Airway and metabolic resistance to intravenous salbutamol: a study in normal man

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Summary
1. The airway and metabolic responses to an intravenous \( \beta \)-agonist salbutamol have been investigated in normal subjects before and after chronic administration of inhaled salbutamol, 1600 \( \mu g \) daily for 2 weeks.
2. Before chronic inhalation of salbutamol there was a dose-dependent increase in specific airway conductance after intravenous salbutamol in cumulative doses from 25 to 300 \( \mu g \).
3. Measurement of concentrations of blood glucose, lactate, pyruvate, glycerol, ketone bodies, non-esterified fatty acids, insulin, plasma cyclic AMP and cyclic GMP were made after each increment of salbutamol and all showed an increase apart from cyclic GMP.
4. After chronic inhalation of salbutamol there was a decrease in the airway, metabolic and insulin response to intravenous salbutamol. The cyclic AMP response showed little change.
5. This study confirms the development of \( \beta \)-adrenergic resistance in the airways of normal subjects after large doses of inhaled salbutamol and shows that this is associated with widespread metabolic \( \beta \)-adrenergic resistance.

Key words: adrenergic resistance, airway resistance, asthma, salbutamol.

Introduction
Patients with asthma have a diminished cardiovascular, metabolic and lymphocyte adenylate cyclase response to \( \beta \)-adrenergic agonists (Cookson & Reed, 1963; Inoue, 1967; Middleton & Finke, 1968; Smith & Parker, 1970; Logsdon, Middleton & Coffey, 1972). Szentivanyi (1968) suggested that this diminished \( \beta \)-adrenoreceptor responsiveness might be related to the development of asthma. This view was later challenged when it was shown that an impaired response to \( \beta \)-adrenergic agonists (adrenergic resistance) could be produced in both normal subjects and subjects with asthma by taking large doses of these drugs (Paterson, Conolly, Davis & Dollery, 1968; Nelson, 1973; Spaulding, Nelson, Branch, Pfuetze & Wood, 1975; Conolly & Greenacre, 1976; Morris, Rusnak & Barzens, 1977; Jenne, Chick, Strickland & Wall, 1977; Nelson, Raine, Doner & Posey, 1977). However, in patients with asthma the majority of studies on airway responsiveness to \( \beta \)-agonists have failed to demonstrate the adrenergic resistance seen in lymphocyte, cardiovascular and metabolic studies after large doses of \( \beta \)-agonists. This may be due in part to the problems associated with longer term pharmacological studies in patients with asthma when small drug-induced changes may be masked by spontaneous and unpredictable variations in airway calibre.

In normal subjects we demonstrated a progressive reduction in the airway response to a \( \beta \)-adrenergic agonist, salbutamol, after chronic inhalation of the drug for 4 weeks (Holgate, Baldwin & Tattersfield, 1977). We have investigated this reduction in \( \beta \)-adrenergic responsiveness further by comparing the airway
and metabolic responses to intravenous salbutamol in normal subjects before and after regular, large doses of inhaled salbutamol were given.

**Subjects and methods**

**Subjects**

Six healthy men without asthma aged 22–30 years were studied. The subjects gave their informed consent and the protocol was approved by the local Ethical Committee. None was taking any medication before the study. Their weights ranged from 92 to 114% of ideal body weight.

**Protocol**

After fasting overnight a Venflon venous catheter was inserted into an antecubital vein of each arm and catheter patency was maintained with small injections of sodium chloride solution (9 g/l : 150 mmol/l, saline).

Subjects then rested for 40 min. Baseline blood samples were taken and measurements of airway resistance made before the subjects received 25 µg of salbutamol sulphate in 2.5 ml of saline in the inflow cannula in the opposite arm. Previous studies on four subjects had shown that maximum bronchodilatation occurs within 5 min of intravenous salbutamol. 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 50 µg of salbutamol was injected before the last blood sample was taken and measurement of airway resistance made.

**Salbutamol resistance**

After completing control dose–response studies, subjects inhaled salbutamol regularly from a specially prepared pressurized metered aerosol (400 µg/inhalation), four times daily for 2 weeks, to provide a total daily dose of 1600 µg. Airway and metabolic dose–response studies were repeated 12 h after the last regular dose of salbutamol.

**Methods**

**Airway dose–response studies**

Airway resistance was measured in a whole-body plethysmograph and results were expressed as specific airway conductance (sGaw) to take account of variations of lung volume at which measurements were made. Each measurement was made from at least 10 plethysmograph tracings, recorded on light-sensitive paper, coded and later read blind by an independent observer, to obtain a mean value of airway resistance, thoracic gas volume and sGaw.

**Measurement of metabolites**

(a) Concentrations of blood glucose, glycerol, lactate, pyruvate, acetoacetate and 3-hydroxybutyrate were measured by automated or semi-automated fluorimetric techniques based on enzyme systems requiring reduced or oxidized NAD or NADP as coenzymes (Price, Lloyd & Alberti, 1977; Lloyd, Burrin, Smythe & Alberti, 1978). Total ketone bodies refers to the sum of the 3-hydroxybutyrate and acetoacetate concentrations.

