Airway and metabolic responsiveness to intravenous salbutamol in asthma: effect of regular inhaled salbutamol

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Summary

1. Airway, metabolic and cyclic nucleotide responses to intravenous salbutamol were measured in five patients with mild asthma who had taken no medication in the week before the study. The studies were repeated after the patient had taken regular inhaled salbutamol for 4 weeks, in doses increasing to 2000 \( \mu \)g daily in week 4.

2. The pretreatment airway, metabolic and cyclic nucleotide responses to salbutamol were similar to those previously reported in normal subjects. These patients therefore did not show evidence of partial \( \beta \)-adrenoceptor blockade.

3. After 4 weeks' salbutamol therapy the airway response to intravenous salbutamol was unchanged.

4. The glucose, pyruvate and adenosine 3':5'-cyclic monophosphate (cyclic AMP) responses to intravenous salbutamol were depressed after regular salbutamol administration. The dose–response curve for non-esterified fatty acids and insulin, though displaced downwards, did not indicate an impaired response to salbutamol since the shape was unchanged. There was no significant change in the lactate, glycerol and total ketone response.

5. This study confirms that tissues differ in the ease with which they develop resistance to \( \beta \)-adrenoceptor agonists. Asthmatic airways appear to be relatively protected from developing resistance when compared with other tissues in asthmatic patients and when compared with the airways of normal subjects.

Key words: adrenergic resistance, airway resistance, asthma, cyclic AMP, adenosine 3':5'-cyclic monophosphate, salbutamol.

Abbreviations: cyclic AMP, adenosine 3':5'-cyclic monophosphate; FEV\(_1\), forced expiratory volume in 1 s; cyclic GMP, guanosine 3':5'-cyclic monophosphate.

Introduction

Patients with asthma often take \( \beta \)-adrenoceptor agonists on a regular basis. It has been suggested that patients may become tolerant or resistant to larger doses (Van Metre, 1969; Reisman, 1970) and that resistance to effects on the airways may account for some problems in the control of asthma and might be a factor in asthmatic deaths (Conolly, Davies, Dollery & George, 1971). Normal subjects develop progressive resistance in the airway response and in certain metabolic responses to salbutamol after large doses of regular inhaled salbutamol (Holgate, Baldwin & Tattersfield, 1977; Holgate, Stubbs, Wood, McCaughey, Alberti & Tattersfield, 1980). It is important to know whether similar changes occur in patients with asthma. We report the airway, metabolic and plasma cyclic nucleotide responses to intravenous salbutamol in five patients with mild asthma studied before and after increasing doses of salbutamol for 4 weeks.

Subjects and methods

Subjects

Studies were carried out on five male patients with mild asthma. Their age ranged from 23 to 28...
years, weight from 91 to 112% of ideal body weight and their forced expiratory volume in 1 s (FEV₁) from 74 to 117% of predicted. The subjects gave informed consent and the protocol was approved by the Local Ethical Committee. All patients had a history of intermittent wheezing for at least 5 years, immediate positive skin tests to at least two common allergens and a minimum 15% change in FEV₁ or peak expiratory flow rate, either spontaneously or after 100 μg of inhaled salbutamol. All had mild asthma, which had never required hospital admission nor steroid therapy and had required only an occasional salbutamol inhalation, less than one per week. None had taken any medication in the week before the study.

**Study design**

A control salbutamol dose–response study was carried out on each subject, the change in airway resistance and blood metabolites being measured after increasing doses of intravenous salbutamol. The subjects then inhaled salbutamol regularly from a pressurized metered aerosol for 4 weeks. The dose was increased each week: 100 μg four times a day in week 1, then 300 μg, 400 μg and 500 μg four times a day in weeks 2, 3 and 4 respectively. A second salbutamol dose–response study was carried out at the end of week 4, 12 h after the last dose of inhaled salbutamol.

**Salbutamol dose–response study**

After an overnight fast, the antecubital vein of each arm was catheterized with a Venflon venous catheter. Catheter patency was maintained with small injections of sodium chloride solution (9 g/l, 150 mmol/l: saline). The subject rested in a seated position for 40 min, after which baseline blood samples were taken and airway resistance was measured. The subject then received 25 μg of salbutamol sulphate in 2.5 ml of saline into the inflow cannula in the opposite arm. Preliminary studies showed that maximum bronchodilatation occurred within 5 min of an intravenous bolus of salbutamol so plethysmograph recordings, blood samples and salbutamol injections (25 μg) were carried out at 10 min intervals until a cumulative dose of 250 μg had been given. A final dose of salbutamol (50 μg) was injected before the last blood sample and airway resistance measurement.

