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The effects of chronic inhalation of salbutamol on the acute airway responsiveness to salbutamol and ipratropium bromide in the conscious and the anaesthetized guinea-pig

P. V. WOODSFORD[1], D. R. TOMLINSON[2]*, M. J. WARD[2], JULIE P. PALMER[1] AND T. R. POOLE[1]

[1]*Department of Surgery (Anaesthesia) and* [2]*Department of Physiology and Pharmacology, Medical School, Queen's Medical Centre, Clifton Boulevard, Nottingham, UK*

The effects of chronic exposure to a nebulized mist of salbutamol on the capacity of systemic salbutamol to prolong the time taken for inhaled histamine to produce bronchospasm in guinea-pigs have been examined. Initially a reproducible cough time to inhalation of histamine acid phosphate (0·5 ml ml^{-1}) in 100% O_2 was established. Antagonism of this response by intraperitoneal salbutamol or ipratropium Br was assessed to establish submaximal responses to these drugs. A fresh group of animals was then exposed to a persistent mist of nebulized water for 16 days, before and during which each animal was tested by exposure to histamine mist either alone or shortly after salbutamol (10 μg kg^{-1} i.p.) or ipratropium Br (5 μg kg^{-1} i.p.). The nebulized water had no effect on the response to the drugs. The same animals were rested for 7 days and then exposed to nebulized salbutamol solution (5 mg ml^{-1}) for 15 days, during which time tachyphylaxis developed to salbutamol (i.p.) but not to ipratropium Br. At the end of the 15 days the animals were anaesthetized and total lung resistance (RL) measured. At this time, the protective effect of intravenous salbutamol was also diminished by comparison with untreated guinea-pigs while the response to ipratropium Br was unaffected. A separate group exposed to 1 mg ml^{-1} of nebulized salbutamol for 20 days developed selective tachyphylaxis to intraperitoneal salbutamol. The animals were then allowed to breathe room air and the reponse to intraperitoneal salbutamol after 13 days returned to normal as did the effects of intravenous salbutamol on the RL response to histamine.

Asthmatic patients develop tachyphylaxis to β_2-adrenoceptor agonists (Jenne et al 1975) and in the lungs of experimental animals chronic stimulation of β_2-adrenoceptors reduces the functional response to stimualtion in-vitro (Benoy et al 1975), reduces the responsiveness of lung adenylate cyclase (Elfellah et al 1976) and down-regulates β-adrenoceptor density (Mukherjee et al 1975). However, the pulmonary effects of β_2-adrenoceptor stimulation may be more resistant to tachyphylaxis development than those in other tissues (Holmberg et al 1981; Harvey et al 1981). Furthermore, recent clinical studies indicate some disagreement as to whether tachyphylaxis to β_2-adrenoceptor agonists develops a (see review by Jenne 1982). None of the studies on animals used inhalation as a means of chronic stimulation of pulmonary β_2-adrenoceptors. We have therefore examined the development and reversal of tachyphylaxis to salbutamol in animals exposed chronically to a mist of nebulized drug. The selectivity of the tachyphylaxis was studied by examining responses of salbutamol-tolerant guinea-pigs to the muscarinic blocking drug ipratropium Br (Engelhardt & Klupp 1975).

* Correspondence.

MATERIALS AND METHODS

Male Duncan Hartley guinea-pigs, initial weight 450 to 550 g were used. The responsiveness to acute salbutamol or ipratropium Br was assessed in conscious animals by measurement of the increase in time taken for nebulized histamine to provoke bronchospasm (Herxheimer 1955). The animals were pretreated with a single intraperitoneal dose of salbutamol or ipratropium Br as detailed later. When the animals had developed full tachyphylaxis or, in other experiments, recovered therefrom, they were anaesthetized and measurements made of airway resistance and the responsiveness to the same drugs given intravenously in order to correlate the effects on histamine cough time with those on airway resistance.