(b) Plasma non-esterified fatty acids were estimated by a modified radiochemical 5°C0-binding assay (Ho & Meng, 1969).

(c) Serum concentration of insulin was measured by a double-antibody radioimmunoassay with guinea pig anti-insulin as the first antibody and rabbit anti-(guinea pig globulin) as the second antibody (Soeldner & Slone, 1965).

(d) Plasma concentration of cyclic adenosine 3':5'-monophosphate (cyclic AMP) was measured by a competitive protein-binding technique (Brown, Albano, Ekins, Sgherzi & Tampion, 1971). Cyclic AMP-free plasma was used in standard tubes to compensate for the effect of protein on the assay.

(e) Plasma cyclic guanosine 3':5'-monophosphate (cyclic GMP) was measured by a double-antibody radioimmunoassay (Wood & Marks, 1978).

**Statistical methods**

Regular inhalations of salbutamol caused small increases in baseline sGaw, so the airway response to intravenous salbutamol was expressed as increments from control baseline values (ΔsGaw). Mean dose–response curves were constructed. Individual control sGaw values were compared with values at the same salbutamol dose after regular treatment by the non-parametric Wilcoxon's paired, signed rank test (Siegel, 1956).

Mean cumulative dose–response curves were also constructed for the metabolites, non-esterified fatty acids and cyclic nucleotides. Student's t-test for paired samples was used to compare individual values at each salbutamol
Results

Control dose–response studies

The control mean resting sGaw for the six subjects was 1.67 s⁻¹ kPa⁻¹. After intravenous salbutamol administration there was a progressive rise to 2.55 s⁻¹ kPa⁻¹ (53% increase) (Fig. 1).

Resting, fasting concentrations of all metabolites fell within the normal range for healthy young adults (Table 1) (Foster, Alberti, Hinks, Lloyd, Postle, Smythe, Turnell & Walton, 1978). Significant increments in the concentrations of blood lactate were detectable after 25 µg of salbutamol, of glucose and plasma non-esterified fatty acids after 50 µg, of blood pyruvate after 100 µg and of glycerol after 125 µg (Fig. 2). Total blood ketone bodies only showed a significant increase from baseline values after 225 µg of salbutamol. Both serum insulin and cyclic AMP concentrations increased significantly after 50 µg of salbutamol (Fig. 3). Salbutamol had no effect on plasma cyclic GMP concentrations.

There were wide differences in the magnitude of the response achieved with each metabolite. After 300 µg of salbutamol the greatest response was seen with glycerol (81%), total ketone bodies (75%) and lactate (70%), and the least response with glucose, pyruvate and non-esterified fatty acids (17, 27 and 30% respectively). The lactate/pyruvate ratio increased linearly with the dose of intravenous salbutamol (r = 0.927). With 300 µg of salbutamol, the mean concentration of serum insulin had risen by 60% and of cyclic AMP by 100%.

Dose–response studies after regular administration of salbutamol

After the subjects had taken inhaled salbutamol regularly for 2 weeks, there was a marked attenuation of the airway response to intravenous salbutamol, P < 0.001 (Fig. 1). After 300 µg of intravenous salbutamol the mean increase in sGaw was 20% of the control value (P < 0.05). The results were significantly different from the control response at all salbutamol doses between 50 and 300 µg.

![Graph showing change in sGaw after intravenous salbutamol before (O--O) and after (●——●) 2 weeks regular inhalation of salbutamol. The doses shown are cumulative and each point represents the mean change in sGaw from control baseline for six subjects (+1 SEM). *P < 0.05.](image)

Although there was no change in the basal circulating concentrations of metabolites, non-esterified fatty acids and insulin after regular salbutamol inhalation, all showed a diminished response to intravenous salbutamol (Figs. 2, 3) and a significant downward displacement of the dose–response curve (Table 1). After 300 µg of salbutamol significant differences between the control response and that after regular administration were found for lactate, pyruvate, glycerol, non-esterified fatty acids and insulin.

The plasma cyclic AMP dose–response curve to intravenous salbutamol was displaced downwards (P < 0.01) although the response to
intravenous salbutamol was maintained (Fig. 3). The only value to differ significantly from the control was at the 300 µg dose ($P < 0.005$). Plasma concentration of cyclic GMP showed no significant change with intravenous salbutamol, before or after regular administration of salbutamol.

**Discussion**

A previous study has shown that the airway response to inhaled salbutamol is progressively reduced when normal subjects take increasing doses of inhaled salbutamol regularly for 4 weeks (Holgate *et al.*, 1977). We have now shown a similar reduction in the airway response to intravenous salbutamol in normal subjects after inhalation of large doses of salbutamol for only 2 weeks, and this is associated with an impaired metabolic response.

Adrenergic drugs administered by aerosol are said to have few systemic effects because the drug concentrations achieved in the circulation are low (Paterson & Shenfield, 1974), though large doses can cause tremor, restlessness and anxiety (Svedmyr, Larsson & Thiringer, 1976; Larsson, 1977; Edwards & Holgate, 1979). Salbutamol by oral or intravenous administration has profound effects on carbohydrate and lipid metabolisms (Riley, 1978) and moderate doses of inhaled salbutamol (500 µg) or isoprenaline (800 µg) increase plasma concentrations of cyclic AMP.

