamine release from rabbit blood. These early negative results are not surprising since we now know that cortisone acetate is inactive *in vitro*, its *in vivo* glucocorticoid effect depending on metabolic conversion to cortisol (Briggs and Brotherton, 1970).

The mode of action of prednisolone in suppressing antigen-evoked histamine release in skin is uncertain. Prednisolone did not interfere with antigen–antibody union in the present experiments and must therefore have acted at a later stage. Surprisingly little work has been done on the mode of action of glucocorticoids at a cellular level, although a membrane stabilizing effect has frequently been suggested. This could be brought about by an inhibitory action on formation of lipid peroxides by mitochondria (Weissmann and Thomas, 1964). There is some evidence that glucocorticoids may increase cellular concentrations of cyclic AMP (Logsdon, Middleton and Coffey, 1972) and cyclic AMP is known to inhibit IgE hypersensitivity in human skin *in vitro* (Yamamoto, Greaves and Fairley, 1973a). Antigen-evoked histamine release from mast cells is triggered by an energy-requiring multistep enzyme cascade which, in human skin, probably also involves calcium-dependent activation of cytoplasmic contractile microfilaments (Mongar and Schild, 1962; Yamamoto and Greaves, 1973; Yamamoto, Greaves and Fairley, 1973b). Thus the possible sites of action are numerous.

Suppression of human reaginic hypersensitivity in the respiratory tract and skin by glucocorticoids has long been recognized (Hench, Kendall, Slocumb and Polley, 1950; Carey, Harvey, McGehee, Howard and Wagley, 1950; Carey, Harvey, McGehee, Howard and Winkenwerder, 1950). This action has previously been attributed mainly to a non-specific reduction in reactivity of blood vessels to histamine and other mediators (Bangham, 1951; Humphrey, 1951). Thus it is of especial interest that neither hydrocortisone nor prednisolone reduced the contractile responses of guinea-pig smooth muscle to histamine in our histamine bioassay procedures.

We have now demonstrated the existence of a specific inhibitory action of prednisolone in acute hypersensitivity in human skin which should stimulate a full reappraisal of the pharmacology of glucocorticoids in immunological reactions.

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