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Effects of Topically Applied Clobetasol-17-propionate on Histamine Release in Human Skin

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The effects of topical glucocorticoid treatment on histamine responses and histamine release induced by the histamine liberating agent compound 48/80 were studied in 17 healthy volunteers. The potent glucocorticoid ointment clobetasol-17-propionate was applied on one upper arm of each individual 14, 4 and 2 hours before testing. The other arm was treated in the same way with the corresponding vehicle. Solutions of histamine and compound 48/80 were injected intradermally in both arms. The size of the flare reaction and the duration of the itch response were recorded. It was found that the flare reactions evoked by histamine were slightly ($p<0.05$) reduced on the steroid-pretreated arm whereas the responses to compound 48/80 were much more suppressed ($p<0.01$). Glucocorticoid treatment did not influence the itch responses to histamine while the itch duration following injection of compound 48/80 was significantly reduced in steroid-treated skin compared to control skin. Our results indicate that topical glucocorticoid treatment can suppress histamine release from dermal mast cells in man. *Key words: Flare reaction; Itch; Compound 48/80.* (Received September 16, 1983.)

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The potent anti-inflammatory effects of glucocorticoids are well known, but in spite of their extensive use the mechanisms of action are still not sufficiently understood. Although clinically effective in the treatment of bronchial asthma, allergic rhinitis and urticaria, a common opinion has been that glucocorticoids do not influence the immediate allergic reactions mediated by histamine and other mast-cell derived inflammatory substances. However, some recent in vitro experiments have shown inhibition of histamine release in human basophils (1) and in mouse mast cells (2) following glucocorticoid incubation, whereas others have failed to demonstrate any effect of glucocorticoids on histamine release in human (3) or guinea pig lung (4).

In the present experiments, which were performed in vivo, we have studied the influence of topical glucocorticoid treatment on cutaneous histamine release. The resposes to the histamine liberator compound 48/80 were compared with those induced by exogenous histamine. The results suggest that histamine release from dermal mast cells can be reduced by topical glucocorticoid treatment.

MATERIALS AND METHODS

Seventeen non-atopic, drug-free volunteers, aged 20–56 years, participated in this investigation. Clearly outlined areas 14×5 cm on the lateral aspect of the upper arms were chosen for the experiment. Ointment containing 0.05 % of clobetasol-17-propionate (Dermovate, Glaxo) was applied topically on one arm on three occasions 14, 4 and 2 hours before testing. The other arm was treated in the same manner with the corresponding vehicle (Glaxo). The subjects themselves applied the ointment from plastic syringes containing 0.8 g of the ointment, the same amount for each application. Solutions of histamine (2, 3.3 and 10 μg/ml) and of compound 48/80 (3.3 μg/ml) were used for testing. Small volumes, 0.01 ml, of the solutions were injected intradermally within the pretreated areas in a double-blind fashion. All injections were given by the same person. In order to obtain accurate

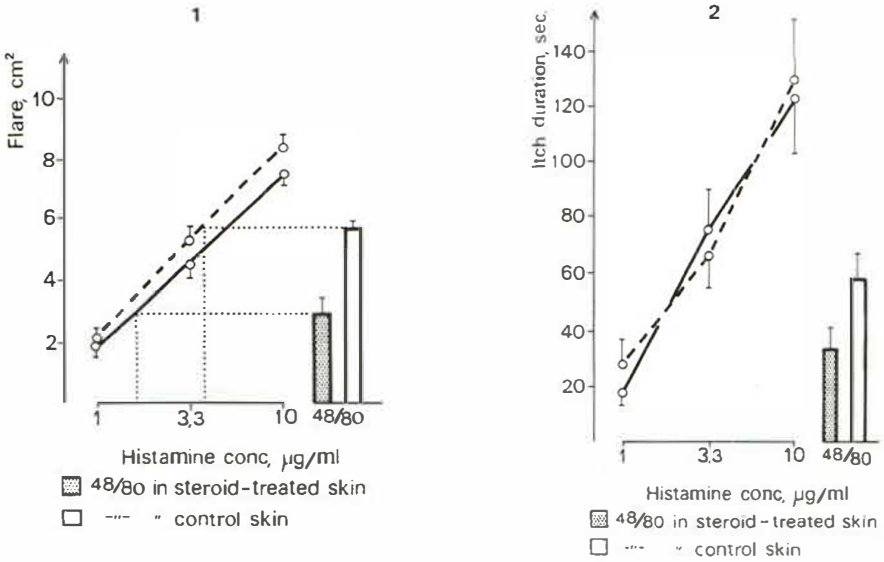

Fig. 1. Dose–response curves for flare reaction induced by histamine in steroid-treated skin (O—O) and control skin (O- - -O). The included bars show flare response after injection of compound 48/80 (3.3 µg/ml) in steroid treated and control skin (means±SE; $n=17$). The dotted lines indicate the intrapolation procedure performed in the individual dose–response curves (see text for explanation).