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**Fig. 2.** Change in serum concentrations of intermediary metabolites after intravenous salbutamol before (○---○) and after (●---●) 2 weeks regular inhalation of salbutamol. The doses shown are cumulative and each point represents the mean increment from control resting values for six subjects (+ 1 SEM). *P < 0.05.*
Resistance to intravenous salbutamol

22
20
18
16
14
12
10
0

Cyclic AMP

Salbutamol (pg)

25 50 100 200 300

Inhaled in the present study we found no significant change in resting airway resistance or blood metabolites after subjects had inhaled large doses of salbutamol for 2 weeks. There was, however, a marked attenuation of both airway and metabolite responses to intravenous salbutamol. This suggests widespread impairment of β-adrenergic responsiveness. A number of studies have shown an impaired metabolic response to adrenergic challenge after chronic administration of oral β-adrenergic drugs (Nelson, 1973; Spaulding et al., 1975) and the present study shows similar changes after inhalation. A loss of the lymphocyte cyclic AMP response to isoprenaline has previously been demonstrated in normal subjects and subjects with asthma after inhaling 3000 µg of salbutamol daily for 8–10 days (Greenacre & Conolly, 1978).

The mechanism underlying the development of adrenergic resistance is uncertain. β-Adrenergic agonists increase mucous secretion in animals (Gallagher, Kent, Passatore, Phipps & Richardson, 1975) and mucosal ciliary activity in man (Yeates, Aspin, Levison, Jones & Bryan, 1975). Impaired access of salbutamol to receptors could provide a mechanism for resistance to inhaled salbutamol but could not account for resistance to intravenous salbutamol. A decrease in local bronchial drug concentrations is also unlikely since salbutamol is not metabolized by catechol O-methyltransferase or monoamine oxidase (Nilsson, 1974) and unlike isoprenaline it does not compete for neuronal and extraneuronal tissue uptake (Levy, 1972). A compensatory increase in bronchoconstrictor influences mediated by α-adrenergic or vagal stimulation (Fleish, Maling & Brodie, 1970; Alston, Patel & Kerr, 1974) would be expected to cause a decrease in baseline sGaw and possibly an increase in basal plasma concentrations of cyclic GMP (Ball, Kaminsky, Hardman, Broadus, Sutherland & Liddle, 1972; Honma & Ui, 1978), neither of which occurred in this study. One explanation for the flattened dose-response pattern is non-competitive antagonism. This has been observed in isolated fat-cells where repeated noradrenaline stimulation causes an increase both in intracellular cyclic AMP and an inhibitory factor, adenosine, which decreases the subsequent cellular response to cyclic AMP (Manganiello, Murad & Vaughan, 1971). A similar mechanism could account for diminished airway and metabolic responsiveness to salbutamol in the presence of a nearly normal response of plasma cyclic AMP. Most of the cyclic AMP measured in plasma is the result of cell leakage and represents 1% or less of the concentrations measured in solid tissues (Broadus, Hardman, Kaminsky, Ball, Sutherland & Liddle, 1971). Thus the response of plasma cyclic AMP to salbutamol in resistant subjects may be a poor reflection of intracellular changes.

Finally, a direct decrease in the number of functional β-adrenoreceptors should be con-

Fig. 3. Change in concentrations of serum insulin and plasma cyclic nucleotides after intravenous salbutamol before (O—O) and after (●—●) 2 weeks regular inhalation of salbutamol. The doses shown are cumulative and each point represents the mean increase from control resting values for six subjects (+ 1 SEM). *P < 0.05.
considered. A diminished cyclic AMP response to 
β-adrenergic agonists has been clearly dem-
onstrated after prolonged high-dose exposure to 
isoprenaline, both in tissue culture (Makman, 
1971; Franklin & Foster, 1973; Remold-
O'Donnell, 1974) and in tissues obtained from 
the intact animal (Kebabien, Zatz, Romero & 
Axelrod, 1975; Mukherjee, Caron & Lefkowitz, 
1975). Further studies with radioactive ligand-
labelling of β-adrenergoreceptors have shown that 
diminished isoprenaline responsiveness is 
associated with a proportional decrease in the 
numbers of ligand binding sites (Mickey, Tate 
& Lefkowitz, 1975). A similar mechanism might be 
involved in the production of salbutamol resis-
tance, and would be supported by the shape of the 
dose–response curves in resistant subjects. It 
would also fit with our previous findings that 
hydrocortisone will restore responsiveness to 
β-agonists (Holgate et al., 1977), possibly by 
induction of a cyclic AMP-dependent protein 
kinease or by increasing the density of functionally 
active β-adrenergoreceptors (Fuller, Byus & Russell, 
1978; Mano, Abbarzadeh, Koesnadi, Sano, 
Bewtra & Townley, 1979).

Whatever the precise mechanisms involved, 
this study has shown widespread impairment of 
β-adrenergoreceptor responsiveness in normal sub-
jects after chronic administration of inhaled 
salbutamol. It remains to be shown whether patients with asthma behave in a similar way.

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