ADRENOCEPTOR RESPONSIVENESS IN BRONCHIAL ASTHMA: EFFECT OF ISOPRENALINE INHALATION ON PLASMA CYCLIC 3',5'-AMP LEVELS

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- 1 The effect of isoprenaline inhalation on plasma levels of cyclic AMP has been studied in a group of normal subjects and compared to a group of asthmatics.
- 2 The normal subjects had a prompt rise in their plasma cyclic AMP level, and when compared to the asthmatic group, had a significantly greater percentage rise in plasma cyclic AMP from the basal levels.
- 3 The markedly attenuated response to isoprenaline inhalation in the asthmatic group is consistent with the concept that asthmatics have a relative impairment of β -adrenoceptor function.
- 4 These results support the *in vitro* studies demonstrating that lymphocytes from asthmatic subjects incubated with isoprenaline produce less cyclic AMP than those from normal subjects.

Introduction

A number of studies have suggested that asthmatic subjects have a diminished physiological and metabolic responsiveness to adrenergic agents. The subject has recently been extensively reviewed by Parker (1973). In particular, asthmatic subjects have been postulated to have an impaired bronchodilator response to β -adrenoceptor agonists, possibly due to a relative impairment of β -adrenoceptor function (Szentivanyi, 1968). This would result in a diminished degree of adenylyl cyclase activation such that, following stimulation, the expected rise in cyclic AMP production would be attenuated. Bronchodilation is thought to be related to a rise in intracellular cyclic AMP concentration following β -adrenoceptor stimulation (Kaliner, Orange, Koopman, Austen & Laraia, 1971; Mathé, Åström & Persson, 1971). The stabilizing effect of C'AMP on the release of mediators such as histamine from mast cells (Lichtenstein & Margolis, 1968) is probably also of major importance in the effective treatment of an asthmatic attack.

The presence of altered responsiveness has been demonstrated *in vitro* by a number of workers including Parker & Smith (1973), Logsdon, Middleton & Coffey (1972), and Gillespie, Valentine & Lichtenstein (1974). However, there has been little published on the *in vivo* production

of cyclic AMP in asthmatic subjects following the administration of sympathomimetic agents, although Bernstein, Linarelli, Facktor, Friday, Drash & Fireman (1972) reported an attenuated rise in the urinary excretion of cyclic AMP following the subcutaneous injection of adrenaline in a group of eight asthmatic children. Urinary cyclic AMP excretion has been widely studied, particularly in relation to endocrine diseases such as hyperparathyroidism. However, studies on plasma cyclic AMP concentrations have been considerably fewer, probably due to the greater technical difficulties in measuring plasma concentrations, which are approximately 1% of the urine concentration. We report the results of studies on the effect of isoprenaline inhalation on plasma cyclic AMP levels in a group of asthmatic subjects compared to a group of control subjects.

Methods

Ten patients under treatment for asthma agreed to participate in this study. A summary of their clinical details is given in Table 1. All had symptoms of asthma at the time of the study. Skin tests had been performed on eight patients, and

seven had positive immediate responses to two or more allergens. Ten healthy volunteers were also studied, and consisted of eight males and two females, mean age 28.5 years (range 22-35 years). None of the volunteers was on medication. The asthmatics continued on their usual medication, except for their inhaled sympathomimetic bronchodilator treatment, which was suspended for at least 6 h prior to commencement of the study. During the study period, subjects were resting either semi-recumbent or sitting. Electrocardiograph and heart rate were monitored on a Hewlett Packard 7700 Recorder. The protocol was approved by the Board of Medical Research of the Royal Melbourne Hospital.

At time zero, isoprenaline in a metered aerosol (Medihaler Iso Forte, Riker Laboratories) was administered in two puffs, 20 s apart. Each puff is claimed by the manufacturer to deliver 400 µg of isoprenaline. The inhaler was used by a technique similar to that described by Connolly (1975): the aerosol was released 4 cm from the open mouth at the commencement of a full inspiratory manoeuvre. Blood samples were collected from a cannulated cubital vein at intervals during the 10 min prior to administration of the aerosol, and during the 30 min afterwards. The samples were kept in an ice-water bath in tubes containing EDTA until centrifugation, which was usually within minutes of collection. Previous experiments have demonstrated that in the presence of 6 mM EDTA the samples could be left at ambient

temperature for up to 1 h without a significant alteration in cyclic AMP content (Murad, 1973). The plasma obtained was stored at -20°C until assayed. Plasma cyclic AMP levels were determined by the protein-binding radio-assay technique of Brown, Albano, Ekins & Sgherzi (1971). Individual standard curves were prepared for each patient, using the patient's own plasma made free of cyclic AMP by allowing a sample of heparinized blood to stand overnight at ambient temperature.

