The Lymphocyte β-Adrenoceptor in Normal Subjects and Patients with Bronchial Asthma

THE EFFECT OF DIFFERENT FORMS OF TREATMENT ON RECEPTOR FUNCTION

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ABSTRACT β-adrenoceptor function has been compared in lymphocytes of normal subjects, asthmatic patients taking large doses of β-adrenergic bronchodilators, and comparable asthmatics treated exclusively with nonadrenergic medication. The effect of prolonged administration of β-adrenoceptor agonists on receptor function in normal subjects has also been examined. β-receptor response in each situation was quantitated by changes in levels of cyclic AMP, measured by a protein-binding assay.

Dose response curves to isoproterenol (10 nM–0.1 mM) have been constructed for each group. Maximal increase in cyclic AMP in lymphocytes from normal subjects (393.2±44.0%) and in asthmatics on nonadrenergic preparations (408.3±46.7%) was significantly greater ($P<0.001$) than in asthmatics taking large doses of β-sympathomimetics (67.5±24.2%).

Depression of the cyclic AMP response appeared to correlate with the degree of exposure to β-adrenergic agonists but not with the prevailing severity of the patient's asthma.

Withdrawal of β-adrenergic drugs was followed by a reversion of the cyclic AMP response to normal values, which suggests that the depression was drug-induced rather than an inherent feature of the disease.

This interpretation was confirmed by the finding that prolonged exposure of normal subjects to high doses of a β-adrenergic agonist caused a marked and significant ($P < 0.001$) reduction in the cyclic AMP response, very similar to that seen in asthmatics on large doses of adrenergic bronchodilators.

A possible link between drug-induced changes in the cyclic AMP response and the rise in the United Kingdom asthma death rate in the 1960's is discussed.

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INTRODUCTION

Adrenergic activity is a key part of the physiological and pharmacological response to bronchial asthma. Bronchial smooth muscle relaxation results directly from β-adrenoceptor stimulation, but the same receptor mechanism is also important in inhibiting the release of histamine, as shown by Schild (1), slow-reacting substance of anaphylaxis, reported by Orange et al. (2), and prostaglandins (Liebig, et al. [3]), presumably from mast cells in the lung parenchyma. For this reason, factors that impair this mechanism (for example injudicious use of nonselective β-blocking drugs) can have serious consequences in asthmatic patients. The hypothesis advanced by Szentivanyi (4, 5), that an inherent defect of the β-adrenoceptor was a major etiological factor in atopic diseases such as asthma, has therefore attracted considerable attention and provoked a great deal of research.

There are obvious difficulties that hinder direct investigation of cellular mechanisms in human lung. An ingenious solution to this problem, first suggested by Smith and Parker (6), has been to use peripheral blood leukocytes, which themselves possess β-adrenoceptors. This approach presupposes a pharmacological identity between the lung and leukocyte receptors. We have recently confirmed that both have the characteristics of a β_2 receptor.[1] It is also necessary to assume that changes affecting the leukocyte β-receptors will affect those of mast cells in lung tissue. This is less certain, though the suggestion of Burnet (7, 8), that mast cells and basophils may be postmitotic derivatives of thymus-derived lymphocytes, gives some support to

[1] Conolly, M. E., and J. K. Greenacre. 1976. The β-adrenoceptor of the human lymphocyte and human lung parenchyma. *Br. J. Pharmacol.* **58**: In press.

TABLE I
Patients on High Doses of β-Adrenergic Bronchodilators

Patient	Age	Sex	Asthmatic type	Spirometry	Best	Worst	At time of study	Medication at time of study
	yr							
1	62	M	Intrinsic	PEFR	370	60	On respirator	Isoproterenol, 3 mg/24 h; epinephrine 300 μg + 700 μg s.c.; aminophylline, 1.0 g; hydrocortisone, 750 mg.
2	63	F	Intrinsic	$\frac{FEV_1}{FVC}$	$\frac{1.0}{1.5}$	$\frac{0.35}{0.75}$	$\frac{0.4}{1.0}$	Isoproterenol, 10 mg/24 h (aerosol); prednisone, 15 mg/day
3	63	M	Intrinsic	Not recorded		Not recorded	$\frac{0.35}{0.95}$	Salbutamol, 2.5 mg/24 h (nebulizer); hydrocortisone, 800 mg; aminophylline, 1.0 g.
4	26	F	Extrinsic	$\frac{FEV_1}{FVC}$	$\frac{2.6}{3.6}$	$\frac{0.5}{1.0}$	$\frac{0.5}{1.0}$	Salbutamol, 4.0 mg/24 h (aerosol); prednisone, 40 mg/day; disodium cromoglycate (spinhaler); aminophylline suppository
5	47	F	Intrinsic	$\frac{FEV_1}{FVC}$	$\frac{2.0}{3.4}$	$\frac{1.0}{2.5}$	$\frac{1.0}{2.5}$	Salbutamol, 4.0 mg/24 h (aerosol)
6	53	F	Intrinsic	$\frac{FEV_1}{FVC}$	$\frac{1.9}{3.0}$	$\frac{0.6}{1.2}$	$\frac{0.6}{1.2}$	Salbutamol, 4.0 mg/24 h (aerosol) and 16 mg/24 h (tablets)
7	47	F	Intrinsic	$\frac{FEV_1}{FVC}$	$\frac{2.6}{3.3}$	$\frac{1.0}{1.6}$	$\frac{1.0}{1.6}$	Salbutamol, 2.0 mg/24 h (aerosol) and 16 mg/24 h (tablets)

