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Basophil Histamine Release in Atopic Dermatitis and Its Relationship to Disordered Cyclic Nucleotide Metabolism

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Maximal histamine release (HR) from leucocytes, in response to Concanavalin A (Con A) was significantly higher in a group of 16 adults with moderate to severe atopic dermatitis (AD) when compared to 13 non-atopic adults. In a further 4 adults with AD, HR was similar to that in the normals, suggesting the existence of 'high releaser' and 'low releaser' subsets within the AD group. Leucocyte cyclic AMP phosphodiesterase (PDE) activity was significantly higher in the 'high releaser' group compared to the 'low releaser' and normal groups. High and low HR responses showed strong correlations with high and low PDE. Pre-treatment of leucocytes from 'high releasers' with the experimental PDE inhibitor RO-20-1724 reduced the HR to normal levels. These findings suggest that increased histamine 'releasability' in AD is related to abnormalities in cyclic nucleotide regulation. No significant HR could be demonstrated in response to a range of concentrations of methacholine in 'high releaser' atopics and normals. Methacholine also did not affect HR in response to maximal Con A stimulation in 'high releaser'' atopics. Basophil percentages within the leucocyte preparation and the histamine content per basophil, were not significantly different between the atopics and normals. Con A-stimulated histamine release did not correlate significantly with serum IgE levels. *Key words: Histamine release; Concanavalin A; Basophils; Phosphodiesterase activity.*

Previous studies have shown increased leucocyte HR in some patients with AD (1, 2). Cyclic AMP (cAMP) is one mechanism by which HR from basophils and mast cells is controlled. PDE is increased in mononuclear leucocytes in AD (3). We examined HR in AD and normal leucocytes in response to Con A and cholinergic stimulation and questioned whether increased breakdown of cAMP by PDE in basophils might account for the excessive HR in AD.

SUBJECTS

AD subjects fulfilled previously published, strict clinical criteria (4). Their dermatitis was moderate to severe. Age-matched normal subjects had *no personal* or *family* history of AD, asthma or hayfever. All systemic medications were ceased 72 hours prior to investigations, no caffeine was taken in the previous 14 hours.

METHODS

Histamine release

Leucocytes. Venous blood samples anticoagulated with EDTA, were drawn in parallel from AD and normal subjects and were coded for blinding. Leucocyte suspensions were prepared using hydroxyethyl starch solution twice, washed and resuspended in Hepes buffered saline containing calcium and magnesium (5). Duplicate 0.5 ml. Aliquots were incubated with Con A for 45 min at 37°C. Total histamine was extracted from one duplicate by boiling. In separate experiments (i) leucocytes were stimulated with methacholine (10^{-4} M and 10^{-2} M), (ii) methacholine (10^{-6} M to 10^{-2} M) was

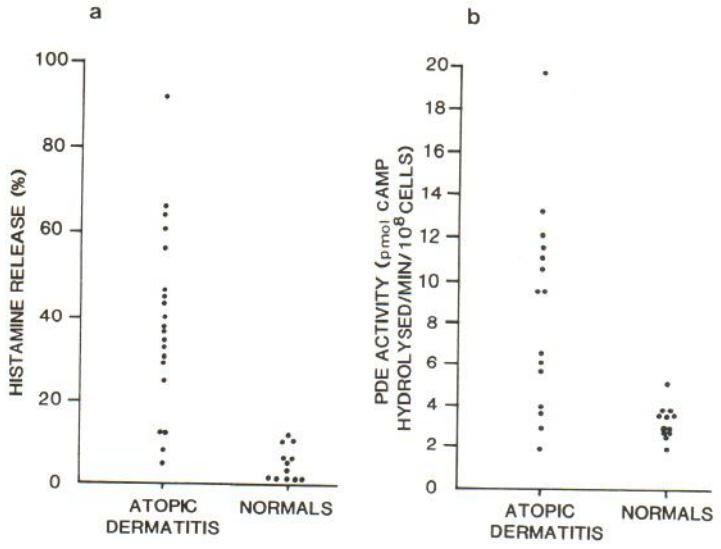


Fig. 1. (*a*) Leucocyte HR, in response to Con A (1.25 μg/ml) in 20 AD subjects and 13 normals, expressed as a percentage of the total histamine content. (*b*) Leucocyte PDE activity expressed as pmol cAMP hydrolysed/min/10^8 cells in 15 AD subjects and 12 normals.