Histamine-induced bronchospasm test

The method used was based on that of Herxheimer (1955). Single guinea-pigs were placed in a wedge-

shaped purpose-built airtight Perspex chamber (Fig. 1) that permitted the animal to maintain normal posture without being able to turn around. A small plastic nebulizer (C. R. Bard International Ltd, Sunderland, UK) was fitted to a port at the rostral end of the chamber, the lid of which was fitted with a single 'quick release' catch to facilitate rapid removal.

Initially each animal was placed in the chamber for a few minutes each day and distilled water was nebulized using 100% oxygen. Bronchospasm was then induced with an aqueous solution of 0·5 mg ml^{-1} histamine acid phosphate, a concentration found to give a latency of about 1 m from onset of histamine nebulization to attainment of bronchospasm. This timed period is referred to as the histamine cough time (HCT). The onset of bronchospasm was signalled by a characteristic paroxysmal cough which was a well-defined and reproducible end point. The animal was then placed in a second Perspex chamber flushed continuously with 50% oxygen in nitrogen to permit recovery without hypoxia.

Chronic exposure to inhaled salbutamol

For certain periods the guinea-pigs were housed in a chamber (Fig. 2) constructed from a polyethylene animal holding box (1·0 × 0·5 × 0·2 m) fitted with a sealing Perspex lid incorporating 2 × 500 ml water bottles and a feeding hopper as well as a ventilation stack fitted with a gauze filter to allow air exchange and to trap nebulized droplets of drug. The box was ventilated by a water powered venturi-vacuum pump so that air was constantly drawn in via the ventilation stack and removed via the water pump. This acted as a failsafe against animal asphyxia due to failure of the nebulizer (see below) and obviated contamination of the room air. Nebulization of drug or placebo solutions was by using an Inspiron Disposable Nebulizer (C. R. Bard International Ltd, Sunderland, UK) driven by an electronically controlled air-compressor. The purpose built control circuit was set to deliver nebulized solution for 20 s every 15 min throughout 24 h. The nebulizer was set at 50% dilution (i.e. drawing ambient air to dilute the driving air) and delivered nebulized solution, via a 3 cm corrugated hose, to 3 entry ports in the box lid. This distributed a mist of drug to all parts of the box.

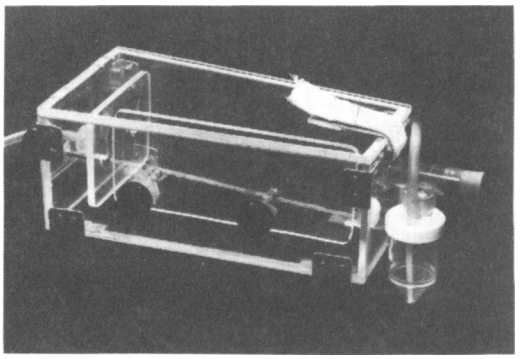

FIG. 1. Perspex box used for measuring the time taken for induction of bronchospasm by histamine.

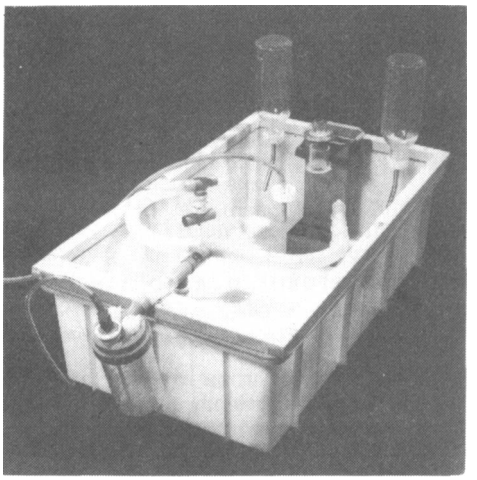

FIG. 2. The chamber for housing up to three guinea-pigs during chronic exposure to nebulized salbutamol (see text for details).