Fig. 2. Dose–response curves for itch duration induced by histamine in steroid-treated (O—O) and control skin (O- - -O). The included bars show itch response after injection of compound 48/80 (3.3 µg/ml in steroid treated and control skin (means±SE; $n=17$).

volumes we used a special plastic adaptor with the syringe. The adaptor was copied from a prototype developed and kindly lent to us by Dr Spector (5). It was made to fit our disposable tuberculine syringes, one turn of the screw delivering 0.01 ml. The duration of the itch response was recorded. The area of the flare reaction was outlined 5 min after injection, traced on a plastic film and then measured with a planimeter (model 317, Gebrüder Haff GmbH, Pfronten, West Germany).

Analysis of covariance was used to evaluate the difference between the histamine dose–response curves for the two arms. Student's *t*-test for paired samples was used to estimate the significance of differences in itch and flare responses between the arms after injection of compound 48/80.

RESULTS

Due to steroid-induced vasoconstriction (for refs. see 6) all skin areas treated with clobetasol-17-propionate showed a distinct pallor in comparison with untreated skin or skin treated with vehicle only. Both histamine and compound 48/80 induced wheal and flare responses and itching, i.e. there was no qualitative difference between the reactions.

Flare reaction

The flare reactions evoked by histamine were generally smaller in steroid-treated skin than in control skin, most probably an effect of the above-mentioned steroid-induced vasoconstriction. Also following injection of compound 48/80 the flare reactions were significantly reduced in steroid-treated compared to control skin. However, the reduction in flare size was more pronounced after compound 48/80 than after histamine (Fig. 1).

In order to compensate for the influence of steroid-induced vasoconstriction on the flare responses we made the following intrapolation procedure. Dose–response curves for the

flare reactions induced by histamine were plotted for each individual. Into each curve the corresponding response induced by compound 48/80 (0.3 μg/ml) was intrapolated. The procedure is indicated by the dotted lines in Fig. 1 (which is based on the means of all 17 subjects and therefore not used for the statistical analysis). From intrapolation in the individual dose–response curves histamine release values were calculated.The release values, expressed as μg histamine/ml (means±SE), obtained in steroid-pretreated skin (1.97±0.39) were lower than those from control skin (5.28±1.19). The difference was statistically significant ($p<0.01$).

Itch duration

The itch responses following injections of histamine were unaffected by glucocorticoid treatment, whereas 48/80-induced itching was markedly reduced in skin pretreated with the steroid (Fig. 2). The difference between the arms was statistically significant ($p<0.025$) confirming our data from the flare reactions that pretreatment with clobetasol can inhibit histamine release induced by compound 48/80.

DISCUSSION

The present results suggest that histamine release in vivo in human skin can be suppressed by a topically applied glucocorticoid. To our knowledge corticosteroid-induced inhibition of histamine release has not previously been demonstrated in vivo.

Our deductions are based upon the assumption that the responses after intradermal injection of compound 48/80 were caused by histamine released from the dermal mast cells and that compound 48/80 had no direct effects *per se*. Such an assumption seems justified since we found that the responses after histamine and compound 48/80 were qualitatively identical and since it is well known that after depletion of the local histamine stores, compound 48/80 does not elicit itch and flare reactions whereas the responses to histamine are uneffected (7).

The corticosteroid-induced inhibition of the release seemed obvious when studying the itch responses, which were inhibited only when induced by compound 48/80, but unaffected when elicited by histamine. The situation was less apparent for the flare reactions where clobetasol pretreatment inhibited both the histamine- and the 48/80-induced reactions, althought the latter was more inhibited. To compensate for the inhibition of the histamine responses, we intrapolated the 48/80 responses into the histamine dose–response curves and could thus calculate the concentration of histamine which would be necessary to give the same flare reactions as 3.3 μg of compound 48/80.

Recent publications suggest that in order to exert their effects the glucocorticoids must be added to the medium at least several hours before the antigenic challenge. Both Schleimer (1) and Daëron (2) showed that inhibition of histamine release started only 3–4 hours after glucocorticoid incubation and then increased linearly for 24 hours. Pipcorn et al. (8) reported a blockade of anti-IgE-mediated histamine release in human nasal mucosa following one week of steroid treatment. These in vitro observations thus are consistent with our results where the pretreatment period was 14 hours.

Indeed, the traditional concept that glucocorticoids lack effects on the immediate allergic response is being challenged partly as a consequence of increasing knowledge about arachidonic acid metabolism. Mast cell degranulation probably requires an intact metabolism of arachidonic acid (9, 10). Arachidonic acid is generated from membrane phospholipids through the action of phospholipase A_2. According to new theories many effects of the glucocorticoids may be linked to arachidonic acid metabolism and evidence is accumulating that glucocorticoids inhibit the activity of phsopholipase A_2 possibly by

inducing the synthesis of a blocking factor—the so-called lipomodulin (11, 12, 2). However, phospholiphase A_2 also is believed to be a mast cell secretagogue and blockade of phospholipase A_2 effectively inhibits histamine secretion induced by both immunological and non-immunological stimuli (9). These findings are as yet difficult to fully evaluate, but they might help to explain the anti-inflammatory actions of glucocorticoids both in the immediate and late inflammatory responses.

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