Acta Derm Venereol (Stockh) 1985; Suppl. 114: 93–97

# Adrenoceptor Function in Atopic Dermatitis: in Vitro and in Vivo Observations

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Impaired beta-adrenergic and enhanced alpha-adrenergic reactivity have been implicated in the pathogenesis of atopic dermatitis. We have measured the elevation of cyclic AMP in peripheral blood leukocytes in response to isoprenaline, histamine and prostaglandin E2, in the presence and absence of a phosphodiesterase inhibitor. An impaired response to beta-adrenergic stimulation was demonstrated in subjects with atopic dermatitis but impaired responses were also observed with histamine and prostaglandin E2. In vivo, both noradrenaline and salbutamol caused significant inhibition of the histamine-induced weal response in atopic and normal subjects. However, there was no significant difference between the two groups when alpha- and beta-adrenoceptor responses were compared.
*Key words: Cyclic AMP; Phosphodiesterase; Histamine weal.*

Following the proposal by Szentivanyi (1) that impaired beta-adrenergic reactivity is a primary determinant of atopy, numerous efforts have been made to substantiate this hypothesis. Studies of peripheral blood leukocytes in atopic dermatitis have frequently demonstrated impaired adrenergic reactivity as revealed by a loss of regulatory effects on lysosomal enzyme secretion and reduced formation of cyclic adenosine monophosphate (cyclic AMP) (2), by decreased affinity of binding sites for radiolabelled beta-adrenoceptor agonists (3) and by an increased ratio of alpha-adrenoceptor to beta-adrenoceptor binding sites (4). In some studies, normal reactivity to prostaglandin El has accompanied reduced reactivity to beta-adrenoceptor stimulation (2). However, the selectivity of this impaired reactivity can be disputed, since it has been reported that cyclic AMP responses of mononuclear leukocytes are reduced in atopic dermatitis, not only to beta-adrenoceptor stimulants, but also to histamine (5) and to prostaglandin El (6). Such non-selective impairment of responses may be secondary to increased cyclic AMP-phosphodiesterase activity, which has been observed in mononuclear leukocytes from patients with atopic dermatitis (7).

We have compared the effects of isoprenaline, histamine and prostaglandin E2 on cyclic AMP production in peripheral blood mononuclear leukocytes, both in the presence and absence of a potent phosphodiesterase inhibitor (PDEI), to establish whether there is selective impairment of the response to beta-adrenoceptor agonists. In addition, the association between altered adrenoceptor function and atopy has prompted us to evaluate in atopic dermatitis a simple skin test, known to detect beta- and alpha-adrenergic reactivity in cutaneous vessels of normal subjects (8).

## PATIENTS, MATERIALS AND METHODS

### *Mononuclear leukocyte cyclic AMP responses*

14 patients with active atopic dermatitis, 12 patients with typical psoriatic plaques and 16 normal volunteers were studied. The diagnosis of atopic dermatitis was established be standard clinical criteria, including physical examination, a positive personal history and/or family history of atopy, elevated serum IgE concentration and histopathological features. Prior to investigation, patients had not received systemic therapy (e.g. antihistamines) for a period of at least four days, although some

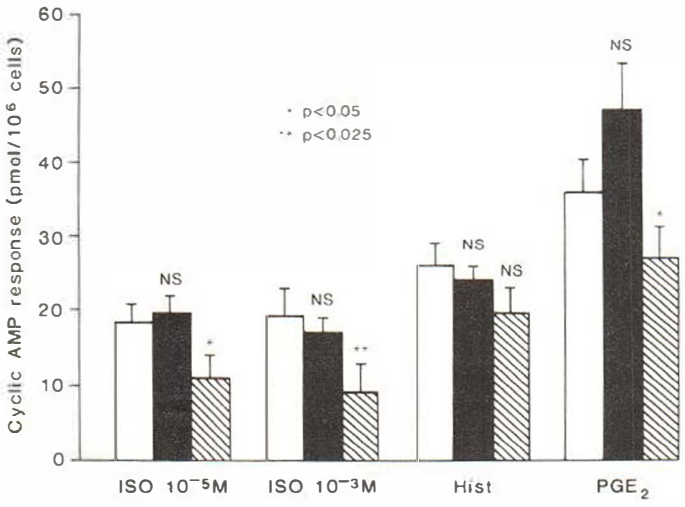


*Fig. 1.* Cyclic AMP responses: mean of stimulated less basal levels (pmol/$10^6$ cells ± SEM) in atopic ▩, psoriatic ■ and normal [] subjects in the *presence* of the phosphodiesterase inhibitor.

had received limited application of topical corticosteroids. None had received adrenergic drugs. Normal volunteers were not atopic and were taking no concurrent medications.

