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DESENSITIZATION OF THE β-ADRENOCEPTOR OF LYMPHOCYTES FROM NORMAL SUBJECTS AND PATIENTS WITH PHAEOCHROMOCYTOMA: STUDIES *IN VIVO*

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1 Following the observation that lymphocyte β-adrenoceptor responsiveness was not depressed in asthmatics treated only with non-adrenergic drugs we have explored the effects of prolonged exposure to β-adrenoceptor agonists in normal subjects.

2 Treatment with oral salbutamol (12–16 mg/kg/day for 10 days), or with inhaled salbutamol (3000 μg/day for 8–10 days) resulted in a significant reduction in lymphocyte β-adrenoceptor responsiveness.

3 A 48 h infusion of isoxsuprine (10 mg/h) resulted in a marked depression of lymphocyte β-adrenoceptor responsiveness ($P < 0.001$).

4 Prolonged elevation of endogenous catecholamines caused by phaeochromocytoma was also associated with a marked depression of lymphocyte β-adrenoceptor responsiveness ($P < 0.001$).

5 There was no evidence that an increase in phosphodiesterase activity could explain the reduced cyclic AMP response.

6 It is concluded that diminished β-adrenoceptor response occurs as a response to prolonged exposure to β-adrenoceptor agonists. It is likely that the diminished response seen in asthmatic subjects can be explained on a similar basis and does not indicate an inherent cellular defect.

7 The possible clinical significance of such changes in asthmatics are discussed.

Introduction

The hypothesis (Szentivanyi, 1968) that an inherent defect of β-adrenoceptors might underlie a range of atopic diseases, including bronchial asthma, has stimulated a great deal of research. Reports of depressed β-adrenoceptor function from *in vivo* studies in asthmatic subjects (Cookson & Reed, 1963; Inoue, 1967; Lockey, Glennon & Reed, 1967) and more recent studies using the lymphocyte as a source of readily accessible β-adrenoceptors (Smith & Parker, 1970), appeared to support this idea (Logsdon, Middleton & Coffey, 1972; Parker & Smith, 1973; Alston, Patel & Kerr, 1974). However, despite other work which failed to confirm these findings (Grieco, Pierson & Pi-Sunyer, 1968; Zaid, Beall & Heimlich, 1968) the possibility that the depressed response could have been due to prolonged exposure to large doses of adrenergic bronchodilators has not been fully explored.

We have demonstrated that lymphocyte β-adrenoceptor responsiveness is depressed in asthmatics taking large doses of adrenergic bronchodilators, but that it is not in asthmatics taking non-adrenergic medication (Conolly & Greenacre, 1975, 1976).

We now report the effects of β-adrenergic agonists, when given in large doses to normal subjects, on lymphocyte β-adrenoceptor responsiveness. The drugs have been given orally, by inhalation, and by intravenous infusion. Preliminary data on the effect of such an infusion of a β-adrenoceptor agonist on the level of phosphodiesterase are also given. In addition, observations on the level of β-adrenoceptor responsiveness in lymphocytes from four patients with phaeochromocytoma are described.

Methods

Clinical studies

Lymphocyte β-receptor responsiveness was measured in peripheral blood lymphocytes isolated as described below from the following subjects:

1. Three normal volunteers studied before (4–5 times) and after (1–4 times) taking salbutamol tablets, 12–16 mg/day for 10 days.

2. Five normal volunteers studied twice each before and twice after taking salbutamol from a pressurized aerosol (30 inhalations/day for 8 to 10 days).

3. Three obstetric patients studied once before and once after a 48 h infusion of isoxsuprine. This long acting β-adrenoceptor agonist was given to prevent the onset of premature labour following intrauterine transfusion for severe rhesus incompatibility.

In one other patient receiving this treatment the cyclic AMP response to prostaglandin E_1 (PGE_1) and the level of phosphodiesterase (PDE) activity was measured before and after the infusion and PDE activity alone was measured in three further patients.

In group I, the dose used was a conventional clinical dose. In group II the dose of aerosolized salbutamol exceeded the manufacturers recommended dose about 4-fold, but was chosen because it approximated a dose not uncommonly used by asthmatic patients in relapse.

