

Effect of Terbutaline on Cutaneous Responses in Man to Rechallenge with Allergen and Compound 48/80

REIDAR GRÖNNEBERG & KJELL STRANDBERG

Department of Thoracic Medicine, Section of Allergology, Karolinska Hospital, Stockholm, and Division of Clinical Pharmacology, Uppsala University Hospital, Uppsala, Sweden

Beta-adrenoceptor stimulating agents possess anti-allergic effects *in vitro* and *in vivo*. To study the site of action further 15 atopic subjects were pretreated with 1 μ g terbutaline injected intradermally (i.d.) followed by allergen challenge. Other skin sites challenged without such pretreatment served as controls. At different time intervals the skin was rechallenged with the same allergen, dissimilar allergen, or the histamine liberating agent, compound 48/80. In addition terbutaline was injected i.d. alone followed by allergen challenge at different time intervals to determine the duration of the anti-allergic effect.

Pretreatment of the skin with 1 μ g terbutaline inhibited skin reactions to subsequently injected allergen with approximately 80% (flare) and 60% (wheal) ($P < 0.001$) as compared with control. The inhibitory effect of terbutaline on the wheal and flare response to allergen was found to last up to 8 h ($P < 0.01$). Rechallenge with the same allergen or a dissimilar challenging agent resulted in a reduced skin reaction compared with control challenge ($P < 0.01$). Skin challenged with allergen in the presence of terbutaline gave a diminished response on rechallenge with the same allergen 24 h later, whereas rechallenge with an unrelated allergen or compound 48/80 produced a response similar to that of the control. The results suggest that allergen challenge in the presence (or absence) of terbutaline desensitizes the mast cells to further challenge with the same allergen and that terbutaline is capable of preventing mediator depletion of skin mast cells.

Key words: allergen; challenge; desensitization; histamine release; terbutaline.

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Previous investigations have shown terbutaline injected intradermally (i.d.) to be a potent inhibitor of flare and wheal responses to allergen in atopic skin (8, 9). In contrast terbutaline did not inhibit the flare reaction to histamine or compound 48/80 and only weakly suppressed whealing following injection of these agents. Experiments with beta-adrenoceptor stimulating compounds with differences in receptor specificity suggested that the anti-allergic effect is mediated via activation of beta₂-adrenoceptors possibly on the mast cell resulting in inhibition of mediator release (9). In addition, beta₂-adrenoceptor stimulants seem to possess an

anti-whealing effect by antagonizing permeability changes in the microvasculature (11).

The aim of the present investigation was to further study the site of action of terbutaline injected i.d. on reactions to subsequently injected allergen. The concept that terbutaline inhibits mediator release was tested by injecting the drug i.d. in atopic individuals followed by allergen challenge and rechallenge once the anti-allergic effect was resolved.

MATERIAL AND METHODS

Skin responses to allergen were studied in 15

volunteering atopic subjects, aged 20–32 years, with a history of allergic rhinitis and/or bronchial asthma, as well as a positive skin test to horse dander (15/15) and birch pollen (13/15). A RAST test to one or both of these allergens was positive in the majority of the subjects. No subject exhibited obvious dermographism or suffered from atopic dermatitis and they were not allowed any oral medication 48 h prior to day of experiment and were also instructed to avoid xanthine-containing beverages on days of skin testing. The study was approved by the Karolinska Hospital ethics committee.

The allergens and compound 48/80 were injected i.d. in doses producing approximately a flare response corresponding to that of the ED₅₀ for histamine as previously determined in each individual (8). All injection volumes were 0.02 ml. Terbutaline, histamine and saline were injected with a tuberculin syringe and allergens and compound 48/80 with a micrometer syringe with standard 20-gauge Luer type needles. The injections were given according to a randomized scheme. The resulting flare and wheal responses to all challenging agents were outlined with a marking pen on the skin 5 and 15 min after injection and traced on a transparent plastic film for later planimetric measurements.

In the first part of the experiments all reactions at the retest sites and simultaneous control reactions were read by an independent observer with no knowledge of the conditions of the experiment. The early reactions were read by one of the investigators (R.G.). This was permitted since the first injections only served the purpose of producing optimal retesting conditions.