FEV_1, forced expiratory volume in 1 s (liters); FVC, forced vital capacity (liters); PEFR, peak expiratory flow rate (liters/min).

this assumption. The relevance of leukocyte β-adrenoceptors to those of bronchial smooth muscle remains conjectural. Several groups have studied asthmatic patients using this approach: Logsdon et al. (9), Parker and Smith (10), and Alston et al. (11). They have reported depressed β-adrenoceptor function, at least during the active phases of the disease, and have interpreted these data as supporting Szentivanyi's hypothesis. Gillespie et al. (12), however, were unable to find a significant difference between normal subjects and most asthmatic patients.

In the light of other data (13–15), an alternative explanation is that the depressed β-adrenoceptor response observed in asthmatics could have been caused by intensive therapy with β-adrenergic bronchodilator drugs, and this could obviously relate to the increase in the asthma death rate linked to the abuse of such preparations. We have therefore examined lymphocytes from normal subjects and from asthmatic patients treated either with adrenergic bronchodilators or with nonadrenergic antiasthmatic drugs available in the United Kingdom, namely disodium cromoglycate (cromolyn sodium, marketed as Intal (Fisons Corporation, Pharmaceutical Division, Loughborough, England, and Bedford, Mass.) or Aarane (Syntex (F. P.) Inc., Humacao, P. R.), and beclomethasone dipropionate (Becotide, Allen and Hanburys Ltd., Ware, England).

METHODS

The following groups of subjects were studied: (*a*) 11 normal volunteers. (*b*) 7 asthmatic patients studied at the end of a period (up to 10 days) of heavy use of adrenergic bronchodilators, which comprised inhalations of isoproterenol (up to 1.5 mg/day) or salbutamol (2.5–4.0 mg/day by inhalation). Two patients also took salbutamol tablets in a dose of 16 mg/day. Clinical details are given in Table I. (*c*) 12 asthmatic patients treated at the time of study exclusively with nonadrenergic antiasthmatic drugs (disodium cromoglycate or beclomethasone dipropionate). These patients were comparable to the first group in that all had clinically well-established asthma, five had a previous history of status asthmaticus, and six had required at least one course of systemic steroids in the recent past. Clinical details are given in Table II. (*d*) Five asthmatic patients studied before and after treatment and changed from adrenergic to nonadrenergic drugs. (*e*) Four nonasthmatic obstetric patients receiving 48-h infusions of another β-agonist

TABLE II
Patients on Nonadrenergic Medication

Patient	Age	Sex	Asthmatic type	Spirometry	Best	Worst	At time of study	Medication at time of study
	yr							
2	63	F	Intrinsic	$\frac{FEV_1}{FVC}$	$\frac{1.0}{1.5}$	$\frac{0.35}{0.75}$	$\frac{0.5}{1.1}$	Beclomethasone; prednisone 10 mg/24 h
6	53	F	Intrinsic	$\frac{FEV_1}{FVC}$	$\frac{1.9}{3.0}$	$\frac{0.6}{1.2}$	$\frac{1.1}{2.1}$	Beclomethasone
7	47	F	Intrinsic	$\frac{FEV_1}{FVC}$	$\frac{2.6}{3.3}$	$\frac{1.0}{1.6}$	$\frac{2.3}{3.1}$	Beclomethasone
8	53	F	Intrinsic	PEFR	120	80	85	Cromoglycate
				$\frac{FEV_1}{FVC}$			$\frac{0.38}{0.51}$	
9	23	M	Extrinsic	$\frac{FEV_1}{FVC}$	$\frac{3.1}{5.3}$	$\frac{2.0}{4.2}$	$\frac{3.1}{5.3}$	Cromoglycate
10	36	M	Extrinsic	$\frac{FEV_1}{FVC}$	$\frac{2.0}{3.9}$	$\frac{1.1}{3.0}$	$\frac{2.2}{3.7}$	Cromoglycate
11	70	M	Intrinsic	$\frac{FEV_1}{FVC}$	$\frac{1.6}{3.3}$	$\frac{0.7}{1.35}$	$\frac{0.7}{1.35}$	Cromoglycate
12	52	F	Intrinsic	PEFR	330	240	310	Cromoglycate
13	60	F	Intrinsic	PEFR	390	210	390	Beclomethasone
14	20	M	Extrinsic	$\frac{FEV_1}{FVC}$	$\frac{3.7}{4.2}$	$\frac{2.7}{3.6}$	$\frac{2.9}{4.1}$	Cromoglycate
15	29	M	Intrinsic	$\frac{FEV_1}{FVC}$	$\frac{2.7}{5.0}$	$\frac{0.6}{1.1}$	$\frac{1.8}{3.5}$	Cromoglycate
16	13	F	Extrinsic	PEFR	400	130	400	Cromoglycate
				$\frac{FEV_1}{FVC}$		$\frac{0.7}{1.4}$		

Abbreviations as in Table I.