In all ten controls, and in the last six asthmatics studied, peak expiratory flow rate (PEFR; litres/min) was measured using a Wright peak flow meter (Airmed, Harlow). The PEFR measurements obtained were then expressed as a percentage of the predicted, based on a regression equation incorporating the patient's age and height (Ferris, Anderson & Zickmantel, 1965). At least two sets of measurements were recorded in the 10 min prior to administration of isoprenaline, and at least three further sets of measurements were made in the subsequent 5 to 30 minutes. Each set consisted of three determinations of PEFR, the highest of which was recorded.

Results

The mean basal PEFR in the six asthmatics was $48.1 \pm 6.6\%$ of predicted, and the mean PEFR following isoprenaline was $61.7 \pm 6.3\%$ of

Table 1 Details of the ten subjects with asthma

Patient	Sex	Age (years)	Length of history (years)	Inpatient or outpatient	Current treatment
H.Sm.	F	28	1	OP	Isoprenaline aerosol
B.J.	M	21	18	IP	Salbutamol aerosol, ephedrine, prednisolone 5mg/day
J.Br.	M	19	16	OP	Salbutamol, Quibron
R.C.	M	24	21	OP	Salbutamol aerosol
A.H.	F	21	20	IP	Terbutaline (oral and s.c.), theophylline, prednisolone (15 mg/day), amoxycillin
R.Z.	M	19	11	OP	Salbutamol aerosol, Quibron
I.D.	M	32	12	IP	Salbutamol aerosol, Quibron, cromoglycate, prednisolone (15 mg/day)
J.Be.	M	24	22	OP	Salbutamol aerosol, Quibron, Amesec
R.S.	M	45	26	IP	Salbutamol aerosol, Tedral
H.St.	M	30	28	IP	Salbutamol aerosol, Quibron
Mean		26.3	17.5		

Quibron contains guaiphenesin (90 mg) and theophylline (150 mg) per capsule; Amesec contains aminophylline (130 mg), ephedrine HC1 (25 mg), and amylobarbitone (25 mg) per capsule; Tedral contains theophylline (130 mg), ephedrine HC1 (24 mg), and phenobarbitone (8 mg) per tablet.

Figure 1 Heart rate response (mean $\pm$ s.e. mean) to two puffs of isoprenaline (administered at arrow) from a metered aerosol in ten asthmatics ($\bullet$) and ten control subjects ($\circ$).

predicted (s.e. diff = 2.8% of predicted, $P < 0.005$). The mean basal PEFR in the control group was $96.3 \pm 3.63\%$ of predicted.

The resting heart rates in the normal and asthmatic subjects (Figure 1) were 71.0 ± 2.2 and 87.5 ± 5.4 beats/min respectively ($P < 0.02$). The higher resting heart rate in the asthmatic group probably reflects the cardiovascular changes accompanying bronchospasm. The peak rise in heart rate following isoprenaline was 32.5 ± 6.2 beats/min in the normals, and 16.9 ± 7.2 beats/min in the asthmatics, and occurred between 0.5 and 2 min following the inhalation of the drug. The heart rate response followed the same time course in both groups.

The plasma cyclic AMP levels in the normal and asthmatic subjects prior to inhalation of the drug were 15.5 ± 1.7 and 18.5 ± 2.3 pmol/ml respectively, and did not differ significantly from one another (Table 2). However in the normal subjects, the rise in cyclic AMP after inhalation of isoprenaline was prompt and sustained. In contrast, there

Figure 2 Plasma cyclic AMP response to inhaled isoprenaline (administered at arrow) in ten asthmatics ($\bullet$) and ten control subjects ($\circ$). Each individual's response has been expressed as a % of his own mean basal level. The mean $\pm$ s.e. mean for each group are plotted.

was little rise in plasma cyclic AMP in the asthmatic subject, although it followed the same time course. The differences in the responses of the two groups are highlighted in Figure 2 where the changes in plasma cyclic AMP concentrations are expressed as percentages of each individual's mean basal levels. The differences at 7, 12 and 20 min after inhalation are all highly significant ($P < 0.005$).