added to the Con A (1.25 μg/ml) stimulation reaction, (iii) aliquots of AD leucocytes were preincubated with isoproterenol (10^{-4} M) for 15 min prior to Con A stimulation. Basophil percentages were determined using a modified Alcian blue stain and accounted for around 1% of the leucocytes.

Foreskins. Three foreskins were collected and skin sections prepared according to the method of Tharp et al. (6). No data were available with regard to family history of atopy in donors. 200 μm tissue sections, suspended in Hepes buffered saline containing Ca++ and human serum albumin, were incubated for 30 min at 37°C to determine spontaneous histamine release, and for a further 30 min at 37°C with a range of concentrations of carbachol (10^{-6} M to 10^{-2} M). Morphine sulphate was used as a control HR stimulant in parallel samples. The total histamine was extracted from each sample by boiling and homogenization. The histamine content of the supernatants was determined using the radio-enzyme assay of Shaff & Beaven (7), employing histamine methyl transferase derived from rat kidney. Results were expressed as a percentage of the total histamine content of the specimen.

Phosphodiesterase activity

Leucocyte preparations were examined for their PDE activity using the method of Appleman & Thompson (8). Results were expressed in pmol cAMP hydrolysed/min/10^8 cells.

Effect of phosphodiesterase inhibitor

Leucocytes from 8 'high releaser' AD subjects were incubated with the specific PDE inhibitor, RO-20-1724, for 45 min at 37°C. Parallel samples incubated with buffer alone were used as controls. Con A stimulated HR and PDE activity were then determined in both cell preparations.

Cyclic AMP responses

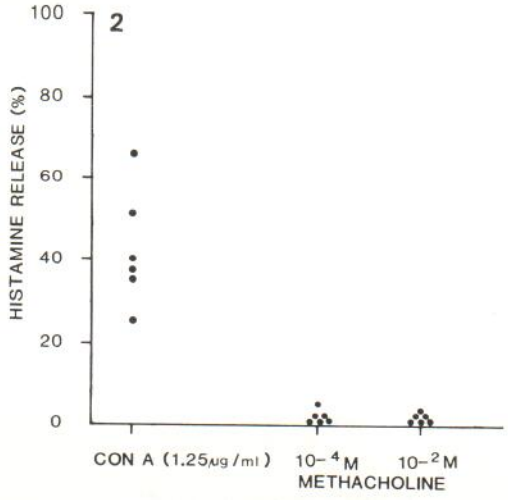
Leucocytes from 6 AD and 5 normal subjects were incubated with isoproterenol (10^{-4} M) and cyclic AMP levels were determined by radio immunoassay, prior to, after 15 sec and 15 min of stimulation.

RESULTS

Leucocyte and foreskin histamine release

Leucocytes. A dose response curve for Con A was determined in leucocytes from 7 atopic and 3 normal subjects. Maximal HR occurred with a concentration of 1.25 μg/ml. Con A in both groups and this concentration was used in subsequent assays. Spontaneous release was less than 5% in all experiments.

Con A stimulated leucocyte HR was measured in 20 atopics and 13 normals (Fig. 1*a*). The mean HR in the atopic group was 38.1% which was significantly higher than the 3.4%



