

Sustained Inhibition of Antigen-Induced Histamine Release from Human Lung by the β_2 -Adrenoceptor Agonist Terbutaline

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Antigen-induced histamine release from passively sensitized human lung tissue was inhibited in the presence of the β_2 -adrenoceptor agonist, terbutaline. A sustained and statistically significant suppression was detected in the concentration interval 3×10^{-8} – 1×10^{-6} M. Fifty per cent inhibition IC_{50} , was obtained at an interpolated concentration of $5.3 \times 10^{-8} \pm 0.4 \times 10^{-8}$ M ($n = 13$), when the histamine secretion was elicited with optimum concentration of antigen. Histamine release induced with a suboptimum concentration of antigen was inhibited to a greater extent than release initiated with optimum concentration. The data in the present investigation support the concept that terbutaline-induced inhibition of mediator release from human lung tissue can contribute to the clinical effectiveness of the drug during treatment of allergic asthma.

Key words: β_2 -adrenoceptor agonists; histamine release; human lung; inhibition; terbutaline.

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CLINICAL ASPECTS

Pretreatment of passively sensitized human lung tissue *in vitro* with the β_2 -agonist terbutaline resulted in a pronounced and sustained inhibition of antigen-induced histamine release. A statistically significant suppression was induced at terbutaline concentrations obtainable *in vivo* at clinical use of the drug. The data in the present investigation support the concept that terbutaline-induced inhibition of mediator release from human lung tissue can contribute to the clinical effectiveness of the drug during treatment of allergic asthma.

Patients suffering from allergic asthma develop symptoms of the disease, such as airway smooth muscle contraction, oedema formation, increased bronchial mucosal permeability and production of tenacious mucus following exposure to relevant antigens. These pathological changes are considered to be elicited by biologically active substances secreted from sensitized mast cells and basophilic leukocytes during an immediate hypersensitivity reaction (1, 3, 4, 12, 17, 20). Induced bronchoconstriction can be counteracted by β -adrenoceptor stimulating drugs, which also have the capacity to stimulate the mucociliary transport (16, 25). Besides inducing symptomatic relief, the β -adrenoceptor agonists have the potential capa-

city to exhibit a prophylactic anti-allergic effect. Thus, it was shown in previous investigations that isoprenaline inhibits antigen-induced mediator release from human lung tissue by interacting with β -adrenoceptors (2, 19). Data have been presented suggesting that the suppression of mediator release from human tissue is mediated by β_2 -adrenoceptors (8). The purpose of this investigation was to evaluate the inhibitory effect of the selective β_2 -adrenoceptor agonist, terbutaline, on immunologically induced histamine release from human lung tissue. A pronounced and sustained inhibition of histamine release was detected at terbutaline concentrations obtainable *in vivo* at clinical use of the drug.

MATERIAL AND METHODS

Chemicals. Terbutaline sulphate; a gift from AB Draco, Sweden, was dissolved in Tyrode solution immediately before use. Other chemicals were of analytical grade from commercial sources.

Antigen. Birch pollen extract from Vitrum AB, Sweden, diluted 1:100 was dialyzed against Tyrode solution for 18 h at 4°C before use.

Passive sensitization of human lung tissue

Human lung tissue was obtained from 14 non-allergic patients undergoing cancer surgery. Only macroscopically normal areas of the lung tissue were used for these experiments. The tissue was chopped into small pieces, which were washed fourfold with calcium-magnesium free Tyrode solution (incomplete Tyrode solution). Serum from a patient allergic to birch pollen antigens was diluted (1:5) with incomplete Tyrode buffer, and 100-mg samples were incubated with 0.5 ml of diluted serum for 16–18 h at 20–22°C and finally for 1 h at 37°C. The sensitized lung tissue fragments were then washed three times with 1.5 ml incomplete Tyrode solution before suspension in complete Tyrode buffer.