Rechallenge with allergen

In eight subjects the back was divided into 20 sections from the upper level of the scapules to the second lumbar vertebra giving four injection sites at each level. Two skin sites were pretreated at each level with 1 µg terbutaline and 1 µg terbutaline followed 5 min later by injection of horse dander respectively. The third site was challenged with horse dander 5 min after injection of physiological saline, leaving one site at each level for later injection of control. All injection

sites were carefully outlined with a marking pen to enable later rechallenge with horse dander after a time interval of 1, 2, 4, 8 and 24 h respectively, i.e. all sites were only rechallenged once and the resulting skin reactions were compared with those of simultaneously injected control sites at each injection level.

Rechallenge with dissimilar challenging agents

In 10 subjects, including three from the first part of the study, two levels of injection sites were used with two sites each injected with 1 µg terbutaline and two sites each with saline followed by challenge with birch pollen ($n = 5$) or horse dander ($n = 5$). After a time interval of 24 h the sites were rechallenged with a dissimilar allergen at one level and the histamine liberator compound 48/80 at the other level. Comparisons of the resulting reactions were made with control reactions, i.e. the reactions of non-pretreated skin sites to the same agonist. The latter reactions were elicited in skin sections next to the sections used for rechallenge.

Terbutaline (Bricanyl, Draco, Lund, Sweden), histamine dihydrochloride, compound 48/80 (Leo, Helsingborg, Sweden) were prepared and dissolved in sterile physiological saline, containing 10% (v/v) Sörensen phosphate buffer ($\text{Na}_2\text{HPO}_4 + \text{KH}_2\text{PO}_4$, 67 mM) pH 7.4. Fresh dilutions of birch pollen allergen standardized by the RAST-technique (Pharmacia, Uppsala, Sweden) and horse dander allergen (Vitrum, Stockholm, Sweden) were prepared by the hospital pharmacy each week using physiological saline with 0.3% phenol. The same batches of allergens were used throughout the study.

Statistical analysis

Results are derived from a comparison of responses (mm^2) at retest and control sites injected at the same time and at the same injection level. Differences were analyzed by three- and four-way analysis of variance (1). In the experiments with different challenging agents responses at retest sites represent the mean of two tests. The reproducibility, expressed as mean coefficient

of variance, c.v. (%), for skin reactions to simultaneously injected allergen at *different levels* of the back was calculated from the formula

$$\frac{1}{n} \sum_{i=1}^n \frac{S_{di}}{\bar{X}_i}$$

whereas the formula for the calculation of c.v. for reactions at the *same level* was

$$\frac{1}{n} \sum_{i=1}^n \frac{\sqrt{\frac{(di)^2}{2}}}{\bar{X}_i} \quad di^2 = \left[\frac{X_1 - X_2}{\left(\frac{X_1 + X_2}{2} \right)} \right]^2$$

RESULTS

The concentrations of allergens needed to produce a flare reaction of a size equal to that produced by the ED₅₀ for histamine varied between 10⁻⁹ – 10⁻⁴ w/v (horse dander) and 10⁻⁷ – 10⁻⁴ w/v (birch pollen) due to differences in reactivity of the allergic subjects. The flare and wheal reactions (mm²) of the non-pretreated sites to horse dander were 1001 ± 27 and 60 ± 3 respectively (mean ± s.e.m., *n* = 101). Corresponding reactions elicited by injection of birch pollen were 1148 ± 44 and 64 ± 5 (*n* = 25) respectively. Compound 48/80 elicited reactions of 1173 ± 85 (flare) and 73 ± 12 (wheal) (*n* = 10).

The mean coefficient of variance, c.v., for all reactions to horse dander injected approximately simultaneously at five levels of the back of eight subjects in the absence of terbutaline pretreatment was 18.7% (flare) and 22.1% (wheal) with no significant differences in size of reactions between the levels of injection sites.

In the second part of the study the c.v. for the two reactions to birch pollen injected simultaneously at the same level in non-pretreated skin of 10 subjects was 11.8% (flare) and 19.6% (wheal). Corresponding data on reactions to horse dander were 18.4% and 32.0% respectively. All data on areas of skin reactions presented

are based on measurements 15 min after injection of the agonists.

Rechallenge with allergen

I.d. pretreatment with 1 µg terbutaline 5 min prior to injection of horse dander significantly inhibited flare and wheal responses by 92 ± 1% and 55 ± 3% respectively (mean ± s.e.m.; *n* = 38) (*P* < 0.001) as compared with control injections.