Measurement of total lung resistance (RL)

The method used was of described by Amdur & Mead (1958). Guinea-pigs were anaesthetized with a mixture of pentobarbitone Na (25 mg kg^{-1}) and α-chloralose (50 mg kg^{-1}) given intraperitoneally. A tracheal cannula was connected to a Fleisch pneumotachograph the side arms of which were connected across a differential micromanometer (DMD FC001 Furness Controls Ltd, Bexhill, England), which drove one channel of a Grass polygraph. The tracheal cannula was also connected to one side of a differential low pressure transducer (Pye Ether Ltd, Stevenage, England) the other side being open to atmosphere, to register transpulmonary pressure (TPP). The transducer drove a second channel of the Grass polygraph. A midline thoracotomy was performed to equate intrapleural pressure with atmospheric pressure and to negate the animal's ventilatory movements. The lungs were ventilated by means of a respiratory pump (C. F. Palmer Ltd, London) set to deliver a stroke volume of 4·0 ml at a

frequency of 1·0 Hz. Total lung resistance (RL) was computed from tracheal flow (F) and transpulmonary pressure (TPP) according to the formula RL = TPP/F × calibration factor (see Amdur & Mead 1958) using a purpose built electronic signal processor. RL was also displayed on the Grass polygraph.

Increases in RL were effected by administration of test doses of histamine acid phosphate (3 μg kg^{-1}) via a jugular venous cannula. Responses to salbutamol (i.v.) were effected by giving the drug 2 min before the standard histamine dose and measuring the degree of inhibition of the bronchoconstrictor effects.

Experimental organization

Four groups of guinea-pigs were studied. The first group (n = 6) was used to establish i.p. doses of salbutamol and of iptratropium Br that gave graded increases in the time taken for histamine to induce bronchospasm, thereby establishing standard doses for subsequent use. The second group (n = 6) was used to establish reproducible responses to inhaled histamine and its inhibition by salbutamol. These animals were then housed in the polyethylene chamber for 16 days whilst distilled water was nebulized (see above). During this time their response to inhaled histamine was measured every 2–3 days. They were then rested for 7 days and returned to the chamber when salbutamol respiratory solution, 5 mg ml^{-1} (Allen and Hanburys Ltd) was nebulized for a further 15 days. Again the guinea-pigs were removed every 2–3 days for testing with inhaled histamine. Three hours elapsed between removal from the chamber and exposure to histamine to allow plasma salbutamol to fall to an ineffective level. At the end of 15 days the animals' response to i.p. salbutamol was checked for comparison with the starting response. The animals were then anaesthetized for measurement of RL as described above.

The third group (n = 6) was used to examine responses to ipratropium Br as well as salbutamol and to follow the course of recovery from tachyphylaxis to salbutamol. The group was challenged with histamine alone and in combination with i.p. salbutamol and then i.p. ipratropium Br to measure initial responsiveness. The animals were then housed in the chamber for 20 days exposure to nebulized salbutamol (1 mg ml^{-1}) after which responsiveness to i.p. salbutamol and to ipratropium Br was measured. The animals were then removed, re-housed normally and studied whilst they recovered from the tachyphylaxis to salbutamol.

On completion of the studies on the second and third groups, the animals were anaesthetized and prepared for measurement of RL. The responsiveness to i.v. salbutamol was then examined and for comparison a fourth group of hitherto untreated age-matched guinea-pigs was similarly studied.

RESULTS

Dose/response curves for salbutamol and ipratropium bromide

The aim of these experiments was to verify that both drugs offered protection against histamine-induced bronchospasm and also to determine doses for each that approximated to the ED50 and that would be suitable for testing tachyphylaxis to the chronic effects of salbutamol. The results are in Fig. 3 and from them, 5·0 μg kg^{-1} ipratropium Br and 10·0 μg kg^{-1} salbutamol were selected as doses that would give the desired responses.

Development of tachyphylaxis to salbutamol in conscious animals

Guinea-pigs in the chamber and exposed to nebulized water showed neither a change in the duration of HCT without pretreatment nor an alteration in the response to i.p. salbutamol (Fig. 4a). There were no significant differences in the HCT (histamine alone) over 0 to 16 days (paired *t*). There was no significant difference between the HCT following salbutamol on day 2 and day 14 (Fig. 4a; paired *t*).