Histamine release from human lung tissue

Lung tissue samples were preincubated at 37°C with buffer or terbutaline at the specified concentration in a 1.35 ml sample volume for the time intervals indicated under results. After this pretreatment, antigen in 0.15 ml Tyrode solution or vehicle was added and the reaction was allowed to proceed for another 20 min at 37°C. Unless otherwise stated the birch pollen extract was used in a final dilution of 1:10,000, a concentration which gave optimum release in preliminary experiments. In one series of experiments, histamine release was elicited both by optimum and suboptimum concentrations of antigen. Because the histamine release induced with suboptimum concentration of antigen is slower than the secretion elicited with optimum concentration, the antigen treatment in this set

of experiments was performed for 40 min at 37°C (21). After the antigen treatment period the samples were cooled in an icebath and the supernatants aspirated. All samples were run at least in triplicate. The total histamine content in each sample was calculated from the amount present in the supernatant and that extracted from the tissue fragments by boiling in 1.5 ml of Tyrode solution for 10 min. Histamine was assayed spectrophotofluorometrically as modified by Grant et al. (11). The amount of histamine release into the supernatant was expressed as

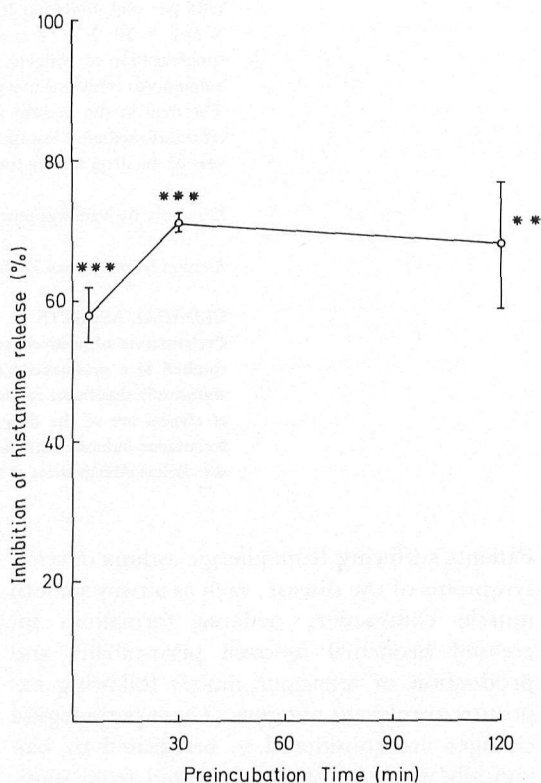


Fig. 1. Time course of terbutaline-induced inhibition of histamine release from passively sensitized human lung tissue fragments. In four separate experiments lung tissue samples were treated at 37°C with terbutaline 10^{-7} M for various time intervals before the addition of optimum amounts of antigen. Each point represents mean per cent inhibition ($\pm$ SEM) of the antigen-induced histamine release. Control histamine release in this set of experiments was $36.0 \pm 2.9\%$ (mean $\pm$ SEM).

per cent of the total content. The percentage inhibition was calculated from the following formula:

$$\left[1 - \frac{HR_D}{HR_C} \right] \times 100$$

where HR_C is per cent antigen-induced histamine release (in excess of the blank) from buffer pretreated cells. HR_D is the corresponding histamine release from cell pretreated with drug. Statistical analyses were undertaken with Student's *t*-test. The probability level of significance is indicated by asterisks in figures and tables: * $P < 0.05$; ** $P < 0.01$; and *** $P < 0.001$.

RESULTS

In the present investigation histamine release elicited with optimum concentration of antigen from buffer-pretreated samples (control release) ranged from 9.2 to 57.7%. The spontaneous release of histamine after 5 or 30 min preincubation with buffer or terbutaline varied between 2 and 6%. When the pretreatment was prolonged to 120 or 240 min the spontaneous release ranged from 4 to 13%.

Time course of terbutaline-induced inhibition of histamine release from human lung tissue

Passively sensitized human lung tissue fragments were incubated with 10^{-7} M terbutaline at 37°C for 5, 30 or 120 min before the addition of optimum amounts of antigen. Pretreatment of human lung tissue with terbutaline for 30 or 120 min resulted in approximately the same degree of inhibition. A 5-min preincubation seemed to be somewhat less effective (Fig. 1). The capacity of terbutaline to suppress histamine release after prolonged (240 min) preincubation was studied in additional experiments. A pronounced suppression was obtained in these experiments and the inhibition after a 240-min preincubation was comparable with that obtained after 30 min pretreatment (Fig. 2B and Table 1).