(isoxsuprine, 5 mg/h) to prevent the onset of labor after intrauterine transfusions for rhesus incompatibility.

Pharmacological response was quantitated by measuring changes in cyclic 3',5' adenosine monophosphate (cyclic AMP)[2] in the lymphocytes, with a Gilman-type protein-binding assay.

Preparation of lymphocytes. Lymphocytes were isolated from heparinized blood by Harris and Ukaejiofo's modification (16) of Böyum's technique (17). 60 ml of heparinized blood was centrifuged at 250 *g* at 15°C (MSE Mistral -4L refrigerated centrifuge, Measuring and Scientific Equipment Ltd., London, England). The platelet-rich plasma was removed and replaced with previously oxygenated Hanks' balanced salt solution (HBSS) (Burroughs Wellcome Ltd., Beckenham, England.[2] 10-ml portions of this diluted "blood" were carefully layered onto 10 ml Lymphoprep (Nyegaard and Co., Oslo, Norway) or Ficoll-Hypaque solution (Pharmacia Fine Chemicals AB, Uppsala, Sweden, and Winthrop Laboratories, Surbiton, England) of specific gravity 1.077. This was centrifuged at 400 *g* for 25 min at 15°C, lymphocytes were harvested, diluted with phosphate-buffered saline (Oxoid Ltd., London, England) and centrifuged at 1,000 *g* for 20 min at 15°C, and the pellet was allowed to disaggregate, with only gentle shaking, in Hanks' balanced salt solution. The cells were counted (Coulter Cell Counter, Coulter Electronics Inc., Hialeah, Fla.) and diluted, to produce a suspension of 2×10^6 cells/ml, with further Hanks' solution, human serum albumin (Sigma Chemical Co., Ltd., Kingston-on-Thames, England) (0.3 mg/ml final concentration), and Tris (5 mM final concentration) at pH 7.35. The isolation procedure took approximately 3 h and yielded a cell preparation of 80–95%) lymphocytes. The remainder of the cells were monocytes with only occasional granulocytes. Trypan blue exclusion tests showed greater than 95%

[2] *Abbreviations used in this paper*: cyclic AMP, cyclic 3',5' adenosine monophosphate; PDE, phosphodiesterase.

viability. Lymphocyte recovery was greatly enhanced by the addition of 10% fetal calf serum (Burroughs Wellcome Ltd.) to all solutions used during the cell separation to reduce lymphocyte adhesiveness.

Incubation. Duplicate or triplicate samples of the cells were incubated with theophylline (BDH Chemicals Ltd., Poole, England), 10 mM, and either the ascorbic acid solutions for the "blank" cells or isoproterenol sulfate, 10 nM to 0.1 mM free base (Sigma Chemical Co., Ltd.; 1 mg/ml ascorbic acid was used as antioxidant in all isoproterenol solutions). After 15 min, the reaction was terminated by placing the tubes in boiling water for 5 min. Then 0.18 pmol [^{3}H]cyclic AMP (5,000 cpm, New England Nuclear Corp., Frankfurt, W. Germany) were added to allow determination of recovery of cyclic AMP through subsequent purification.

Cyclic AMP purification and assay. The cells were homogenized with a teflon pestle and centrifuged, and the supernatant was applied to a 2.0 × 0.5-cm column of Dowex resin (AG 1 × 8 formate form, 200–400 mesh, Bio-Rad Laboratories, Richmond, Calif.). The columns were washed with water, and then the cyclic AMP was eluted with 12 ml 2 N formic acid, which did not elute cyclic 3′5′ GMP from the columns. The eluate was lyophilized, the residue dissolved in 1 ml phosphate buffer, pH 6.5, and cyclic AMP assayed.

A protein-binding assay was chosen in preference to measuring conversion of [^{3}H]adenosine, since the latter provides no data on basal levels of cyclic AMP. The assay was a modification of the Gilman technique (18). The binding protein was extracted from rabbit skeletal muscle with a protamine-Sepharose affinity chromatography column (19).[3] It was eluted with a 0–2 M gradient of NaCl, dialysed, and, after the addition of bovine serum albumin (1 mg/ml), frozen in 1-ml portions at −20°C. Under these conditions it showed no loss of activity for more than 2 yr. This protein differed from that described by Gilman in that its ability to bind cyclic AMP was maximal between pH 5.5 and 7.5. All assays were performed at pH 6.5.

Each assay tube contained 50 μl phosphate-citrate buffer, containing known amounts of unlabeled cyclic AMP (0–10 pmol) or the experimental material derived from the incubations, 50 μl of the binding protein, and 0.5 pmol labeled cyclic AMP in 10 μl. After mixing, the tubes were allowed to equilibrate at 0°C for 3 h. Separation of bound and free cyclic AMP was achieved by filtration through Millipore filters (0.45 μm pore size (Millipore Corp., Bedford, Mass) and then dissolved in 2 ml 2-ethoxy ethanol (BDH Chemicals Ltd.). Instagel (Packard Instrument Co., Inc., Downer's Grove, Ill.) was added and samples were counted in a Packard 3375 liquid scintillation spectrometer. As counting efficiency was very similar in all samples, quenching was not corrected for, and calculations were made from counts per minute.