Discussion

The plasma cyclic AMP response to isoprenaline was attenuated in the asthmatic group compared

Table 2 Mean $\pm$ s.e. mean ($n = 10$) plasma cyclic AMP (pmol/ml) before and after inhalation of two puffs of isoprenaline (Medihaler Iso Forte) in asthmatic and control patients

Time (min)	Plasma cyclic AMP (pmol/ml)			
	Asthmatic Mean	s.e. mean	Control Mean	s.e. mean
-10	18.5	1.8	15.1	1.7
-5	18.2	2.4	15.7	1.8
0	Isoprenaline			
2	20.4	2.1	18.6	2.3
7	21.9	2.6	30.6	4.0
12	20.8	1.9	27.3	3.6
20	17.9	2.2	23.4	2.5
30	18.6	1.9	20.6	2.4

to the control group. This finding is consistent with the results of *in vitro* studies of Parker & Smith (1973), who demonstrated a significantly diminished cyclic AMP production in lymphocytes from asthmatic donors compared to those from normal donors following incubation with isoprenaline.

Similar *in vitro* results have also been reported by Alston, Patel & Kerr (1974) and Logsdon, Carnright, Middleton & Coffey (1973). As well as showing a diminished responsiveness of the leucocytes from asthmatic donors to produce cyclic AMP following isoprenaline incubation, they also showed that the attenuated response could be restored to the normal pattern by treatment with the α -adrenoceptor blocking agent phentolamine. This raises the possibility that the α -adrenoceptor is abnormal in asthma as well as, or instead of, the β -adrenoceptor. These *in vitro* experiments presumably describe responses to stimulation of the post-junctional receptors on the effector cell, but the *in vivo* situation is even more complex due to the modulation of release of catecholamines from sympathetic nerves by pre-junctional α -adrenergic receptors (Rand, McCulloch & Story, 1975).

While there was a clear difference in the response between the normal and asthmatic groups, there was considerable variation in the response within each group. The variation was of similar magnitude in both groups, which suggests that it was not a major determinant of the observed group differences in the response pattern. It was assumed that part of this variation was attributable to differing amounts of inhaled isoprenaline reaching the lower respiratory tract. However, Parker & Smith (1973) have also noted considerable within-group variation in their *in vitro* studies, where drug delivery to the cells is not a problem.

The diminished plasma cyclic AMP response to isoprenaline in the asthmatics may be related to bronchospasm causing a reduction in the dose delivered to the lungs. Such an effect would introduce a systematic bias between the two groups. However, Dashe, Ponto, Ganapes, Drage & Kroneberg (1974) have shown that nebulized isoprenaline tends to be deposited particularly in the perihilar zones, and that this uneven distribu-

tion of the aerosol appears to be independent of the pretreatment distribution of ventilation. Also, there was sufficient drug delivered to the bronchi in our subjects to significantly increase PEFR. Furthermore, the similarity of heart rate response between the two groups would suggest that similar quantities of isoprenaline were passing into the circulation to exert comparable cardiovascular effects.

Another factor which could account for the differences between the groups may be an effect of previous bronchodilator medication. Such an effect might alter the responses both *in vivo* and *in vitro*. Previous *in vitro* work (Parker & Smith, 1973) has demonstrated that a 2 week course of Tedral (ephedrine HCl 24 mg, theophylline 130 mg and phenobarbitone 8 mg-1 tablet 4 times daily) did not alter the lymphocyte cyclic AMP response to isoprenaline in five healthy volunteers. By contrast, Jenne, Strickland, Chick & Wall (1975) reported that a 3 week course of terbutaline (5 mg orally 3 times a day) in a group of six patients suffering from asthma or bronchitis reduced by 70% the plasma cyclic AMP response to a further oral dose of terbutaline (5 mg). Furthermore, the cyclic AMP response to terbutaline (5 mg) orally returned to pre-treatment levels when tested 1 week after stopping continuous administration of the drug. Unfortunately, absolute plasma cyclic AMP levels were not reported in this abstract, and the mixed group of subjects makes the interpretation of the results difficult.

Thus, the differences in cyclic AMP response between the normal and asthmatic group are unlikely to be simply due to within-group variations. Whilst the effects of chronic medication and decreased drug delivery have not been entirely excluded, the current findings would support the hypothesis that the diminished cyclic AMP response in asthmatics may be related to altered adrenergic responsiveness.

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