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ADRENOCEPTOR BINDING STUDIES WITH [^{3}H]DIHYDROALPRENOLOL AND [^{3}H]DIHYDROERGOCRYPTINE ON MEMBRANES OF LYMPHOCYTES FROM PATIENTS WITH ATOPIC DISEASE

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Abstract. Using [^{3}H]dihydroalprenolol (DHA) and [^{3}H]dihydroergocryptine (DHE), that is specific radioligands for the measurement of β- and α-adrenoceptors, respectively, binding studies were performed on membranes of lymphocytes from patients with atopic dermatitis, patients with non-atopic skin diseases, and normal individuals. A shift in the numbers of receptors from β to α was found in the lymphocytic membranes from patients with atopic dermatitis but not in the other 2 groups. That this shift cannot be due to possible variations in the proportion of lymphocyte subpopulations was demonstrated by the lack of difference in α- and β-receptor numbers between B and T lymphocytes, indicating that the adrenoceptor shift is intrinsic to the disease-state itself. Taken together with similar findings in lung tissue and lymphocytes from patients with reversible obstructive airways disease (asthma) the observations suggest that the constitutional basis of atopy may lie in an inherited or acquired adrenergic abnormality caused by a shift in available receptor numbers from β- to α-adrenoceptors.

Key words: Adrenoceptor; [^{3}H]dihydroalprenolol; [^{3}H]dihydroergocryptine; Binding; Lymphocytes; Atopy

In the late sixties, the beta adrenergic theory of the constitutional basis of atopy has reformulated the original concept of atopy. In its first (1), and more explicitly in its later expositions (2, 3), the theory, although primarily designed for human asthma, was stated to be also applicable to atopic dermatitis, since patients with the latter disease have increased serum IgE concentrations, a strong genetic predisposition to asthma, and exhibit qualitatively and quantitatively abnormal responses to pharmacological mediators or others stimuli as asthmatics do.

In the experimental analysis of the beta adrenergic theory, both chronologically and in research strategy, 4 phases can be distinguished. In the wake of the early presentations of the theory (1968–72), reduced asthmatic responsiveness measured by systemic parameters of β-adrenergic reactivity such as less rise in blood sugar, free fatty acids, lactate, pyruvate, pulse rate, urinary and plasma cyclic AMP levels and reduced eosinopenic response to β-adrenergic stimulation were found. In contrast, activities of adenylate cyclase, cyclic AMP phosphodiesterase, and cyclic AMP dependent protein kinase were found to be normal (4–6) in skin biopsies from the affected as well as normal-appearing areas of atopic dermatitis. On the other hand, in the subsequent years of 1972–75, in support of the earlier systemic findings in asthma, the same pattern of reduced β-reactivity was demonstrated in *in vitro* preparations of isolated cells derived from asthmatic individuals. In such studies, leukocytes and lymphocytes from asthmatic donors exhibited a reduced cyclic AMP response to beta adrenergic agents, but a completely intact response to other mediators suggesting that the biochemical defect is specifically beta adrenergic, and may in fact be at the level of the β-adrenoceptors. However, in the third phase of the experimental analysis of the theory (1975–77), it was shown that manifestations of β-adrenergic subsensitivity in asthma can be simulated, at least in part, in non-asthmatic normal individuals by pretreatment with adrenergic medication. These findings raised the question whether the adrenergic abnormalities seen in asthma may not be due to the disease itself, but rather to medication taken by asthmatic patients.

In the subsequent years (1977–79), however, beta adrenergic subsensitivity was found to be demonstrable also without concurrent medication, long after medication has been discontinued, and in lymphocytes derived from patients with atopic dermatitis (7) that is in an atopic condition in which adrenergic medication never has been involved. In addition, recent observations in our laboratory (8) provided conclusive evidence that β-adrenergic

Table I. *Binding of [³H]DHA and [³H]DHE to lymphocytic membranes from patients with atopic dermatitis*

Results of binding studies with [³H]dihydroalprenolol (DHA) and [³H]dihydroergocryptine (DHE) to membranes of lymphocytes from normal controls, patients with nonatopic skin disease (NASD), and patients with atopic dermatitis (AD), at 11 nM DHA and 4 nM DHE concentrations using the methodology described by ref. 12