Confirmation of the identity of assay cyclic AMP was

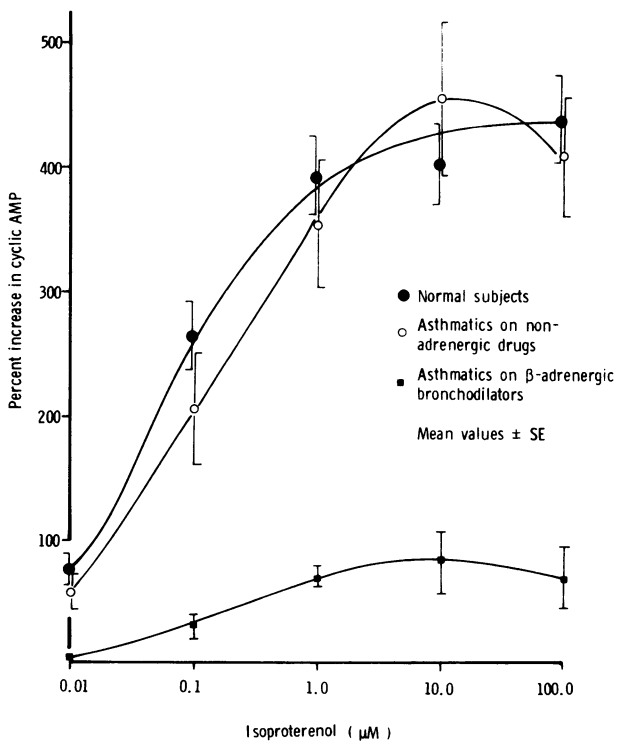

FIGURE 1 Percent increase in cyclic AMP in lymphocytes after a 15-min incubation at 37°C with isoproterenol. Lymphocytes were taken from normal subjects (● —— ●); asthmatic patients receiving only nonadrenergic drugs (○ —— ○); asthmatic patients taking excessive doses of β-adrenergic bronchodilators (■ —— ■).

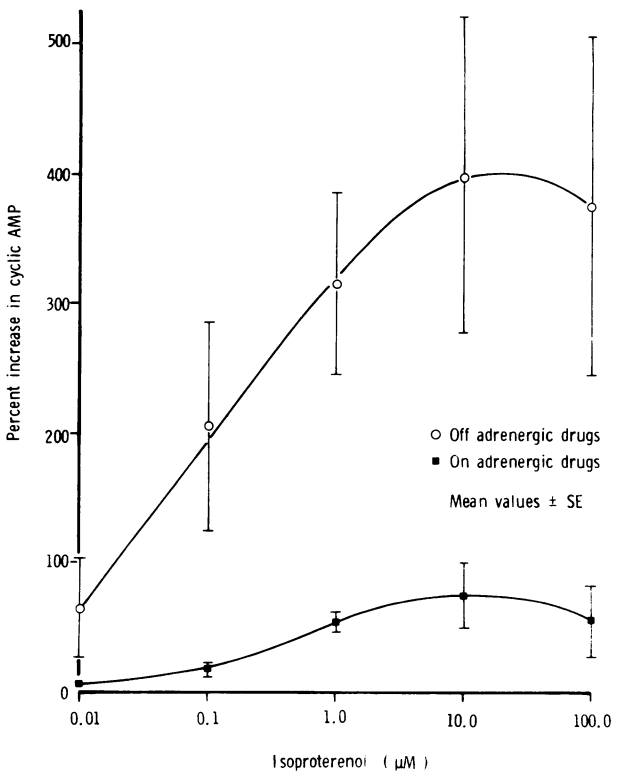

FIGURE 2 Percent increase in cyclic AMP in lymphocytes taken from five asthmatic patients and incubated as described for Fig. 1. The patients were studied initially when taking large doses of β-adrenergic bronchodilators (■ —— ■) and subsequently when changed to entirely nonadrenergic medication (○ —— ○).

[3] Johnson, R. A., and G. Schultz. 1972. Personal communication.