Venous blood samples were collected between 9 and 10 a.m., heparinised, diluted with buffer and layered onto Ficoll-Paque. After centrifugation, the mononuclear cell layer was removed, suspended in Hanks' buffered salt solution and the cells counted. 70–90% of the cell population comprised lymphocytes, with the remainder being mainly monocytes.

Duplicate aliquots ($10^6$ cells/ml) were stimulated, in the presence and absence of a potent phosphodiesterase inhibitor (ICI 63197) at 37°C for 10 min. Freshly prepared agonists were added to incubation tubes to the following final concentrations: isoprenaline ($10^{-5}$ M and $10^{-3}$ M), histamine ($10^{-4}$ M) and prostaglandin E2 ($1.1\times10^{-4}$ M). Samples without added agonist were included to provide basal cyclic AMP levels. Tubes were immersed into boiling water for 10 min to terminate the reactions and stored at −20°C, prior to assay. Total cyclic AMP levels were determined, in duplicate, by radioimmunoassay.

*Actions of locally administered adrenoceptor agonists on histamine-induced cutaneous responses*

A double-blind design was employed in which twenty patients with atopic dermatitis and twenty non-atopic subjects were studied. Weal responses were induced by intradermal injection of coded solutions into randomly allocated sites on the flexor aspect of the forearm. Each solution (50 µl) was freshly prepared in 0.9% saline and contained 0.75 µg histamine base alone, mixed with 0.3 µg noradrenaline (predominantly and alpha-adrenoceptor agonist), or mixed with 100 µg salbutamol (predominantly a beta-2-adrenoceptor agonist). Skin-fold thickness was measured using a spring-loaded thickness gauge (Mitutoyo, Japan) immediately prior to injection and 12 min after injection. Two weal diameters at right angles were measured and weal volume calculated (8).

Student's *t*-test was employed for statistical comparison, unpaired observations being used for comparison between groups and paired observations for within-subject analysis.

## RESULTS

### *Mononuclear leukocyte cyclic AMP responses*

There were no significant differences between basal cyclic AMP levels in cells from atopic and control (normal and psoriatic) groups and, as anticipated, higher cyclic AMP levels were recorded in each group in the presence of the phosphodiesterase inhibitor. In normal and psoriatic subjects, each agonist produced a significant elevation of cyclic AMP, and responses were more pronounced in the presence of the phosphodiesterase inhibitor. Corresponding data for patients with atopic dermatitis showed an essentially similar pattern, although the magnitude of responses to stimulation was generally less than in the control groups.

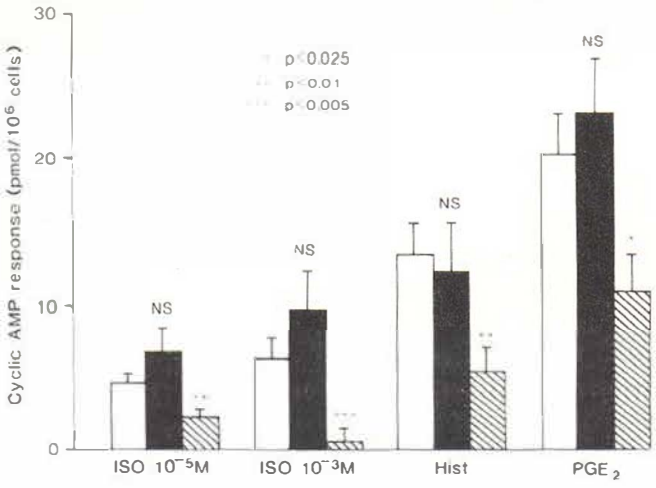


*Fig. 2.* Cyclic AMP responses: mean of stimulated less basal levels (pmol/$10^6$ cells ± SEM) in atopic ■, psoriatic ■ and normal [] subjects in the *absence* of the phosphodiesterase inhibitor.

The differences between the atopic and control groups can more readily be appreciated, when reponses are expressed as net cyclic AMP response to stimulation (that is, the mean of stimulated less basal cyclic AMP levels). In the presence of the phosphodiesterase inhibitor, responses in cells from atopic patients were significantly less than in cells from normal and psoriatic subjects for both concentrations of isoprenaline and for prostaglandin E2 (Fig. 1). In the absence of the phosphodiesterase inhibitor, the impairement of cyclic AMP responses to isoprenaline and prostaglandin E2 in cells from atopic patients was more pronounced, responses to all three agonists, including histamine, being significantly less than in cells from control subjects (Fig. 2).