Concentration-effect relationship of terbutaline-induced inhibition of histamine release from human lung tissue

Passively sensitized human lung tissue fragments were incubated for 30 min at 37°C with various concentrations of terbutaline prior to the addition of optimum amounts of antigen. Five experiments were performed in which complete concentration-response curves were determined. A concentration dependent and statistically significant inhibition of the histamine release was documented in the concentration interval 3×10^{-8} M to 1×10^{-6} M, and an almost complete inhibition was obtained at 3×10^{-7} and 1×10^{-6} M (Fig. 2A). The interpolated concentration of terbutaline inducing 50% inhibition of the histamine release, IC_{50} , ranged from 2.5×10^{-8} M to 7.3×10^{-8} M with a mean ($\pm$ SEM) of $5.3 \times 10^{-8} \pm 0.4 \times 10^{-8}$ M ($n = 13$) (Table 1). The capacity of terbutaline to suppress histamine release from different lung specimens showed a rather low degree of variability (Fig. 2A and Table 1). However, in one of 14 experiments terbutaline completely failed to inhibit the histamine secretion. Also a high concentration (1×10^{-6} M) of terbutaline was without effect. The unresponsiveness persisted also when the histamine release was elicited with a suboptimum concentration of antigen (see below). Because of this complete unresponsiveness, the data from this experiment were not included in the results summarized in the upper part of Table 1.

Inhibitory effect of terbutaline on histamine release induced by different antigen concentrations

Experiments were also performed in order to compare the inhibitory effect of terbutaline on histamine secretion triggered with suboptimum or optimum concentrations of antigen. In these experiments sensitized human lung tissue fragments were pretreated with buffer or terbutaline for 30 min at 37°C before the addition of optimum amounts of antigen (final dilution 1:10,000) to one set of samples and suboptimum amounts (final dilution 1:100,000) to another. The results presented in Fig. 3 show that

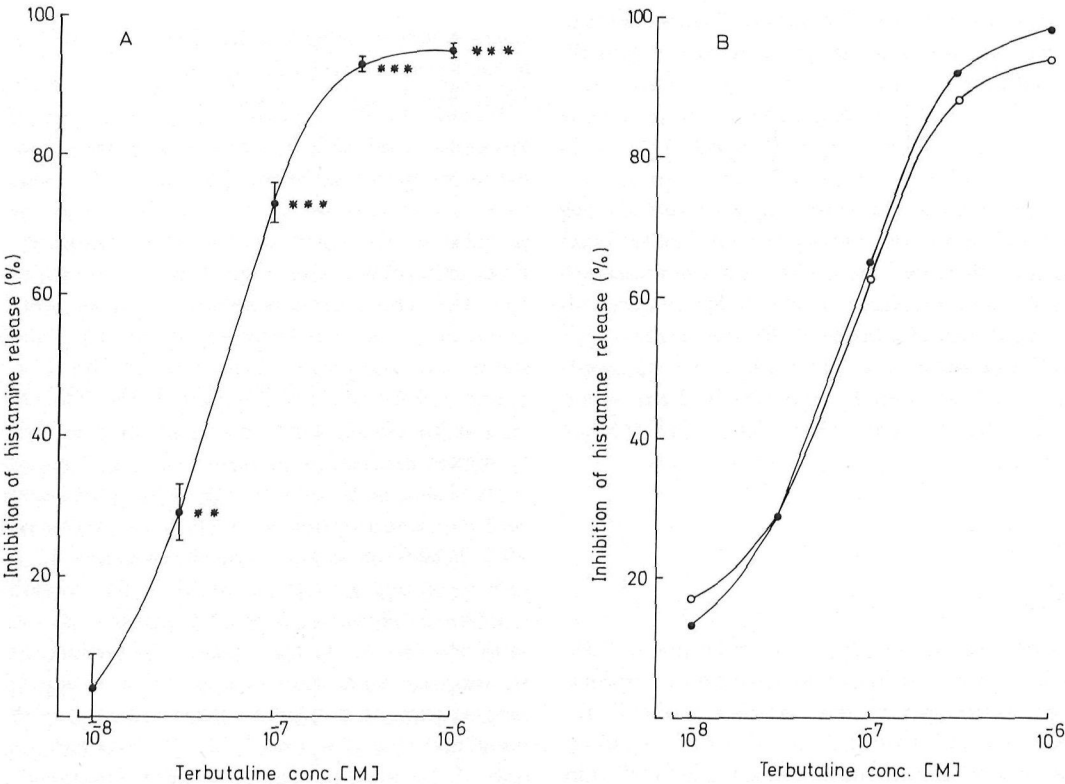


Fig. 2. A. Inhibition of antigen-induced histamine release from passively sensitized human lung tissue. Lung tissue samples were incubated with various concentrations of terbutaline at 37°C for 30 min before the addition of optimum amounts of antigen. Five experiments were performed and the points represent mean per cent inhibition ($\pm$ SEM) of the antigen-induced histamine release. The control histamine release in these experiments was $37.6 \pm 4.6\%$ (mean $\pm$ SEM).
B. Inhibition of antigen-induced histamine release from human lung. Passively sensitized lung tissue samples from one donor were incubated with various concentrations of terbutaline at 37°C for 30 min (●—●) or 240 min (○—○) before the addition of optimum amounts of antigen. The control histamine release in this experiment was 48%.

the capacity of terbutaline to inhibit the histamine liberation increased when the mediator release was decreased by the use of a suboptimum concentration of antigen.