**Measurements**

**Airway resistance.** This was measured by whole-body plethysmography (Dubois, Botelho & Comroe, 1956) and the results were expressed as specific airway conductance (sGaw) to take account of variations in the lung volume at which measurements were made. Each result was calculated from at least 12 plethysmograph tracings recorded on light-sensitive paper, coded and read blind by an independent observer.

**Metabolites.** Blood concentrations of glucose, glycerol, lactate, pyruvate, acetoacetate and 3-hydroxybutyrate were measured by automated or semi-automated enzymatic fluorimetric techniques (Price, Lloyd & Alberti, 1977; Lloyd, Burris, Smythe & Alberti, 1978). Total ketone bodies refer to the sum of the 3-hydroxybutyrate and acetoacetate concentrations. Plasma non-esterified fatty acids were estimated by a modified radiochemical 57Co binding assay (Ho & Meng, 1969). Serum insulin was measured by a double-antibody radioimmunoassay (Soeldner & Slone, 1965). Triglycerides were estimated after hydrolysis with an enzymatic fluorimetric assay for glycerol (Postle & Goodland, 1978). Plasma adenosine 3':5'-monophosphate (cyclic AMP) was measured by a competitive cyclic AMP-dependent protein kinase-binding technique (Brown, Albano, Ekins, Sgherzi & Tampion, 1971). Plasma guanosine 3':5'-cyclic monophosphate (cyclic GMP) was measured by a double-antibody radioimmunoassay (Wood & Marks, 1978).

**Statistical methods**

Mean cumulative dose–response curves were constructed for sGaw, metabolites, insulin and cyclic nucleotides with increasing doses of salbutamol. Control baseline values were compared with baseline values during week 4, by the Mann–Whitney U-test (Siegel, 1956). An analysis of variance was carried out on the airway and metabolic dose–response curves and the differences in slope and position of the paired regression lines were tested for significance. In all statistical tests probability values of < 5% were accepted as significant.

**Results**

**Control dose–response studies**

The mean baseline sGaw for the five subjects was 1·77 s⁻¹ kPa⁻¹. This increased by 50% after intravenous salbutamol to 2·65 s⁻¹ kPa⁻¹ (Fig. 1), an increase of 0.88 ± SEM 0.13 s⁻¹ kPa⁻¹ (P < 0·01).

Baseline fasting levels of all metabolites fell within the normal range for healthy young adults.
Response to intravenous salbutamol in asthma

(Foster, Alberti, Hinks, Lloyd, Postle, Smythe, Turnell & Walton, 1978). With increasing doses of intravenous salbutamol there was an increase in the levels of glucose (9%), insulin (58%), glycerol (57%), non-esterified fatty acids (70%), total ketones (11%), lactate (53%), pyruvate (28%) and lactate/pyruvate ratio (29%) (Fig. 2). Cyclic AMP levels showed a 77% rise (Fig. 3). There was no significant change in triglycerides or cyclic GMP.

Dose–response studies after regular salbutamol (Table 1)

After the subjects had taken regular inhaled salbutamol for 4 weeks, mean baseline sGaw, 1.45 s⁻¹ kPa⁻¹, had not changed significantly (P < 0.2). sGaw increased by 59% to 2.3 s⁻¹ kPa⁻¹ after 300 μg of intravenous salbutamol, an increase of 0.85 ± SEM 0.18 s⁻¹ kPa⁻¹ (P < 0.02). The whole dose–response curve was displaced downwards (P < 0.01), but the slope was unchanged (Fig. 1).

After 4 weeks’ regular salbutamol, none of the metabolites showed a significant change in baseline value. There was a reduction in the slope of the salbutamol dose–response curve for glucose and pyruvate after regular salbutamol. The dose–response curves for insulin and non-esterified fatty acids were displaced downwards and that for triglycerides upwards, though the slopes of all three were unchanged.

The cyclic AMP dose–response curve showed a reduction in slope after regular salbutamol and that for cyclic GMP showed no change.

Discussion

The main problem in studies of adrenergic resistance in asthma has been to distinguish the
effects of previous treatment from those of asthma itself. We therefore studied patients with mild asthma taking only an occasional inhalation from a salbutamol aerosol and no treatment at all in the week before the first study. Our findings are therefore unlikely to be due to previous therapy.