The inhibitory effect of terbutaline on horse dander allergen challenge was demonstrable also when the pretreatment period was extended to 8 h (*P* < 0.01). Figs. 1 and 2 portray the duration of the inhibitory effect of 1 µg terbutaline on skin reactions to horse dander allergen. When the skin sites were rechallenged with horse dander there was a significantly reduced response compared with control injections at all time intervals (*P* < 0.01). There was no significant difference in response to rechallenge between sites pretreated with terbutaline compared with untreated challenged sites (Figs. 1, 2).

Rechallenge with dissimilar challenging agents

I.d. pretreatment with 1 µg terbutaline 5 min prior to injection of horse dander significantly inhibited flare and wheal responses by 91 ± 2% and 54 ± 5% respectively (mean ± s.e.m.; *n* = 20) (*P* < 0.001) as compared with control injections. Corresponding data on reactions to birch pollen were 70 ± 4% and 62 ± 3% respectively (*n* = 20, *P* < 0.001).

Table 1 presents the results of experiments in which 24 h after allergen challenge and treatment as described the effect of rechallenge with the same allergen, a dissimilar allergen, or compound 48/80 was studied. The flare and wheal responses to a dissimilar allergen and the flare response to compound 48/80 were significantly reduced compared with responses at control sites (*P* < 0.01) whereas compound 48/80-elicited whealing was only slightly attenuated by the first challenge. However, skin sites pretreated with 1 µg terbutaline prior to first challenge with allergen produced almost

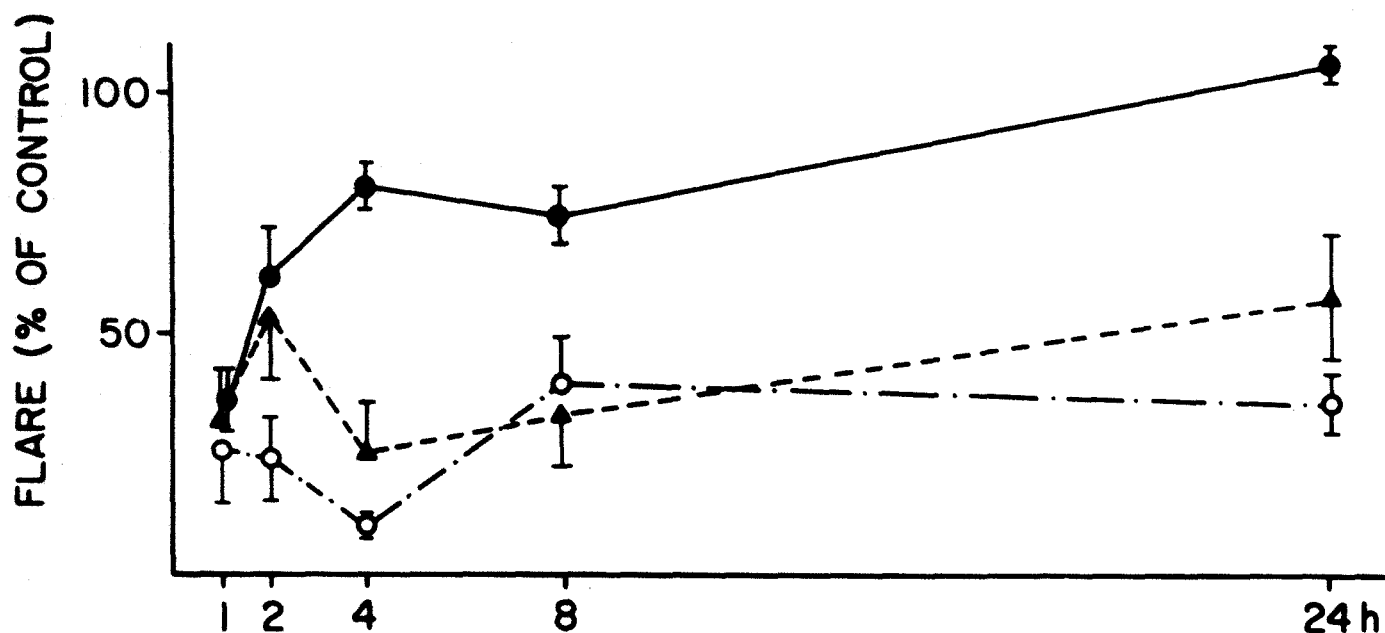


Fig. 1. Flare responses, expressed as % of control (mean $\pm$ s.e.m.; $n = 8$), of skin sites pretreated with 1 µg terbutaline (●—●—●), sites injected with 1 µg terbutaline followed by allergen (▲---▲) and sites injected with saline followed by allergen (○---○) to rechallenge with the same allergen at different time intervals.