TABLE III

Normal Subjects' Lymphocyte Response to Isoproterenol

Subject	Age	Sex	Basal level of cyclic AMP	Increase in response to isoproterenol: mol/liter				
				10^{-8}	10^{-7}	10^{-6}	10^{-5}	10^{-4}
	yr		*pmol cyclic AMP/4 × 10^6 cells*					
19	33	M	13.3	10.6	47.7	69.6	70.2	73.5
20	41	M	24.5	11.3	39.4	77.1	80.9	86.6
			29.1	16.0	57.2	125.5	—	159.8
			61.4	0.0	84.2	152.4	—	139.3
21	32	M	28.0	0.0	63.5	58.3	55.4	139.4
22	33	M	24.7	9.4	25.8	72.3	120.9	—
			100.6	34.2	122.9	179.3	220.0	371.6
			42.7	108.2	269.4	321.4	317.6	334.5
			135.2	88.2	295.8	845.8	773.5	837.2
			92.7	—	245.8	436.2	303.8	275.6
23	31	F	82.1	50.8	235.2	316.8	—	—
			79.7	37.2	236.4	292.4	—	—
			34.2	0.6	19.4	116.3	230.9	—
			26.1	7.0	116.3	202.6	—	—
			42.9	33.7	103.5	169.8	170.7	210.0
24	24	M	62.9	3.5	57.1	148.1	167.7	165.4
			82.9	48.3	175.6	203.3	258.3	234.7
25	30	M	49.9	74.9	272.6	275.3	291.3	323.9
			49.6	129.4	248.7	290.2	321.3	329.9
			12.6	15.4	43.9	71.5	80.2	69.2
			37.4	18.6	72.9	99.5	97.3	93.2
26	30	F	51.1	35.8	193.6	181.0	222.0	237.8
27	28	M	51.7	6.4	65.1	97.8	99.9	114.4
			52.4	26.8	62.1	93.7	104.7	136.3
28	29	M	8.6	4.0	8.7	12.2	15.2	12.5
			16.8	10.9	27.2	57.5	39.1	47.9
29	28	M	55.3	34.3	144.1	259.1	259.8	323.1
			21.6	25.3	79.4	93.6	72.8	83.0
Mean			48.9	31.1	121.9	189.9	190.2	208.6
±SE			5.7	6.5	17.3	31.0	33.4	36.1

Data are given as basal cyclic AMP levels, and increase above this level in response to the indicated concentrations of isoproterenol.

obtained by comparing results in some samples before and after incubation with beef heart phosphodiesterase (Boehringer Mannheim, London, England).

Full dose-response curves of the different groups were compared by two-way analysis of variance with replication (20).

RESULTS

There is a highly significant difference ($P < 0.001$) in the response of lymphocytes from normal subjects and asthmatics on large doses of adrenergic drugs. In contrast, the response of asthmatics on nonadrenergic drugs was almost identical to that seen in normal subjects, and therefore also differed significantly ($P < 0.001$) from the other group of patients. These results are summarized in Fig. 1.

If the data are expressed in terms of absolute levels of cyclic AMP rather than as percentage increases, the average response for asthmatics on nonadrenergic drugs is somewhat lower than the mean for normal subjects ($P < 0.001$). However, as shown in Tables III and V, all these values fall within the range spanned by normal subjects, and the difference between the two groups of asthmatics (Tables IV and V) remains significant ($P < 0.001$).

TABLE IV

Asthmatic Patients on Large Doses of β-Adrenergic Bronchodilators: Lymphocyte Response to Isoproterenol

Patient	Age	Sex	Basal level of cyclic AMP	Increase in response to isoproterenol: mol/liter				
				10^{-8}	10^{-7}	10^{-6}	10^{-5}	10^{-4}
	yr			*pmol cyclic AMP/4 × 10^6 cells*				
1	62	M	18.0	0.0	3.6	—	3.6	—
2	63	F	62.5	0.0	7.6	37.8	99.3	90.4
3	63	M	12.2	0.0	5.1	—	18.7	—
4	26	F	16.0	—	1.6	10.2	13.0	10.2
5	47	F	52.1	2.1	46.8	59.0	—	52.2
6	53	F	27.0	—	6.4	18.4	4.8	6.8
7	47	F	54.9	10.8	14.4	29.2	25.7	4.7
Mean			34.7	2.6	12.2	30.9	27.5	32.9
±SE			8.0	2.1	6.0	8.4	14.8	16.8

Data are given as basal cyclic AMP levels and increase above this level in response to the indicated concentrations of isoproterenol.
These results are significantly different from normals in Table III ($P < 0.001$) and significantly different from asthmatics on nonadrenergic medication, as in Table V ($P < 0.001$).

TABLE V

Asthmatic Patients on Nonadrenergic Drugs: Lymphocyte Response to Isoproterenol

Patient	Age	Sex	Basal level of cyclic AMP	Increase in response to isoproterenol: mol/liter				
				10^{-8}	10^{-7}	10^{-6}	10^{-5}	10^{-4}
	yr			*pmol cyclic AMP/4 × 10^6 cells*				
2	63	F	38.3	35.7	69.3	112.5	122.6	98.3
6	53	F	16.6	—	43.4	68.4	107.7	101.1
7	47	F	65.7	0	37.5	89.2	60.8	64.1
8	52	F	14.6	3.6	1.8	17.8	32.2	49.4
9	23	M	10.7	0.9	4.1	19.5	45.6	49.4
10	36	M	20.0	9.0	52.7	100.0	106.0	144.5
11	70	M	10.3	5.3	26.7	33.3	49.3	56.3
12	52	F	24.3	—	3.9	18.7	53.7	92.2
13	60	F	37.0	7.4	42.2	82.2	131.2	94.5
14	20	M	74.6	62.9	177.1	262.9	273.1	343.3
15	29	M	16.5	35.2	—	109.6	128.0	—
			16.0	5.0	46.5	69.0	79.0	58.0
16	13	F	41.1	37.9	134.0	161.0	208.0	—
Mean			29.7	18.4	53.3	88.0	107.5	104.0
±SE			5.8	6.3	15.3	18.8	19.2	25.4

Data are given as basal cyclic AMP levels and increase in cyclic AMP above this level in response to the indicated concentrations of isoproterenol.
These results are significantly different from normals (Table III) ($P < 0.001$) and significantly different from asthmatics on adrenergic drugs (Table IV) ($P < 0.001$).