*Actions of locally administered adrenoceptor agonists on histamine-induced cutaneous responses*

Mean weal volume in response to histamine was not significantly different in atopic patients from that in control subjects (Table I). The histamine-induced weal response was inhibited both by noradrenaline and by salbutamol in all 40 subjects. In the presence of noradrenaline, weal responses were reduced by 60% in patients with atopic dermatis and by 61% in the control subjects. In the presence of salbutamol, weal responses were reduced by 61% in the patients with atopic dermatitis and 60% in the control subjects. In each group these reductions in weal volume were statistically significant both for noradrenaline ($p<0.001$) and for salbutamol ($p<0.001$). There was no significant difference between atopic and control groups for either alpha-adrenergic or beta-adrenergic reactivity.

Table I. *Mean weal volume (μl ± SEM) following intradermal injection of histamine (0.75 μg base) alone, or mixed with noradrenaline (0.3 μg) or salbutamol (100 μg)*

| Group | Histamine | Histamine + noradrenaline | Histamine + salbutamol |
|---|---|---|---|
| Atopic dermatitis | 150.9±27.9 | 57.1±11.6 | 51.0±7.9 |
| Control | 109.6±9.3 | 43.0±4.2 | 40.7±3.8 |

## DISCUSSION

The observation that patients with atopic dermatitis exhibit impaired cyclic AMP responses of peripheral blood mononuclear leukocytes to prostaglandin E2 and histamine, as well as to isoprenaline, implies that impaired reactivity is not confined to the beta-adrenoceptor, but lies at a site common to all three agonists. The present study does not exclude the possibility that there is an impairment of adenylate cyclase activation. However, differences between cells from atopic patients and control subjects were exaggerated by the omission of a phosphodiesterase inhibitor, and therefore could be a consequence of increased leukocyte phosphodiesterase activity which has been reported in atopic dermatitis (7). It is reasonable to attribute these abnormalities to atopic dermatitis and not to cutaneous inflammation *per se*, since in the patients with psoriasis, mononuclear leukocyte cyclic AMP responses to all stimulants were normal. In atopic asthma, impaired leukocyte cyclic AMP responses to isoprenaline have been frequently observed but are often attributed to prolonged beta-adrenoceptor agonist therapy (9, 10). In this in vitro investigation, particular care was taken to ensure that patients had no previous history of adrenergic medication, so that drug-induced tachyphylaxis can be discounted.

Studies of skin reactions in experimental animals have shown that mediator-induced plasma protein extravasation depends upon an interaction between increased vessel wall permeability and increased cutaneous blood flow (11). In guine-pig skin, bradykinin-induced responses can be inhibited both by alpha-adrenergic and by beta-adrenergic agents (12). Alpha-adrenoceptor agonists appear to act by reducing cutaneous blood flow, whereas beta-adrenoceptor agonists, which are vasodilator in skin, appear to oppose increased permeability of the vascular endothelium by an anti-porosity effect. Corresponding observations of alpha-adrenergic and beta-adrenergic actions have been reported for human skin where propranolol blocks the inhibitory action of salbutamol on histamine weal formation (8).

The present in vivo study has confirmed the observation that local administration of an alpha-adrenoceptor agonist, or a selective beta-2-adrenoceptor agonist, reduces the histamine-induced weal response in normal subjects, and further has demonstrated that patients with atopic dermatitis exhibit comparable sensitivity both to histamine and to the inhibitory effect of adrenergic agents. Support for defective beta-adrenoceptor function in atopic dermatitis has been inferred largely from studies of peripheral blood leukocytes, and it has been assumed that corresponding functional defects exist in atopic skin. Equally, increased reactivity to alpha-adrenoceptor stimulation has been inferred from in vitro studies (4). It is possible that our in vivo methods of measurement are too insensitive to detect very subtle abnormalities in adrenoceptor function but then one might question the clinical importance of such abnormalities.

Impaired beta-adrenergic reactivity in peripheral blood mononuclear leukocytes from patients with atopic dermatitis has been confirmed in vitro, but this is not selective since impaired cyclic AMP responses to both histamine and to prostaglandin E2 have also been established. An in vivo method for assessing adrenoceptor function in the skin, however, has not confirmed the existence of altered adrenoceptor reactivity in atopic dermatitis. These results serve to emphasise the need for caution, when interpreting the results of in vitro studies and in extrapolation to the in vivo situation.

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