Salbutamol caused an increase in sGaw and all metabolites apart from triglyceride, which appears not to respond to β₂-adrenoceptor agonists. Our asthmatic patients showed similar baseline values for sGaw and metabolites, and airway and metabolic responses to intravenous salbutamol similar to those of normal subjects studied previously (Foster et al., 1978; Holgate et al., 1980). These patients therefore showed no evidence of impaired β-adrenoceptor responsiveness and the results do not support the hypothesis advanced by Szentivanyi (1968) that partial β-adrenoceptor blockade is causally related to asthma.

The present results appear to conflict with some previous studies in asthma, where impaired metabolic, plasma or lymphocyte cyclic AMP responses to β-adrenoceptor agonists were demonstrated. In many, however, the effects of previous treatment could not be distinguished from the effects of asthma (Cookson & Reed, 1963; Inoue, 1967; Fireman, Palm, Friday & Drash, 1970; Parker & Smith, 1973; Alston, Patel & Kerr, 1974; Makino, Ikemori, Kashima & Fukuda, 1977). When treatment was stopped it was usually for less than 24 h, although resistance to the effects of β-adrenoceptor agonists is known to last up to 10 days in normal

### Table 1. Normal range and significance values for differences in slope and position of dose–response curves for sGaw and metabolites between control and week 4

The position (displacement) of the dose–response curve was tested for significance only when the slope of the dose–response curve showed no significant change between control measurements and week 4.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Normal range*</th>
<th>P for differences in whole dose–response curves (control vs 4 weeks)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Slope</td>
</tr>
<tr>
<td>sGaw</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood glucose (mmol/l)</td>
<td>4.0–6.0</td>
<td>&lt;0.001 N.S.</td>
</tr>
<tr>
<td>Blood lactate (mmol/l)</td>
<td>0.44–1.07</td>
<td>N.S. N.S.</td>
</tr>
<tr>
<td>Blood pyruvate (μmol/l)</td>
<td>28–145</td>
<td>&lt;0.001 N.T.</td>
</tr>
<tr>
<td>Serum insulin (munits/l)</td>
<td>2.0–12.8</td>
<td>N.S. &lt;0.025</td>
</tr>
<tr>
<td>Plasma non esterified fatty acids (mmol/l)</td>
<td>0.19–0.78</td>
<td>N.S. &lt;0.025</td>
</tr>
<tr>
<td>Serum triglycerides (mmol/l)</td>
<td>0.54–2.4</td>
<td>N.S. &lt;0.001</td>
</tr>
<tr>
<td>Blood total ketone bodies (μmol/l)</td>
<td>22–504</td>
<td>N.S. N.S.</td>
</tr>
<tr>
<td>Blood glycerol (μmol/l)</td>
<td>28–108</td>
<td>N.S. N.S.</td>
</tr>
<tr>
<td>Plasma cyclic AMP (mmol/l)</td>
<td>8–18</td>
<td>&lt;0.001 N.T.</td>
</tr>
<tr>
<td>Plasma cyclic GMP (mmol/l)</td>
<td>2–10</td>
<td>N.S. N.S.</td>
</tr>
</tbody>
</table>

* Reference values taken from Foster et al. (1978) and Wood & Marks (1978). N.S. Not significant; N.T., not tested.
subjects (Holgate et al., 1977) and 1 week was needed for the recovery of lymphocyte β-adrenoceptors after the cessation of terbutaline therapy (Galant, Duriseti, Underwood & Insel, 1978). When treatment was discontinued for at least 5 days before patients were studied, the evidence for impaired adrenergic responsiveness was either less or absent (Lockey, Glennon & Reed, 1967; Apold & Aksnes, 1977; Kallenbach, Joffe, Zwi & Seftel, 1979).

Effects of inhaled salbutamol

There was no evidence of adrenergic resistance developing in the airways, since, after 4 weeks’ inhaled salbutamol, the acute response to intravenous salbutamol was similar to the control response. The whole dose–response curve was displaced downwards although none of the individual points including baseline was significantly different, the disparity presumably reflecting the small number of subjects studied. The displacement may therefore be due to chance or may reflect airway narrowing after regular salbutamol. Similar small reductions in peak expiratory flow rates and FEV₁₀ were noted in previous studies (Gibson, Greenacre, König, Conolly & Pride, 1978; Van Arsdel, Scaffrin, Rosenblatt, Sprenkle & Altman, 1978). If long-term salbutamol does cause airway narrowing, the mechanism is unclear, but the changes cannot be attributed to impaired β-adrenoceptor responsiveness since the acute response is clearly maintained (Fig. 1).

