

Effects of local treatment with salmeterol and terbutaline on anti-IgE-induced wheal, flare, and late induration in human skin

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The capacity of terbutaline and the long-acting β_2 -agonist salmeterol to suppress wheal-and-flare reactions (WFRs) to intradermal antihuman IgE and to histamine was evaluated in 36 healthy volunteers. We also examined effects of the two drugs on the subsequent late cutaneous reaction (LCR) to anti-IgE. Salmeterol (10^{-10} – 10^{-6} M) and terbutaline (10^{-10} – 10^{-5} M), injected intradermally 2 or 15 min before anti-IgE challenge, produced a dose-dependent inhibition of the WFRs to anti-IgE (titer 1 : 10 000) with a maximal effect of $\approx 60\%$ (wheal) and $\approx 75\%$ (flare) by both drugs. On a molar basis, salmeterol was approximately 10–100 times more potent than terbutaline in inhibiting the WFRs. Moreover, the wheal response to histamine (4 nmol) was antagonized by $\approx 40\%$ after pretreatment with either salmeterol (10^{-5} M) or terbutaline (10^{-4} M). We found that both salmeterol and terbutaline exhibited anti-WFR activity for up to 24 h, salmeterol being significantly more potent in this regard. When selecting a 15-min pretreatment interval with equieffective anti-WFR doses from the first dose-response experiments (i.e., a salmeterol : terbutaline ratio of 1 : 10), the WFRs to high-dose anti-IgE (titer 1 : 100) were inhibited by terbutaline (10^{-5} M) by 25–30%, but not by salmeterol (10^{-6}). On the other hand, salmeterol attenuated (by up to $\approx 35\%$) the subsequent LCR more effectively than terbutaline. As compared to the pretreatment procedure, infiltration of the drugs (single doses) into the wheals 30 min after challenge with anti-IgE (titer 1 : 100) proved to be less effective on the development of LCRs. Taken together, salmeterol was found to express higher potency and have longer duration of action than terbutaline in inhibition of IgE-mediated inflammation in human skin.

R. Grönneberg¹, J. Raud²

¹Department of Respiratory and Allergic Diseases, Huddinge University Hospital, Karolinska Institute, Huddinge; ²Department of Physiology and Pharmacology, Karolinska Institute, Stockholm, Sweden

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Reidar Grönneberg, MD, PhD
Department of Respiratory and Allergic Diseases
Huddinge University Hospital
S-141 86 Huddinge
Sweden

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β_2 -Adrenoceptor agonists cause relaxation of airway smooth muscle and are widely used in the treatment of obstructive airway disease such as asthma. These drugs have also been shown to inhibit degranulation of mast cells and mediator- and allergen-induced plasma leakage in various animal models (1–4). Moreover, β_2 -agonists, particularly the long-acting ones (i.e., salmeterol, formoterol), reduce inflammatory leukocyte accumulation in rodent tissues (3–6). All these effects of the β_2 -agonists may well contribute to the clinical response when treating allergic asthma, because many of the clinical characteristics of this disease

are believed to depend on ongoing inflammatory processes.

The long-acting β_2 -agonists were developed to meet a clinical need for a prolonged bronchodilator action for use in controlling the symptoms of nocturnal asthma and to provide more convenient maintenance therapy for the asthmatic patient (7, 8). Salmeterol and formoterol, compared with shorter-acting β -agonists (e.g., salbutamol, terbutaline), are more efficacious in reducing late airway responses to antigen inhalation which are characteristically associated with leukocyte accumulation (9–12). Salmeterol and formoterol also provide

prolonged protection against evoked plasma extravasation and mediator release (1, 3, 4, 6, 13). Yet, there is no clear evidence that long-acting β_2 -agonists inhibit the inflammatory mediator release, airway edema, and/or leukocyte recruitment associated with late asthmatic reactions in man (14–16).

The type of mediators released and the sequence of microvascular inflammatory events follow a rather stereotypical pattern regardless of the tissue in which an allergic reaction takes place (17–19). In this context, the accessibility of the skin makes it an attractive tissue for studies of basic mechanisms, including drug intervention, in IgE-dependent inflammatory reactions. For this purpose, cutaneous anti-IgE challenge in nonatopics appears to be a useful and reproducible alternative to allergen provocation in atopics (20, 21). A late cutaneous reaction (LCR), characterized by local edema and perivascular infiltration of inflammatory cells, often follows the early wheal-and-flare reaction (WFR) induced by either allergen or antihuman IgE (22). The LCR has many similarities with the delayed response in the lung and can be attenuated by intradermally (i.d.) administered formoterol or terbutaline (20, 23). In the latter studies, formoterol was more potent and had a longer duration of action than terbutaline in inhibition of anti-IgE-induced skin reactions in healthy subjects. Moreover, the effects of both drugs on the LCR appeared to be partly independent of inhibition of the preceding release of mast-cell mediators (20).