IV. Four patients with proven phaeochromocytomata were studied preoperatively. It was not possible to examine three of these patients postoperatively (two lived abroad, and one had an inoperable tumour), so their results have been compared with our normal population. At the time of study they had persistently elevated urinary vanillyl mandelic acid levels, and their plasma noradrenaline levels (measured radioenzymatically by Dr D.H. Jones) ranged from 1.42–5.20 ng/ml The normal range in our laboratory lies between 0.1 and 0.7 ng/ml.

Lymphocyte isolation

Lymphocytes were isolated by a modification (Harris & Ukaejiofo, 1970) of the method originally devised by Boyum (1968). Our adaptation of this technique has recently been described elsewhere (Conolly & Greenacre, 1977).

Measurement of β-adrenoceptor responsiveness

Dose response curves to isoprenaline were obtained in lymphocytes taken from the different groups of subjects mentioned above. The response measured was the increase in cyclic AMP levels after incubation with isoprenaline ($0–10^{-4}$ mol/l) at 37°C for 15 min. Prior to assay, cyclic AMP was purified by anion exchange chromatography (Dowex AG 1 x 8). After lyophilization, the residue was dissolved in a phosphate buffer (pH 5.5) and then analysed by a modified Gilman (1970) protein binding assay using a protein kinase extracted from rabbit skeletal muscle. Details of these methods have been given previously (Conolly & Greenacre, 1977).

Assay of phosphodiesterase (PDE) activity

Phosphodiesterase activity was measured by a modification of the technique of Thompson & Appleman (1971), in which tritiated cyclic AMP is converted to 5′ adenosine monophosphate by the tissue phosphodiesterase, this product in turn being converted to adenosine by snake venom 5′-nucleotidase added in excess. The rate of production of adenosine thus provides a measure of cellular PDE activity. The experimental details are as follows. The reaction mixture contained, in a 200 µl volume, 5×10^{-2} mol/l Tris/HCl buffer at pH 8.5, [^{3}H] cyclic AMP (400,000 d/min), 4×10^{-4} mol/l unlabelled cyclic AMP, 4×10^{-3} mol/l $MgCl_2$, 0.2 units of 5′-nucleotidase (Sigma Ltd) and 100 µl lymphocyte homogenate. The latter was prepared from a suspension of 40×10^6 cells/ml in 5×10^{-2} mol/l Tris HCl, pH 8.5 by twice rapidly freezing and thawing the sample followed by homogenization in a tightly fitting ground glass homogenizer.

The 15 min incubation at 37°C was initiated by addition of lymphocyte homogenate and terminated by placing the tubes on ice. 50 µl (5000 d/min of [^{14}C] adenosine (Radiochemical Centre, Amersham) were added to quantitate recovery which varied between 80 and 95%. Tris HCl buffer pH 9 (2 ml) added and the reaction mixture placed on a 4 cm column of Dowex AG 1 x 2 (200–400 mesh, chloride form, Biorad Labs). The initial eluate and that obtained after a further 2 ml buffer (pH 9) had been applied to the column was discarded. More of the same buffer (9 ml) was then added to the column and the resulting eluate collected into a scintillation vial. After the addition of 10 ml Instagel (Packard Instrument Company) the sample was counted in a Packard 2650 liquid scintillation spectrometer with automatic quench correction.

Statistical analysis

Dose response curves were compared by two-way analysis of variance with replication (Kempthorne, 1952).

Results

Group I

The isoprenaline dose-response curves showing the per cent increase in cyclic AMP before and after treatment with oral salbutamol are shown in Figure 1. The decrease in response seen after salbutamol treatment, though modest, is statistically significant ($P < 0.05$).