The effect of withdrawal of adrenergic bronchodilators has been studied in five patients. Three patients (2, 6, and 7) were admitted to hospital with severe asthma after several days' excessive use of isoproterenol or salbutamol. The other two, who had only trivial asthma, presented after taking excessive doses of salbutamol. Patient 17 had become psychologically dependent on his aerosol and had been taking 30 inhalations/day for several weeks. Patient 18 had attempted to commit suicide by taking 40 inhalations of salbutamol plus 16 mg salbutamol orally. Her

TABLE VI

Five Asthmatic Patients Studied before and after Changing from Large Doses of Adrenergic Bronchodilators to Nonadrenergic Therapy (Patients 2, 6, 7) or No Therapy (Patients 17, 18)

	Patient	Age	Sex	Basal level of cyclic AMP	Increase in response to isoproterenol: mol/liter				
					10^{-8}	10^{-7}	10^{-6}	10^{-5}	10^{-4}
		yr			*pmol cyclic AMP/4 × 10^6 cells*				
Before	2	63	F	65.0	0.0	7.6	37.8	99.3	90.4
	6	53	F	27.0	—	6.4	18.4	4.8	6.8
	7	47	F	54.9	10.8	14.4	29.2	25.7	4.7
	17*	30	M	69.3	0.0	16.5	45.4	70.0	63.6
	18*	18	F	8.1	0.7	0.0	2.2	2.3	0.0
Mean				44.9	2.9	9.0	26.6	40.4	33.1
±SE				11.8	2.6	3.0	7.6	19.1	18.4
After	2			38.3	35.7	69.3	112.5	122.6	98.3
	6			16.6	—	43.4	68.4	107.7	101.1
	7			67.5	0.0	37.5	89.2	60.8	64.1
	17			100.6	157.8	485.4	536.4	715.2	761.6
	18			41.0	2.2	17.0	83.2	88.0	62.4
Mean				52.8	48.9	130.5	177.9	218.9	217.5
±SE				14.4	37.2	89.1	89.9	124.5	136.3

Data are given as basal cyclic AMP levels and increase above this level in response to the indicated concentrations of isoproterenol.
* Trivial asthmatics.

leukocytes were taken and studied 20 h after the event, when all pharmacological effects had subsided. At the time of study, spirometry in both subjects was only marginally abnormal. The results for this group of patients are summarized in Fig. 2 and Table VI. It can be seen that after the withdrawal of β-adrenergic bronchodilators and, where necessary, their replacement by nonadrenergic medication, there was a significant return ($P < 0.001$) towards a normal response. Serial measurements of respiratory function and lymphocyte responsiveness during the change of therapy were made in patients 2 and 6. Figs. 3 and 4 show that although progressive improvement in lymphocyte responsiveness occurred, this was not accurately matched by an equivalent improvement in respiratory function.

The study of obstetric patients before and after a 48-h infusion of isoxsuprine indicate quite clearly that the cyclic AMP response can be markedly depressed in normal subjects after prolonged exposure to β-adrenergic agonists. The control dose response curve was virtually identical to that seen in the normal subjects shown in Fig. 1. After the infusion, the response was significantly reduced ($P < 0.001$), approaching that seen in patients taking large doses of β-adrenergic bronchodilators. These results are summarized in Fig. 5.

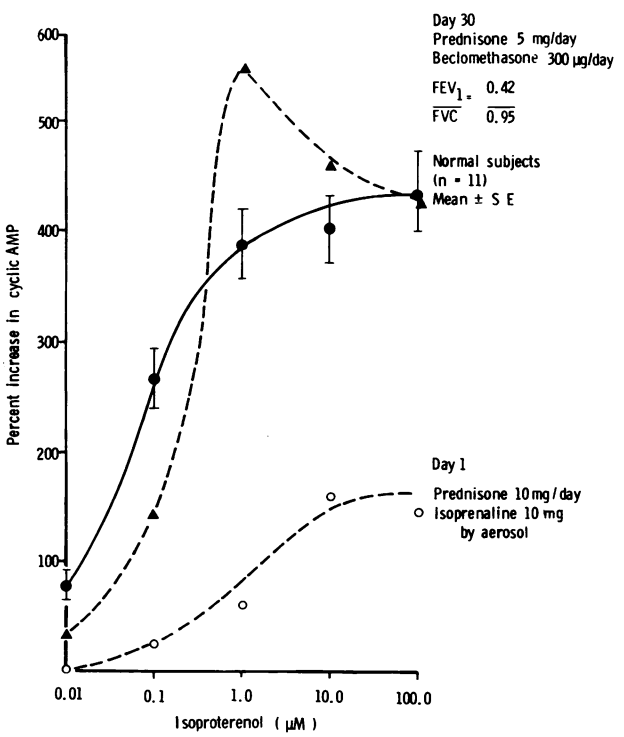

FIGURE 3 Percent increase in cyclic AMP in lymphocytes taken from patient 2, a 63-yr-old woman, presenting with severe asthma, and taking large doses of β-adrenergic bronchodilators (○ - - - ○). The patient was restudied 30 days later when she had been changed over to nonadrenergic therapy (▲ - - - ▲). Dose-response curve for 11 normal subjects is given for comparison (● —— ●). Incubation conditions in each study as described for Fig. 1. FEV_1, forced expiratory volume in 1s; FVC, forced vital capacity.