When the animals were again given salbutamol i.p. (following one week's rest after the nebulized

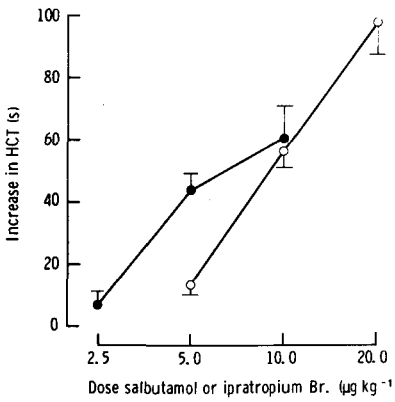

FIG. 3. Dose/response plots for the protection afforded against histamine-induced bronchospasm in guinea-pigs by i.p. salbutamol (○) and ipratropium bromide (●). Limit bars denote s.e.m.; n = 6 guinea-pigs for each point. The same animals were used for both drugs.

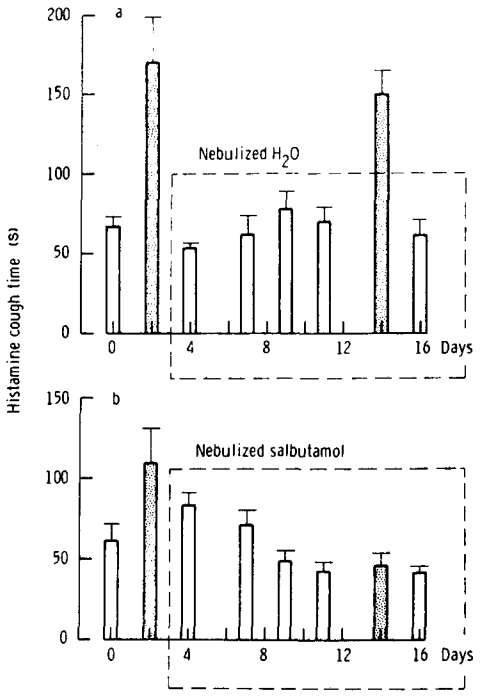

FIG. 4. The effects of housing in an atmosphere of (a) nebulized water or (b) nebulized salbutamol (indicated by the dotted boxes) on the cough time induced by histamine alone (open columns) and by histamine 30 min after i.p. salbutamol (10 μg kg^{-1}; stippled columns). The same group of animals was used for both. Limit bars denote s.e.m.

water; see day 2, Fig. 4b) the protection against histamine was not as great as had been seen earlier (see Fig. 4a). However, salbutamol still produced a significant prolongation of the HCT ($P < 0{\cdot}05$, paired t) for day 0 *vs* day 2; Fig. 4b). After placement in the atmosphere of nebulized salbutamol there was an apparent, but nonsignificant, increase in the HCT for histamine alone (day 4; Fig. 4b) followed by a progressive fall in the HCT for histamine alone which also was not significant when compared with the HCT on day 0. The animals were given salbutamol (10 μg kg^{-1} i.p.) before exposure to histamine on day 14 but this had no effect on the HCT while the response was significantly less than that obtained after salbutamol on day 2 ($P = 0{\cdot}05$, paired t; see Fig. 4b).

Selectivity of salbutamol tachyphylaxis and recovery therefrom

The third group showed similar initial responses to salbutamol (10 μg kg^{-1} i.p.) and to ipratropium Br (5 μg kg^{-1} i.p.; see Fig. 5) as seen earlier. These animals were then exposed to nebulized salbutamol at 1 mg ml^{-1} and during the initial few days this group responded similarly to the 2nd group in the HCT for histamine alone (see Fig. 5). After 1 day there was an apparent but not significant increase in HCT (day 0 or day 4 *vs* day 9; Fig. 5). Thereafter there was a progressive fall in HCT that was significantly less than at day 0 by days 16, 18 and 23 ($P < 0{\cdot}05$, paired t; Fig. 5). Twelve to 15 days after onset of nebulized salbutamol, the animals showed no alteration in the response to ipratropium Br whilst the response to salbutamol (10 μg kg^{-1} i.p.) had disappeared (day 7 *vs* day 25 – $P = 0{\cdot}02$; paired t; Fig. 5).