Group II

Isoprenaline dose-response curves before and after the

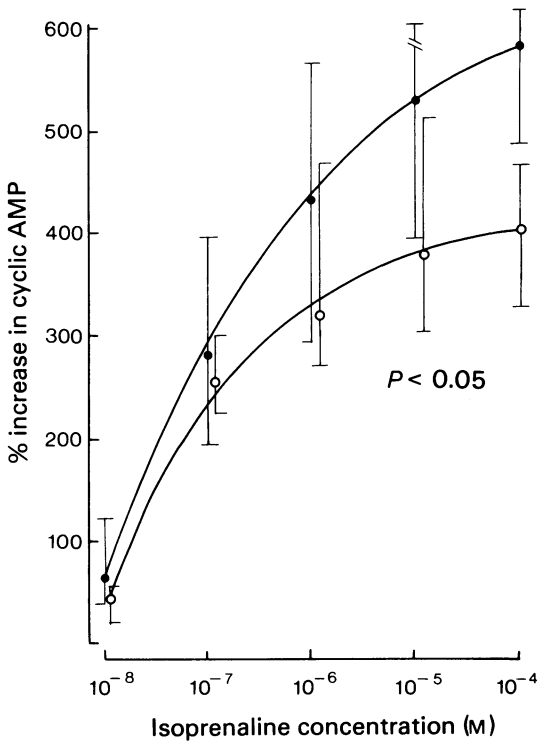

Figure 1 Effect of oral salbutamol (12–16 mg/day for 10 days) on lymphocyte responsiveness in three normal subjects (median values plus interquartile range). ● pre salbutamol; ○ post salbutamol.

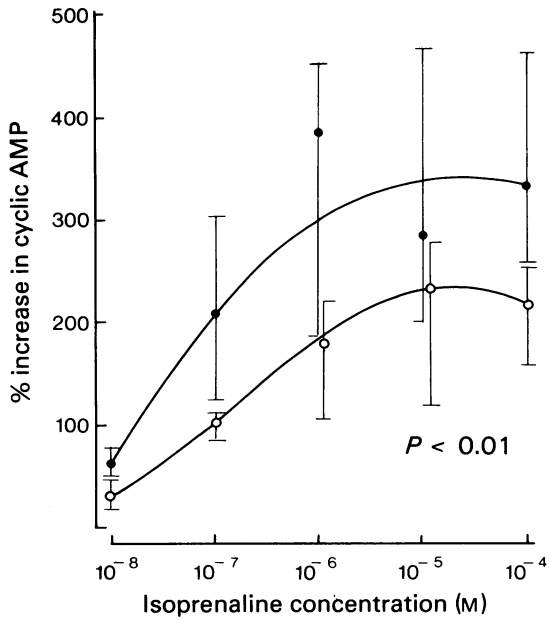

Figure 2 Effect of salbutamol inhalations (30 doses equivalent to 3000 μg/day for 8–10 days) on lymphocyte responsiveness in five normal subjects (median values plus interquartile range). ● pre inhalation; ● post inhalation.

period of salbutamol inhalations are shown in Figure 2. The decrease in response is greater than in the first study, and again is statistically significant ($P < 0.01$).

Group III

The decrease in lymphocyte β-adrenoceptor response after the isoxsuprine infusion is shown in Figure 3. There is a marked depression of the second dose response curve, which is highly significant ($P < 0.001$).

Group IV

The response to isoprenaline (Figure 4) was markedly depressed when compared with a population of normal subjects ($P < 0.001$).

The absolute values (pmol cyclic AMP/4 x 10^6 cells) are given for each group in Table 1. There is considerable between-subject variation in these values, so that in Group II, for data analysed in this form, the difference between the two dose response curves fails to reach statistical significance. None the less, the overall pattern of altered response is preserved, and the difference between the two curves for groups I, III

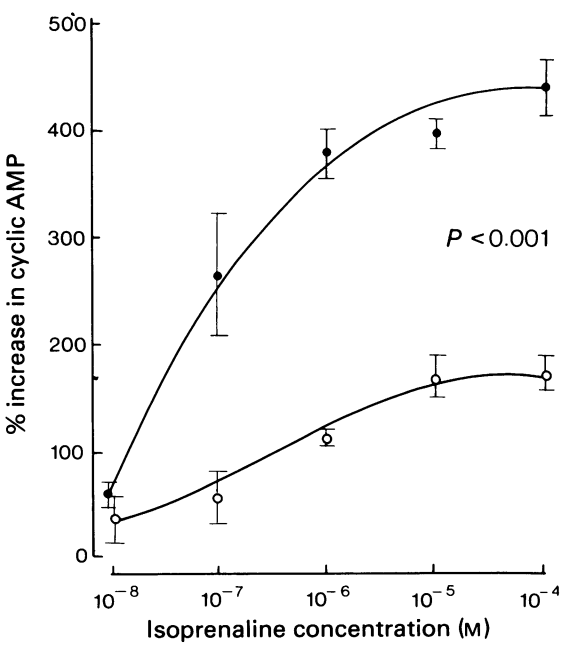

Figure 3 Lymphocyte responsiveness in four obstetric patients before (●) and after (○) a 48 h infusion of isoxsuprine (10 mg/h) (median values plus interquartile range).