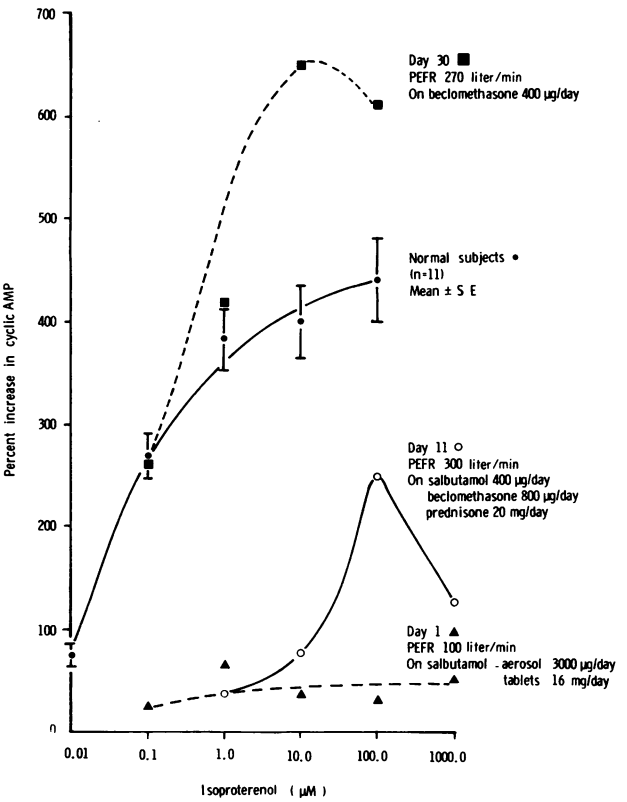

FIGURE 4 Percent increase in cyclic AMP in lymphocytes taken from patient 6, a 53-yr-old woman, presenting with severe asthma, and taking large doses of β-adrenergic bronchodilators (▲ - - - ▲). The patient was restudied on day 11 (○ —— ○) and day 30 (■ - - - ■) during changeover to nonadrenergic therapy. Dose-response curve for 11 normal subjects given for comparison (● —— ●). Incubation conditions in each study were as described for Fig. 1. PEFR, peak expiratory flow rate (liters/minute).

DISCUSSION

These studies show a significant difference between the percent increase in the cyclic AMP response in lymphocytes from asthmatic patients taking excessive doses of adrenergic drugs on one hand, and that from normal subjects or from asthmatics taking only nonadrenergic drugs on the other.[4] Other studies

[4] Although, as pointed out above, the absolute levels of cyclic AMP in lymphocytes from normal subjects was on average higher than the absolute levels in lymphocytes from asthmatics on nonadrenergic medication, the range that this group of patients covered did not fall outside the range of normal values. It seems unlikely, therefore, in view of the close similarity in the percent increase in cyclic AMP in these two groups of subjects, that this difference has any biological significance.

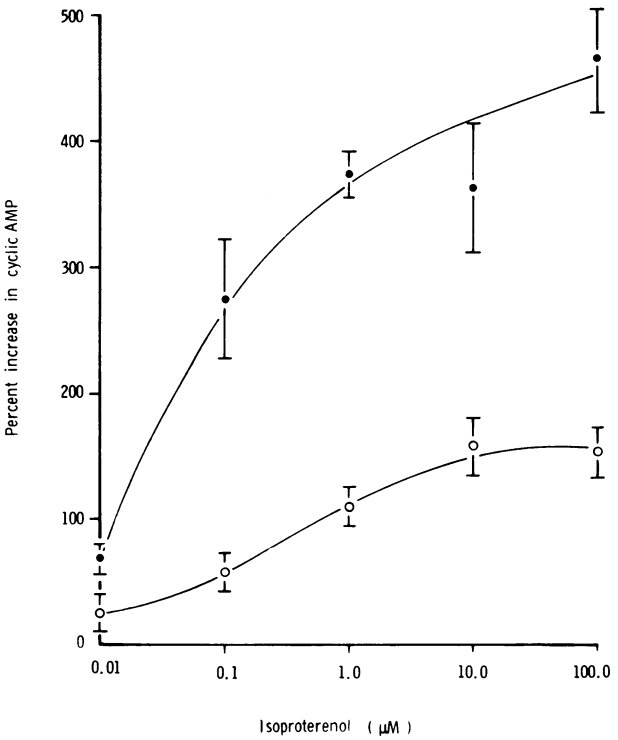

FIGURE 5 Percent increase in cyclic AMP in lymphocytes taken from four obstetric patients before (● —— ●) and after (○ —— ○) a 48-h infusion of isoxsuprine (5 mg/h). Incubation conditions were as described for Fig. 1.

observing such a difference have attributed it either to the disease per se, or to an inherent defect regarded as an etiological factor underlying the asthma. The present data, derived from comparable asthmatics receiving two different forms of treatment, are not compatible with such interpretations, but can be explained as a consequence of excessive exposure to β-adrenergic bronchodilators. Of particular relevance are the observations made in the five subjects in whom therapy was changed from adrenergic to nonadrenergic medication. It was found that their initially depressed lymphocyte response reverted to normal values with this change. There was, however, no consistent relationship between the severity of their asthma and the lymphocyte response during this period.