DISCUSSION

Antigen-induced histamine release from passively sensitized human lung tissue *in vitro* was

Table 1
Inhibition of antigen-induced histamine release from passively sensitized human lung by terbutaline. Histamine release was elicited by an optimum concentration of antigen, and the control histamine release after 30 or 240 min pretreatment was 36.5 ± 3.2 (mean $\pm$ SEM) and $28.3 \pm 8.5\%$ respectively

No. experiments	Time of incubation with terbutaline before antigen challenge	Inhibition in the presence of terbutaline (%) mean $\pm$ SEM		Calculated IC ₅₀ -value (M) mean $\pm$ SEM
		3×10^{-8} M	1×10^{-7} M	
13	30 min	$30 \pm 4^{***}$	$75 \pm 3^{***}$	$5.3 \times 10^{-8} \pm 0.4 \times 10^{-8}$
4	240 min	$32 \pm 3^{**}$	$67 \pm 3^{***}$	$5.7 \times 10^{-8} \pm 0.5 \times 10^{-8}$

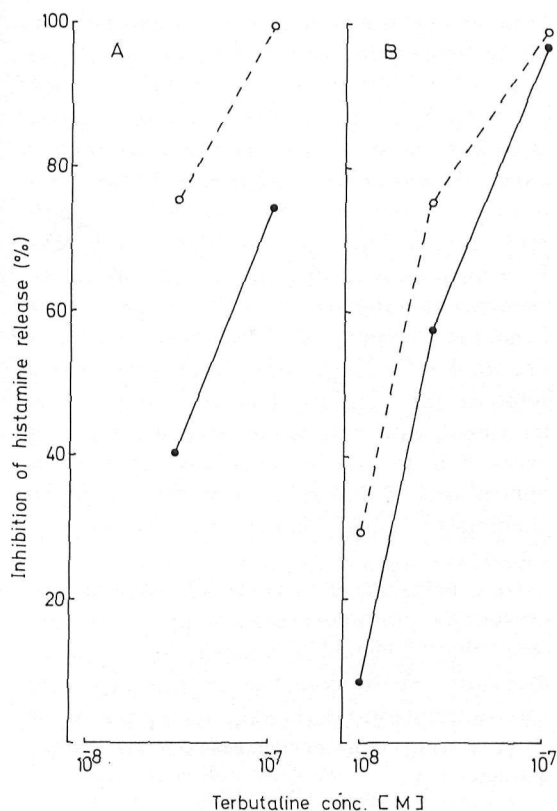


Fig. 3. Inhibitory effect of terbutaline on histamine release from passively sensitized human lung tissue elicited by optimum (●—●) or suboptimum (○—○) concentration of antigen. Before the addition of antigen, the lung tissue samples were incubated with buffer or various concentrations of terbutaline for 30 min at 37°C. Control histamine release from samples treated with optimum (antigen extract diluted 1:10,000) or suboptimum (antigen extract diluted 1:100,000) concentrations of antigen in experiment A was 50.1 and 30.0% respectively. The corresponding figures for experiment B were 57.7 and 19.0%.

inhibited in the presence of the β_2 -adrenoceptor agonist, terbutaline. Maximum inhibition was obtained if the lung tissue was treated with terbutaline for 30 min at 37°C before the addition of antigen. When histamine secretion was elicited by optimum concentration of antigen, a statistically significant suppression was detected in the concentration interval 3×10^{-8} – 1×10^{-6} M (Fig. 2A and Table 1).

Fifty per cent inhibition of antigen-induced histamine release IC_{50} , was obtained at an in-

terpolated concentration of 5.3×10^{-8} M. This value is in good agreement with data reported for two other selective β_2 -adrenoceptor agonists salbutamol ($IC_{50} = 2.2 \times 10^{-8}$ M) and fenoterol (IC_{50} between 10^{-9} and 2×10^{-8} M), but it is more than 10,000 times higher than the value reported for terbutaline by Strandberg et al. (7, 15, 24). However, in the latter study a simultaneously determined IC_{50} value for isoprenaline was about 1000 times lower than the mean IC_{50} value determined by Butcher et al. in a fairly large number of lung specimens (8). At present it is difficult to explain the extremely high potency of terbutaline and isoprenaline noted by Strandberg et al. (24).