After 28 days the animals were removed from the box and allowed to recover. Nine days later the response to injected salbutamol showed an apparent, but non-significant increase, whilst at 13 days it had returned to pretreatment levels (42 days vs 25 days – $P = 0{\cdot}025$, paired t; see Fig. 5). Again the response to ipratropium Br was unchanged.

Effects of salbutamol on RL in anaesthetized guinea-pigs

The data for untreated controls, animals removed 12 h previously from nebulized salbutamol and animals that had recovered from tachyphylaxis to salbutamol are shown in Fig. 6. The untreated guinea-pigs showed a dose-related inhibition to the bronchoconstrictor response to histamine when given salbutamol (1 to 10 μg kg^{-1} i.v.) before the standard histamine dose. The animals that were tolerant to salbutamol, as shown by the lack of effect on the HCT, gave no response to i.v. salbutamol at doses up to 10 μg kg^{-1}. In contrast, the group housed normally for 15 days after exposure to the nebulized salbutamol gave responses to salbutamol which were virtually identical to the untreated controls, indicating full reversal of tachyphylaxis.

DISCUSSION

This study shows that chronic inhalation of salbutamol causes a development of tachyphylaxis to the effects of the drug on the airways that is manifest both in terms of a progressive diminution of its effect on the response to inhaled histamine in conscious guinea-pigs and as reduced antagonism by it towards the effect of intravenous histamine on RL in the anaesthetized guinea-pig.

Tachyphylaxis developed after the inhalation of nebulized salbutamol at 5 mg ml^{-1} and was equally profound after 1 mg ml^{-1}. The protection against histamine afforded by ipratropium bromide was unaltered by salbutamol tachyphylaxis caused by

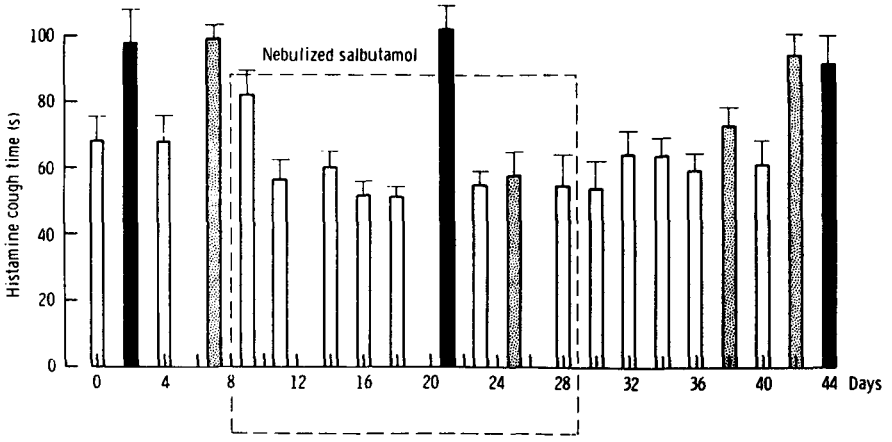

FIG. 5. The effects of housing in an atmosphere of nebulized salbutamol (dotted box) on the cough time induced by histamine alone (open columns), histamine 30 min after i.p. salbutamol (10 μg kg^{-1}; stippled columns) and histamine 30 min after ipratropium bromide 5 μg kg^{-1}; solid columns). From day 28 the animals were allowed to recover in an atmosphere free from salbutamol. Limit bars denote s.e.m.