The infusion studies in the obstetric patients, which extend observations previously made in man and animals (13–15, 21–27) indicate clearly that prolonged exposure of normal subjects to β-agonists leads to a reduced response, whether measured in terms of cyclic AMP levels, or as the final physiologic consequence of β-receptor stimulation. The marked depression seen in the obstetric patients would suggest that asthmatic patients and normal subjects are not inherently different in this respect.

Possible contributions to these results attributable to characteristics of the different groups need to be considered. The average age of the control group is somewhat lower than that of the other two, although there is no significant difference between the ages of the two asthmatic groups. However, no age-related decline in response could be discerned within any of the individual groups, and it seems unlikely that the difference in age between the groups could have made an important contribution to our observations.

The possible mechanisms underlying the reduced responsiveness to β-adrenergic stimulants are of interest in their own right. Several steps lie between the exposure of lymphocytes to isoproterenol and the increase in cyclic AMP measured after a timed incubation. Depletion of cell surface receptors, described in the rat pineal gland by Romero et al. (28) and in frog erythrocytes by Mukherjee et al. (29), is a possible explanation and is currently being investigated in our laboratory. Other mechanisms require consideration, however, and may prove to be of theoretical and therapeutic importance. For instance, Rodbell et al. (30, 31) have shown that various nucleotides appear to play an obligatory role in the hormonal activation of adenylate cyclase, and the possibility that this mechanism may be disordered, or that it may provide a means of correcting the observed desensitization, requires investigation.

Since the response being measured (increase in cyclic AMP) is the net result of two opposing reactions, namely formation by adenylate cyclase and degradation by phosphodiesterase (PDE), increase in PDE activity is another mechanism to be considered. Oleshansky and Neff (32) have shown an increase in PDE activity in rat pineal glands incubated with isoproterenol, and Minneman and Iversen (33) have found diurnal fluctuations in rat pineal PDE activity which relate to known changes in adrenergic tone. Other workers have found PDE activity to increase in mouse fibroblasts cultured with isoproterenol and prostaglandin E_1 (34, 35). Franklin and Foster (36) were unable to confirm this in human fibroblasts cultured with isoproterenol or prostaglandin E_1 for several hours. We have examined human lymphocytes cultured for 24 h with isoproterenol (1 μM) and have observed no consistent change in PDE activity (unpublished data). Furthermore, all the present studies were performed with theophylline at a concentration (10 mM) shown to reduce intracellular PDE activity markedly (37), although complete inhibition cannot be assumed. Finally, there was no statistically significant difference between the basal levels of cyclic AMP in normal subjects and those in either of the two asthmatic groups. For all these reasons it seems unlikely that an increase in PDE activity could have made a sub-

stantial contribution to the changes reported here, although a limited contribution cannot be excluded.

The use of the lymphocyte as a model for studying bronchial asthma requires critical examination. Its constant accessibility regardless of the patients' condition permits clinical studies that would otherwise be impossible. The postulated relationship to mast cells mentioned above lends further justification to its use. However, its limitations must be recognized. Firstly, there is considerable variability both within and between subjects. Other workers have also observed this, and Smith and Parker (6) have suggested that this might be due to variations in thymus (T)- and bone marrow-derived (B) lymphocytes in the samples studied. In other mammals, considerable differences in the response to catecholamines have been noted in the various subsets of the lymphocyte population (38). It is known that separation of lymphocytes by density gradient centrifugation may lead to variable and unpredictable losses of T lymphocytes.[5] This may explain some of the variability that we observed. To a large extent the problem can be overcome by analyzing the data as proportional increases, since cell population with low basal levels of cyclic AMP reach low maxima and vice versa. Nevertheless, the variability encountered robs the method of precision. Secondly, even when it might be expected, there is not a good correlation between respiratory function tests and lymphocyte performance. Clearly, in a multifactorial disease like asthma, perfect correlation will not be obtained, but it also has to be recognized that even in tissues which all possess receptors currently classified as β_2 in type, the susceptibility to desensitization appears to vary (39, 40), and in this respect bronchial smooth muscle and lymphocytes clearly do not correlate well.

The importance of drug-induced adrenergic desensitization in severe asthma remains uncertain. However, the adverse effects of β-blocking drugs, such as propranolol, in some asthmatics are well known, and it is conceivable that drug-induced desensitization could create an analogous situation in asthmatics abusing adrenergic bronchodilators. It is possible that this could have contributed to the rise in asthma mortality linked to the excessive use of aerosols of such preparations (41, 42).

The lymphocyte may provide a useful tool for studying cellular mechanisms in man, and in the evaluation of various pharmacologic interventions. Viewed in this way it has a place in the study of bronchial asthma, although its limitations restrict its usefulness in studying single individuals.

[5] Thompson, A., and S. Vaughan-Smith. 1975. Personal communication.

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