After regular inhaled salbutamol the glucose and pyruvate response to intravenous salbutamol was impaired, suggesting the development of adrenergic resistance. This is similar to the changes seen in normal subjects though they also showed resistance to insulin and various other metabolites (Holgate et al., 1980). In this study the dose–response curve for non-esterified fatty acids and insulin was displaced downwards after treatment and that for triglycerides upwards. These changes are more difficult to interpret, but there is no clear evidence of resistance since the slopes of the dose–response curves did not change significantly. For insulin and non-esterified fatty acids, resistance might have been detected if more subjects had been studied or larger doses of salbutamol had been used; for triglycerides, in view of the lack of response in the control period to salbutamol, the development of resistance would not be expected.

The facility with which different tissues develop resistance to β-adrenoceptor agonists has also varied in previous studies. Impairment of the glucose response to β-adrenoceptor agonists has, in general, occurred more readily than impairment of the response to other metabolites such as glycerol and non-esterified fatty acids (Lockey et al., 1967; Nelson, Raine, Branch & Kalisker, 1976). It has been particularly difficult to demonstrate the development of resistance in the airways despite doses of β-adrenoceptor agonists which have caused impaired tremor, lymphocyte cyclic AMP or metabolic responses (Larsson, Svedmyr & Thiringer, 1977; Morris, Rusnak, Selner, Barzens & Barnes, 1978). These differences are unlikely to be due to differences in drug concentrations at the β-adrenoceptor, since we assume that when salbutamol is given by inhalation, as in our study, the concentration of drug would be highest at the airway receptor. We conclude that tissues vary considerably in the ease with which they develop resistance and that it is particularly difficult to induce adrenergic resistance in airways. The results of studies in one tissue cannot necessarily be extrapolated to other tissues.

Our studies also suggest that asthmatic subjects are protected to some extent from developing resistance, since similar doses of salbutamol in normal subjects produced more widespread resistance of both metabolites and airways (Holgate et al., 1980). The difference in the airway response is probably a true difference, since the increase in sGaw in week 4 was consistent in all our subjects and differed markedly from the previous findings in normal subjects. We have also confirmed these differences by comparing the inhaled salbutamol response in a further group of normal subjects with asthmatic patients after regular inhaled salbutamol (J. E. Harvey, C. J. Baldwin & A. E. Tattersfield, unpublished work). The difference between normal subjects and asthmatic patients may, however, be a quantitative one, since retrospective evidence suggests that asthmatic patients can develop resistance after larger doses of β-adrenoceptor agonist. Both Van Metre (1969) and Reisman (1970) identified some patients taking excessive amounts of isoprenaline, who on testing showed no change in FEV₁₀ after isoprenaline. When regular isoprenaline was reduced or discontinued the patients improved clinically and their FEV₁₀ response to isoprenaline was restored.

The mechanism underlying adrenergic resistance is not clear but it appears to be due to changes in the number or availability of β-adrenoceptors. Radioligand studies have shown a large reduction in the binding of radiolabelled dihydralprenolol in the lymphocytes of patients
taking oral terbutaline for 6 days (Galant et al., 1978), and the impaired plasma cyclic AMP response to salbutamol in our study after regular treatment would be consistent with this. However, despite this impaired cyclic AMP response to salbutamol the airways and most metabolites did not show evidence of resistance. One explanation for this apparent discrepancy would be that asthmatic patients are protected in some way from the effects of a reduction in intracellular cyclic AMP. Increased levels of steroid hormones for example might have this effect, since they appear to act by increasing the cellular response to cyclic AMP (Baxter & Forsham, 1972; Goldberg, 1975) and hydrocortisone is known to restore β-adrenoceptor responsiveness in normal subjects made resistant to salbutamol (Holgate et al., 1977). There is, however, no evidence at present that patients with asthma have increased levels of intracellular steroid; plasma cortisol levels are increased in severe asthma but are normal in patients with mild asthma (Cayton & Howard, 1973).

Although the dose of salbutamol (2000 μg) in week 4 is above that generally recommended for asthma, it is a dose that is taken by some patients and exceeded by a few. Nevertheless, it is relatively unusual to find patients refractory to treatment. Our results show that resistance does not occur in the airways of asthmatic subjects after doses of salbutamol which caused metabolic resistance and in a previous study caused resistance in the airways of normal subjects. We suggest that asthmatic airways are protected to some extent from the development of β-adrenergic resistance, though the mechanism remains unclear.

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**References**