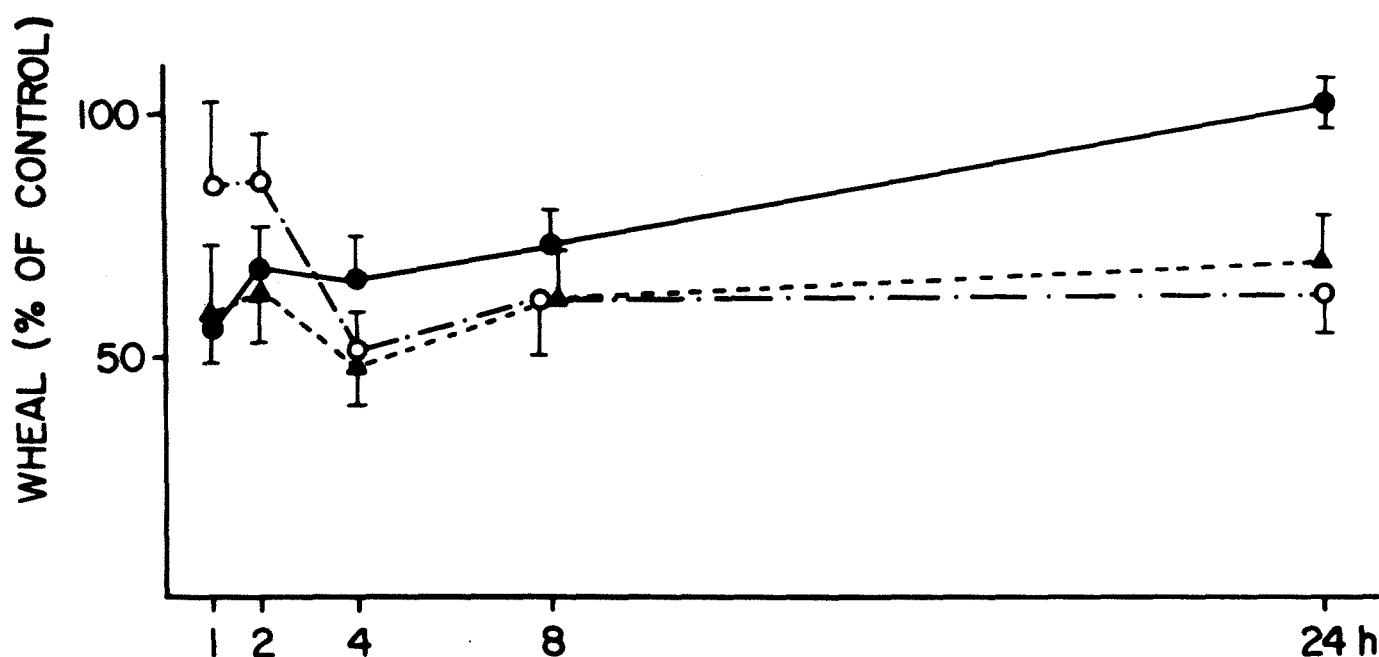


Fig. 2. Wheal responses, expressed as % of control (mean $\pm$ s.e.m.; $n = 8$), of skin sites pretreated with 1 µg terbutaline (●—●—●), sites injected with 1 µg terbutaline followed by allergen (▲---▲) and sites injected with saline followed by allergen (○---○) to rechallenge with the same allergen at different time intervals.

full responses upon injection of dissimilar challenging agents compared with controls.

There was a significant difference in response to rechallenge with dissimilar challenging agents between sites pretreated with terbutaline compared with untreated challenged sites ($P < 0.01$).

The flare response to rechallenge with a dissimilar allergen or compound 48/80 was signifi-

cantly less reduced by the first challenge than the flare response to rechallenge with the same allergen ($P < 0.01$), whereas no such difference was observed for the wheal response.