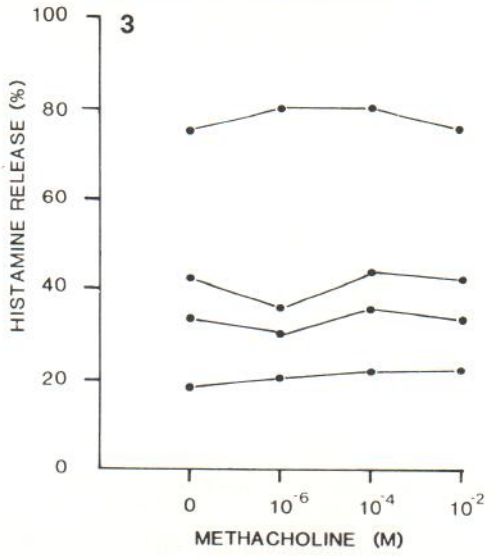


Fig. 2. Leucocyte HR in response to methacholine in 6 AD subjects. Corresponding HR to Con A 1.25 μg/ml also shown.

Fig. 3. Effect of methacholine (M) on leucocyte HR (%) in response to Con A (1.25 μg/ml) in 4 AD subjects.

in normals. 16 of the atopic group, in whom HR exceeded 20%, were designated as 'high releasers'. Their mean HR was significantly higher than the mean of both the 4 remaining 'low releasers' and the normals. We were unable to discern differences clinically or in serum IgE levels between these two subsets.

Repeat release studies in 7 subjects, after periods ranging from 5 to 16 weeks revealed that 'high releasers' remain high and 'low releasers' remain low.

There was no significant difference in either basophil numbers or histamine content per basophil between 'high releasers', 'low releasers' and normals.

Table I. *Histamine release from foreskins in response to carbachol and morphine sulphate stimulation*

Stimulant	Histamine release (%)		
	Spontaneous	Induced	Net
1. Carbachol 10^{-2} M	13.6	9.9	0
Carbachol 10^{-4} M	21.6	14.9	0
Carbachol 10^{-6} M	9.3	7.8	0
Morphine sulphate 1.5×10^{-3} M	18.4	26.2	7.8
2. Carbachol 10^{-2} M	14.9	10.5	0
Carbachol 10^{-4} M	12.0	10.0	0
Carbachol 10^{-6} M	20.0	13.7	0
Morphine sulphate 1.5×10^{-3} M	12.8	46.8	34
3. Carbachol 10^{-2} M	13.6	9.6	0
Carbachol 10^{-4} M	21.8	16.5	0
Carbachol 10^{-6} M	18.7	12.1	0
Morphine sulphate 1.5×10^{-3} M	15.5	40.0	24.5

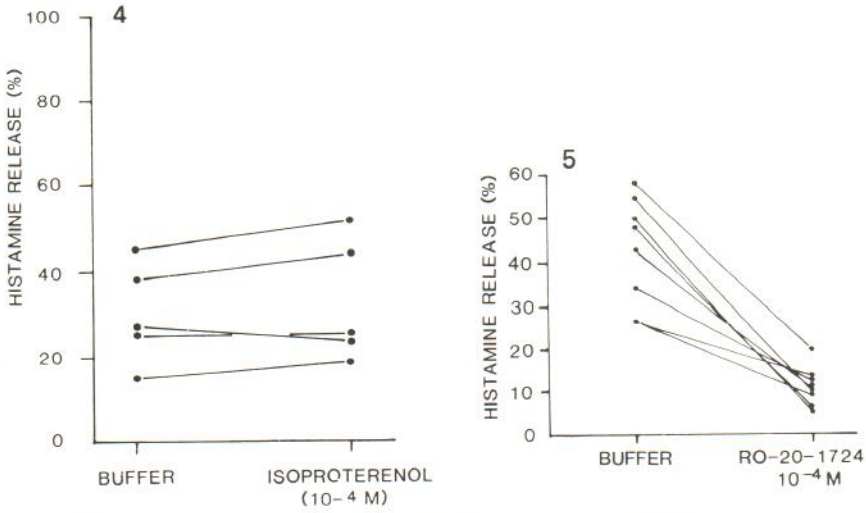


Fig. 4. Effect of pre-incubation with Isoproterenol (10^{-4} M) on leucocyte HR (%) in response to Con A (1.25 μg/ml) in 5 AD subjects.