The histamine secretion in all the experiments summarized in Fig. 2A and Table 1 was elicited by optimum concentration of antigen, and an improved inhibitory effect of terbutaline was detected, when the strength of the immunological challenge was decreased by the use of a suboptimum concentration of antigen (Fig. 3). Therefore the established concentration-response curve and IC_{50} value indicate the minimum inhibitory effect of terbutaline.

The pronounced capacity of terbutaline to suppress histamine release after prolonged preincubation for 120 or 240 min at 37°C shows that this drug does not induce a state of unresponsiveness in the mast cells (Fig. 1 and 2B, Table 1). In this respect, the effect of terbutaline differs from that of another mast cell stabilizing agent, disodium cromoglycate (DSCG). Prolonged preincubation of human mast cells with DSCG prior to the addition of antigen results in a substantial loss of inhibitory effect (10). Furthermore, DSCG-pretreated mast cells are unresponsive, tachyphylactic, to a second treatment with the same or other DSCG-like drugs (10). Also the β_2 -adrenoceptor stimulating drug, isoprenaline, has been reported to lose its suppressive effect, when the lung tissue was pretreated with the drug for 15 to 30 min before the addition of antigen (18). In this case rearrangement of the labile isoprenaline molecule to an inactive derivative during the preincubation period may cause the loss of inhibitory effect. This statement is supported by the observation that the diminished inhibitory

effect only was recognized with low or suboptimum concentrations of isoprenaline (18).

The capacity of terbutaline to inhibit antigen-induced histamine release from different lung specimens showed little variability (Fig. 2A, Table 1). However, in one of 14 tested lung preparations the mast cells were completely unresponsive to treatment with terbutaline. Lung preparations unresponsive to β_2 -adrenoceptor agonist treatment have been described earlier. Thus, Young et al. reported that the mast cells in eight out of 33 lung specimens were completely resistant to treatment with salbutamol (28). For the moment it is unclear why a minority of lung preparations do not respond to β_2 -adrenoceptor agonist treatment.

Inhibition of immunologically induced histamine secretion from human lung tissue *in vitro* was obtained at concentrations of terbutaline approximately five times lower than those reported to relax carbacholine-contracted human bronchial muscle *in vitro* (Fig. 2B, 26). When the *in vitro* histamine release was elicited by optimum concentration of antigen a sustained and statistically significant inhibition was registered down to 3×10^{-8} M, a concentration of terbutaline obtainable *in vivo* at clinical use of the drug (5, 29) (Fig. 2A and Table 1)). In recent years, it has been shown that drug-induced suppression of mediator release from human basophilic leukocytes depends not only on the nature and concentration of the drug, but also on the degree of stimulus applied to trigger the allergic reaction (6, 22, 27). Data presented in Fig. 3 show that the same relationship exists in the human lung system. Thus, histamine release elicited by suboptimum concentration of antigen was suppressed to a greater extent than the secretion induced by optimum concentration (Fig. 3). Similar results have recently been obtained with salbutamol in the dispersed human mast cell system (9). Furthermore, estimates made by March and by Adams et al. indicate that *in vivo* allergic reactions are elicited at low concentration of antigen (1, 13). Under such circumstances, it is likely that inhibition of *in vivo* mediator release can be achieved at terbutaline concentrations even lower than those found to suppress *in vitro*

histamine release from human lung tissue stimulated with optimum concentration of antigen (Fig. 2 and Table 1). That sufficiently high concentrations are available *in vivo* to suppress antigen-induced mediator release from human basophilic leukocytes and mast cells has been shown in experiments performed by Radermecker et al. and by Martin et al. (14, 23). Radermecker et al. detected a long-lasting suppression at antigen-induced histamine release from the basophils of atopic individuals after oral intake of a single dose (5 mg) of terbutaline sulphate (23). Martin et al. showed that oral treatment with terbutaline sulphate (2.5 mg every 6 h for 36 h) decreased the systemic appearance of histamine and the neutrophil chemotactic activity after *in vivo* antigen provocation (14).

In conclusion, the results obtained in the present *in vitro* experiments support the concept that terbutaline-induced inhibition of mediator release from human lung mast cells can contribute to the clinical effectiveness of the drug during long-term treatment to allergic asthma.

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