DISCUSSION

Previous investigations have shown i.d. administered terbutaline to markedly inhibit flare

Table 1

Skin responses to repeated injections of the same allergen or dissimilar challenging agents following allergen challenge in the presence of 1 μ g terbutaline (T) or saline (controls) 24 h earlier

No. of subjects	First challenge and treatment	Second challenge	Response to second challenge expressed as per cent of control at retest time (mean $\pm$ s.e.m.)	
			Wheal	Flare
8	Allergen 1	Allergen 1	63.0 $\pm$ 8.7	34.9 $\pm$ 5.6
8	Allergen 1 + T	Allergen 1	69.5 $\pm$ 10.2	56.7 $\pm$ 13.0
10	Allergen 1	Allergen 2	64.9 $\pm$ 6.3	65.9 $\pm$ 4.8
10	Allergen 1 + T	Allergen 2	93.5 $\pm$ 4.8	94.4 $\pm$ 4.6
10	Allergen	Compound 48/80	84.3 $\pm$ 11.9	64.3 $\pm$ 6.9
10	Allergen + T	Compound 48/80	92.4 $\pm$ 8.2	95.2 $\pm$ 5.7

Skin responses were measured 15 min after injection of agonists

and wheal responses to subsequently injected allergen in atopic subjects (8, 9). The present results reflect our attempts to further determine the site of action of the drug in this respect.

First we investigated the effect of prolonging the pretreatment period with terbutaline on the response to subsequent allergen challenge. A significant inhibitory effect was demonstrable up to 8 h. Thus the duration of the anti-allergic action is similar to that of the bronchodilator effect of the drug (4, 7).

When skin sites pretreated with terbutaline or saline were rechallenged with the same allergen 24 h after a first challenge, i.e. at a time point when the inhibitory action of any terbutaline given was resolved, there was a reduced response to such challenge regardless of type of pretreatment. The fact that a response was elicited at all may indicate that additional mast cells were involved in the reaction due to technical problems of injecting the challenging agent twice exactly in the same tissue compartment. Alternatively, it has been shown that histamine release from mast cells is no all-or-none phenomenon. Thus Thon & Uvnäs (12) demonstrated that when washed and reexposed to compound 48/80 isolated rat mast cells released percentagewise as much histamine as at the first challenge. Furthermore, Diamant et al. (3) showed by a micro-pipette technique that on local application of a releasing agent to a single mast cell degranulation only occurred at that site. The reduced reaction to rechallenge observed in our study

might therefore be due to a diminished mast cell storage of histamine not yet made up by synthesis. However, this view does not account for the fact that although terbutaline inhibited the wheal and flare response at the first challenge, the skin site thus treated did not respond to the rechallenge more than the non-pretreated site. Antagonism by terbutaline of the effect of released mediators including histamine rather than inhibition of mediator release, or allergen desensitization of mast cells participating in the first challenge, would be more likely explanations. Against the former view speaks the data that terbutaline is capable of inhibiting the flare reaction to allergen but has no such effect on the histamine-elicited flare response (8). Furthermore, when in the present experiments skin sites pretreated with terbutaline and challenged with allergen were rechallenged 24 h later with a dissimilar allergen, or the histamine releaser compound 48/80, the wheal and flare response was as large as the control response. This finding supports the view that challenge with one allergen desensitizes the skin mast cells to rechallenge with the same allergen and that terbutaline pretreatment does not interfere with this action. The data also adds considerable weight to the concept that beta-adrenoceptor stimulants execute a major anti-allergic effect at the mast cell level.

Evidence for a local refractory state in hypersensitivity reactions has previously been reported. In 1922 Cooke (2) observed that repeated in-

jections of antigen over a short period of time may lead to a reduction in the flare and wheal response. More recently, Feinberg et al. (6) reported that repeated injections of skin sites with one allergen made the skin unresponsive to a dissimilar allergen and to compound 48/80. Similar observations were made on naturally ascaris-sensitive monkeys rechallenged with ascaris and compound 48/80 (5).

In our experiments, where only one allergen injection was given prior to the retest evaluation, the flare response to a dissimilar allergen or compound 48/80 was found to be less reduced by the first challenge than the flare response to rechallenge with the same allergen. No such difference was noted for the wheal reaction, but this response carries the disadvantage of a somewhat larger methodological error.

Finally, in experiments on rat subcutaneous tissue, passively sensitized with two different sets of reaginic antibodies, histamine was released on challenge with one antigen but there was no release on subsequent challenge with the other antigen (10). When the first challenge with either antigen was performed in the presence of disodium cromoglycate followed by washing and rechallenge with the same antigen there was no release, but when substituting that antigen for a dissimilar antigen histamine release occurred. The results of these *in vitro* studies are in harmony with our data and point to a similar action of the two types of drugs, i.e. interference with allergic reactions by inhibition of mast cell mediator release.

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Address:

Reidar Grönneberg
Department of Thoracic Medicine
Karolinska Hospital
Box 60 500
S-104 01 Stockholm
Sweden

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