Table 1 Increase in cyclic AMP (pmol 4×10^6 cells, mean ± s.e. mean) in response to isoprenaline before and after exposure to β-adrenoceptor agonists

	Before β-adrenoceptor agonist						*After β-adrenoceptor agonist*					
Subjects	*Isoprenaline concentration (mol l^{-1})*						*Isoprenaline concentration (mol l^{-1})*					
	0	*10^{-8}*	*10^{-7}*	*10^{-6}*	*10^{-5}*	*10^{-4}*	*0*	*10^{-8}*	*10^{-7}*	*10^{-6}*	*10^{-5}*	*10^{-4}*
Group I Oral salbutamol	57.8 ±8.1	54.5 ±11.5	173.9 ±24.5	262.6 ±46.5	268.1 ±48.2	312.0 ±60.3	58.5 ±19.6	20.9 ±7.2	127.6 ±38.0	171.1 ±44.2	203.5 ±62.9	171.8 ±47.8
Group II Aerosol salbutamol	36.1 ±6.3	20.2 ±4.7	79.9 ±17.7	142.1 ±39.6	153.1 ±55.0	157.8 ±48.7	85.1 ±34.6	29.8 ±16.9	64.6 ±24.2	104.6 ±41.4	102.0 ±45.8	124.1 ±48.6
Group III Isoxsuprine infusion	49.3 ±17.4	44.2 ±23.6	160.0 ±65.4	173.0 ±55.3	194.6 ±82.6	222.8 ±63.4	20.5 ±8.1	5.8 ±2.1	11.0 ±1.0	27.2 ±6.0	25.5 ±5.2	29.7 ±3.6
Group IV Preoperative values	–	–	–	–	–	–	23.9 ±5.1	42.8 ±2.2	20.3 ±8.3	35.2 ±11.9	54.0 ±9.7	51.3 ±9.1
Normal subjects	47.6 ±5.3	30.7 ±7.0	117.2 ±18.1	173.5 ±21.6	171.2 ±23.1	184.8 ±25.9	–	–	–	–	–	–

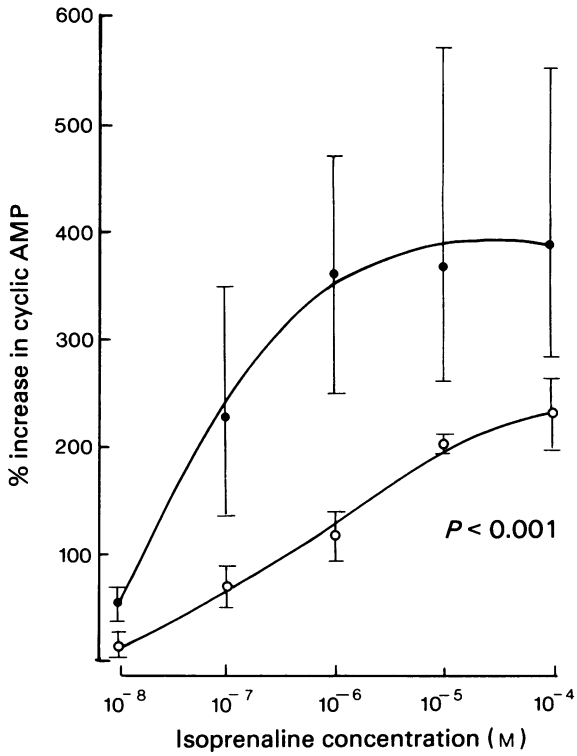

Figure 4 Lymphocyte responsiveness in normal subjects (●) and in four patients with phaeochromocytoma (○) (median values plus interquartile range).

and IV remain statistically significant ($P < 0.01$, < 0.001 and < 0.001 respectively). It is important to note that the baseline values for cyclic AMP in cells studied after prolonged exposure to a β-adrenoceptor agonist do not differ significantly from the baseline values seen in the initial studies (paired *t*-test) under any of these treatment regimes.

Response to PGE_1

The response to PGE_1 was not altered in one subject studied before and after isoxsuprine infusions, in contrast to the response to isoprenaline which was markedly attenuated (Table 2).

Phosphodiesterase activity

No consistent alteration of PDE activity was seen in three patients studied before and after an isoxsuprine infusion (Table 2).