Group (N)	Ligand	Maximal binding fmol/mg protein	Ratio
Normal (70)	[³H]DHA	64±4	5.3
	[³H]DHE	12±5	
NASD (10)	[³H]DHA	58±8	5.3
	[³H]DHE	11±3	
AD (8)	[³H]DHA	36±6	0.5
	[³H]DHE	69±5	

subsensitivity must be regarded as a fundamental characteristic of atopy. In these studies, lung specimens obtained from patients with reversible airways disease (asthma) were shown a reduced capacity to synthesize cyclic AMP to β-adrenergic stimulation. That this abnormality must have lied at an early step of the biochemical sequence of β-adrenergic activation was evidenced by (a) the normal rates of phosphodiesterase-catalyzed cyclic AMP and cyclic GMP breakdowns in the same pulmonary specimens (9) and (b) by the marked shift in the numbers of adrenoceptors from β to α both in lung tissue as well as in membranes of lymphocytes obtained from the same patients with reversible airways obstruction (10).

Table II. *Binding of [³H]DHA and [³H]DHE to T and B lymphocytes from patients with atopic dermatitis*

Results of binding studies with [³H]dihydroalprenolol (DHA) and [³H]dihydroergocryptine (DHE) to membranes of B and T lymphocytes from normal controls, patients with nonatopic skin disease (NASD), and patients with atopic dermatitis (AD), at 11 nM DHA and 4 nM DHE concentrations using the methodology described by ref. 12. Separation of T and B cell-enriched lymphocyte subpopulations was carried out by selective removal of cells forming rosettes with sheep red blood cells (T) or cells adhering to nylon wool columns (B) from whole peripheral lymphocyte populations. Studies were carried out on the non-rosetting (mostly B) or non-adhering (mostly T) cells in each case according to procedures described by refs. 13 and 14

Group (N)	Ligand	Maximal binding (fmol/mg protein) B cells	R	T cells	R
Normal (10)	[³H]DHA	62± 8	4.4	65±10	5.9
	[³H]DHE	14± 6		11± 4	
NASD (10)	[³H]DHA	61±10	5.1	57± 8	4.4
	[³H]DHE	12± 9		13± 4	
AD (8)	[³H]DHA	30± 2	0.4	32± 3	0.5
	[³H]DHE	69± 7		65± 5	

Using the same radioligands, that is [³H]dihydroalprenolol (DHA) and [³H]dihydroergocryptine (DHE), for the measurement of β- and α-receptors, respectively, the same shift from β- to α-receptors was found in membranes of lymphocytes from patients with atopic dermatitis, but not in lymphocytic membranes derived from patients with various non-atopic skin diseases (Table I). That this lymphocytic adrenoceptor shift cannot be due to possible variations in the proportion of lymphocyte subpopulations was demonstrated by the lack of difference in α- and β-receptor numbers between B and T lymphocytes, indicating that the adrenoceptor shift is intrinsic to the disease state itself (Table II). Furthermore, when saturation curves for the binding of DHA and DHE to adrenergically desensitized lymphocytic membranes from patients with asthma or atopic dermatitis were performed, the numbers of both adrenoceptors were quantitatively reduced, but the original ratios between the 2 types of adrenoceptors were preserved indicating that adrenergic desensitization is not the cause of the original adrenergic abnormality in lymphocytes of atopic individuals. Finally, pre-incubation of lymphocytes from atopic donors with hydrocortisone was found to restore the normal ratio between α- and β-receptor numbers (11).

These findings suggest that the constitutional basis of atopy both in asthma and atopic dermatitis may lie in an inherited or acquired (infection) adrenergic abnormality caused by a shift in available receptor numbers from β- to α-receptors possibly by mechanisms similar to those described for the phenomenon of adrenoceptor interconversion (3).

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DISCUSSION

Zachariae (Aarhus). Q: Have you done any longitudinal studies to investigate changes in the patients during longer or shorter periods of their lives?

A: It is possible that the basic status could change with time, especially when major hormonal changes take place in the body such as puberty, pregnancy and climacterium. Thyroid hormons, sexual hormons and insulin are able to shift the momentary balance between alpha/beta receptors, also triggering factors as e.g. viral infections may have a role here.