Fig. 5. Effect of pre-incubation with PDE inhibitor RO-20-1724 on leucocyte HR (%) in response to Con A (1.25 μg/ml) in 8 AD subjects.

Methacholine (10^{-4} M and 10^{-2} M) failed to induce HR from leucocyte preparations from 6 AD (Fig. 2) and 6 normal subjects (not shown) and did not effect Con A induced HR in 4 'high relaser' atopics (Fig. 3). Preincubation with isoproterenol (10^{-4} M) did not inhibit HR from 5 AD leucocyte preparations (Fig. 4).

Foreskins. Spontaneous histamine release was high in the foreskin preparations. Carbachol, however, did not induce significant mast cell HR when compared to morphine sulphate induced HR (Table I).

Phosphodiesterase activity

Leucocyte PDE activity was determined in 15 atopic and 12 normal subjects (Fig. 1 *b*). the mean 8.6 in the atopic group was significantly higher than the mean of 3.4 in normals.

The 4 atopic subjects with the lowest PDE activity (Fig. 1 *b*) were the 4 low histamine releasers (Fig. 1 *a*). This suggests a definite correlation between these two parameters.

The coefficient of correlation (r) of the t tests on the differences in HR and PDE activity between atopic and normal subjects was 0.74 ($p<0.001$, $n=12$). More striking was the 0.94 ($p<0.001$, $n=16$) correlation coefficient between the low PDE activity and low histamine release.

Table II. *Changes in leucocyte cyclic AMP levels (pmol/10^6 cells) in response to isoproterenol (10^{-4} M) stimulation*

	Baseline	15 sec	15 min
Normal ($n=5$)	0.981±0.099	2.536±0.149[a]	2.544±0.217[a]
Atopic dermatitis ($n=6$)	0.896±0.055	2.346±0.278[b]	0.990±0.056[c]

[a] $p<0.005$. [b] $p<0.01$. [c] Not significant.

Effect of phosphodiesterase inhibitor

RO-20-1724 reduced HR in 8 'high releaser' atopics from a mean of 47.6% to 11.71%, which was well within the normal range (Fig. 5). PDE activity was likewise significantly reduced into the normal range during RO-20-1724 incubation. RO-20-1724 did not interfere with the histamine radioenzyme assay or affect spontaneous histamine release or total histamine content.

Cyclic AMP responses to isoproterenol stimulation

Cyclic AMP levels were significantly elevated above baseline levels at 15 sec in both atopics and normals. Stimulated levels were sustained at 15 min in normals, whereas in atopics, cAMP returned to baseline levels by 15 min (Table II).

Based on the measured PDE activity, we have calculated that, in AD leucocytes, negligible amounts of cAMP can be hydrolysed by 15 sec. However, by 15 min, sufficient hydrolysis can occur to return cAMP to baseline levels and thus would explain the lack of inhibition by isoproterenol of leucocyte HR in AD.

DISCUSSION

Using Con A, a readily reproducible and well characterized stimulus to HR, we have confirmed the previous suggested (1) 'high releaser' and 'low releaser' subsets in AD.

Increased 'releasability' of histamine in AD appears to relate to disordered cyclic nucleotide metabolism, in particular, to elevated PDE activity. Differences in HR and PDE activity between AD and normals show good correlation and inhibition of PDE reduces HR to normal levels.

Leucocyte cAMP levels, in response to β agonist stimulation, are not sustained in AD, as a result of the increased PDE activity. Blunting of cAMP responses to β agonists, histamine and other mediators may allow excessive HR from basophils to occur in AD.

Differences in HR could not be explained on the basis of serum IgE levels, percent basophils or histamine content per basophil.

We were unable to confirm previously reported (2) increased HR, in AD, in response to cholinergic stimulation; nor were we able to show an effect of cholinergic stimulation on Con A stimulated HR. Using foreskin preparations, no HR from mast cells in response to cholinergic stimulation could be demonstrated. It would appear from these studies that cholinergic stimulation is not an important factor in leucocyte HR, in AD however, further studies are required to determine its effect on mast cell HR.

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