Discussion

These data indicate that prolonged exposure to endogenous and exogenous β-adrenoceptor agonists leads to a diminished responsiveness to other β agonists (cross tachyphylaxis) in non-asthmatic subjects. Desensitization analogous to that here reported in lymphocytes from patients with phaeochromocytomata has also been observed in fat cells from similar patients with catecholamine secreting tumours (Smith, Isaksson, Jacobson, Nyberg, Sjöström & Stenström, 1975). It seems probable therefore that the impaired β-adrenoceptor responsiveness described in asthmatic patients can be explained on the basis of desensitization induced either by adrenergic bronchodilators or by high levels of endogenous catecholamines secreted as a physiological response to the disease.

It is unlikely that the relatively modest changes seen in the first two groups of subjects reported here have any clinical significance, since many asthmatics take oral salbutamol in the dose used in this study. We have studied a series of asthmatics on this dose and found little significant change in airway responsiveness or severity of asthma (Conolly & Greenacre, unpublished observations). The same, however, may not be true of larger doses. Tattersfield & Holgate (1976) have shown that repeated exposure to inhaled salbutamol led to a progressive reduction in its

Table 2 The response of lymphocytes to isoprenaline (10^{-4} mol l^{-1}) and PGE_1 (10^{-6} mol l^{-1}), and the level of phosphodiesterase (PDE) activity, measured before and after a 48 h infusion of isoxsuprine

	Before isoxsuprine			*After isoxsuprine*		
Patient	*Response to isoprenaline**	*Response to PGE_1**	*PDE activity†*	*Response to isoprenaline**	*Response to PGE_1**	*PDE activity†*
1	420	1000	100%	180	1000	147%
2	–	–	100%	–	–	100%
3	–	–	100%	–	–	87.5%
4	–	–	100%	–	–	113%

* Response measured as % increase in cyclic AMP above unstimulated level.
† Response measured as generation of [^{3}H]-adenosine, expressed as % of pre-infusion value.

bronchodilator effect in normal subjects. Paterson, Courtenay Evans & Prime (1971) observed severe rebound bronchoconstriction after an isoprenaline infusion in some asthmatics, suggesting that desensitization may have deprived the patients of an important defence mechanism.

β-adrenergic receptor activity is of great importance in asthma, mediating both bronchodilatation and, in some patients at least, inhibition of release of bronchoconstrictor substances such as histamine and SRS-A (Schild, 1937; Orange, Kaliner, Laraia & Austen, 1971). The serious effects of the injudicious use of β-adrenoceptor blocking drugs in asthmatics is well known. However, the importance to asthmatic patients of the drug-induced desensitization described here remains conjectural. The fact that it does not appear to be a problem in those many asthmatic patients given large doses of such drugs in the treatment of status asthmaticus may depend on the now universal practice of combining such therapy with large doses of steroids, and this factor may be of crucial importance in ameliorating or reversing any tachyphylaxis (Ellul-Micallef & Fenech, 1975; Shenfield, Hodson, Clarke & Paterson, 1975); Tattersfield & Holgate, 1976).

The extent to which this desensitization can occur, and the mechanisms underlying it are still not clear. The observations made on three patients infused with isoxsuprine suggests that increased PDE activity is unlikely to be a major factor, and the data of Browning, Brostrom & Groppi (1976) suggest that other mechanisms are also involved. In adipocytes the formation of a hormone antagonist has been described (Ho & Sutherland, 1971; Smith, Isaksson, Nyberg & Sjöström, 1976). It has been suggested that this could be an endoperoxide (Gorman, Hamberg & Samuelsson, 1976) but its nature, physiological significance and relevance to the present studies are unknown. Loss (or conformational alteration) of cell surface receptors has been recognized as a component of cholinergic desensitization (Rang & Ritter, 1969, 1970; Miledi & Potter, 1971). Recent evidence suggests that similar changes may take place in β-adrenoceptors in both amphibians and mammals (Romero, Katz, Kebabian & Axelrod, 1975; Mickey, Tate, Mullikin & Lefkowitz, 1976).

Clearly it would be impractical and unethical to pursue studies of this desensitization further in normal subjects or asthmatic patients. In recognition of this, *in vitro* methods of simulating these changes have been devised and some of the results are reported in the companion paper (Greenacre, Schofield & Conolly, 1978).

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