1 mg ml^{-1} sulbutamol. This is not surprising since iprotropium bromide acts by muscarinic receptor blockade (Engelhardt & Klupp 1975) rather than β_2-adrenoceptor stimulation. The antagonism by ipratropium bromide of histamine bronchospasm seen here and blockade of elevated RL induced by histamine (Tomlinson & Ward 1982), indicates that at least part of the effect of histamine on the airways is mediated via cholinergic bronchoconstrictor or secretory mechanisms. The persistence of muscarinic blockade as a means of reversing airway obstruction in a system which has developed tachyphylaxis to β_2-adrenoceptor stimulants is of therapeutic importance. The findings of this study are in accordance with the demonstration that asthmatic patients, who may have a diminished response to β_2-adrenoceptor agonists, benefit from inhalation of ipratropium bromide (Ward et al 1981).

Our results show an enhanced response to histamine after a few days of inhalation of salbutamol. This may indicate that endogenous catecholamines exert a protective role against the bronchoconstriction induced by inhaled histamine in hitherto untreated animals. However, this has not been supported by experiments in which propranolol was given to normal guinea-pigs before histamine treatment (Ward & Tomlinson 1984). Our results also show that the tachyphylaxis to salbutamol is reversible and appears complete over about 14 days free from exposure to the drug.

Our findings must be set against the background of controversy over tachyphylaxis to β-adrenoceptor agonists in animals and especially in normal and asthmatic humans. The human studies have been reviewed by Jenne (1982). To date, the evidence indicates that inhalation of β_2-selective agonists provoke either no tachyphylaxis or less-marked tachyphylaxis than systemic administration. There are also indications that asthmatic subjects are more resistant to tachyphylaxis than normal subjects. Jenne (1982) has argued that the systemic route probably exerts a greater effect on the small airways. If these were the principal site of tachyphylaxis development, then tests of lung function that are predisposed towards particular parts of the bronchial tree might influence the detection of tachyphylaxis.

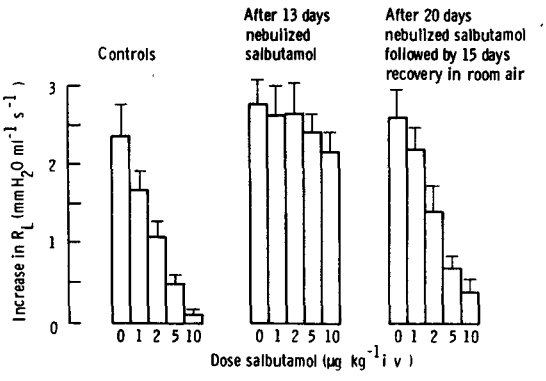

FIG. 6. Responses of 3 groups of anaesthetized guinea-pigs to i.v. histamine (3 μg kg^{-1}) alone and histamine (3 μg kg^{-1}) 2 min after a range of doses of salbutamol. Controls were hitherto untreated, animals given 13 days nebulized salbutamol were those shown in Fig. 4 and the third group were those shown in Fig. 5. Limit bars denote s.e.m.

It is also possible that persistent delivery of a nebulized mist of relatively high concentrations of drug, as are used, might stimulate all bronchial β-adrenoceptors, thereby maximizing the tachyphylaxis.

Other studies have indicated that the β-adrenoceptors of the lung are more resistant to tachyphylaxis than those of other tissues. This phenomenon has been reported both after oral administration in animals (Holmberg et al 1981) and after inhalation in asthmatics (Harvey et al 1981). Our findings offer no further information on this, but our method of generating tachyphylaxis would seem to be of use in examining tissue selectivity of tachyphylaxis after inhalation.

The findings described show only that tachyphylaxis to β_2-selective adrenoceptor agonists can be induced in guinea-pigs by persistent inhalation and that the tachyphylaxis does not extend to a muscarinic blocking drug. This does nothing to resolve the questions about the extent of tachyphylaxis and its therapeutic importance in asthamatics. However, our findings and methods offer a possible means of examining the mechanisms responsible, the tissue selectivity and the extent to which the xanthines and corticosteriods modify the development of β-adrenoceptor tachyphylaxis (see Jenne 1982).

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