Salmeterol is a highly selective β_2 -agonist with a long duration of action, possibly as a result of binding to an exosite within the β_2 -adrenoceptor protein (8). In guinea-pig skin, salmeterol was generally more efficacious than formoterol with respect to inhibition of leukocyte recruitment and leukocyte-dependent plasma protein extravasation (PPE) after i.d. administration of the drugs, whereas both drugs equally attenuated mediator-induced PPE (4). The present study aimed to compare the potency and duration of action of topical salmeterol and terbutaline on IgE-mediated inflammation (diameters of WFRs and LCRs) in human skin.

Material and methods

Subjects

Thirty-six healthy, drug-free volunteers (20–54 years of age, mean 30, 24 women and 12 men), with no history of atopy, negative SPT to a panel of common allergens, and negative reactions to i.d. injection of normal goat IgG were recruited. By history, none of the patients had dermatographism.

All subjects in the study gave their informed consent to participate in the study, which was approved by the ethics committee of the Karolinska and Huddinge hospitals.

Reagents for skin testing

Affinity-purified goat antihuman IgE (ϵ -chain specific) and chromatographically purified normal goat IgG were obtained from AB Kemila-Preparat (Sollentuna, Sweden). The lyophilized antibodies were reconstituted in sterile phosphate-buffered saline (PBS) and dialyzed against PBS for 24 h. The antibodies were then passed through a Millipore filter (0.22 μ m), analyzed for protein content according to Bradford (24), diluted to 0.1 mg/ml (=titer 1:10) in sterile PBS, divided into aliquots, and stored frozen at -20°C . Histamine phosphate (British Drug House, Poole, UK) was dissolved in sterile PBS at a concentration of 200 μ M and passed through a Millipore filter (0.22 μ m). As reported previously, we have no evidence that the subjects become sensitized to the small amounts of animal IgG administered (25).

Salmeterol xinafoate (provided by Glaxo Research and Development, UK) was dissolved in 95% ethanol at a concentration of 10^{-2} M and then further diluted in sterile PBS, with a first dilution step of $\times 100$ (i.e., to 10^{-4} M). Consequently, the highest ethanol concentration injected into skin was 0.01% (i.e., salmeterol 10^{-6} M) in the anti-IgE experiments and 0.1% in the experiments with histamine (i.e., salmeterol 10^{-5} M). In a series of control experiments, we found that ethanol in these concentrations does not interfere with cutaneous responses to anti-IgE or histamine (R. Grönneberg and J. Raud, unpublished observations). Terbutaline sulfate (Bricanyl injection solution 0.5 mg/ml, Draco, Lund, Sweden) was diluted in sterile PBS. Fresh solutions of the two drugs were made each day.

Skin testing

WFR to low-dose anti-IgE and drug treatment. The back of each subject was divided into 30 sections from the upper level of the scapula to the second lumbar vertebra, providing five injection sites at each level. All injection volumes were 20 μ l and were administered by the same investigator by micrometer syringe (Wellcome Laboratories, Beckenham, UK; anti-IgE and histamine) or tuberculin syringe (solutions of drugs, diluents, and normal goat IgG).

To investigate the doses of anti-IgE required to obtain an equivalent response to a predefined dose of histamine, we injected anti-IgE (titer

1 : 33 000–1 : 1000 in 3.3-fold increments) and histamine (4 nmol) i.d. The approximate dose of anti-IgE resulting in a flare reaction similar to that evoked by 4 nmol histamine was selected for further experiments on dose-response relationships and duration of inhibition of WFRs by salmeterol and terbutaline.

To examine the potency ratio (relative potency) of salmeterol to terbutaline, we randomly treated skin sites with coded solutions of salmeterol (10^{-10} – 10^{-6} M) or terbutaline (10^{-10} – 10^{-5} M) in 10-fold incremental doses i.d. 2 or 15 min before injection of the selected dose of anti-IgE. The pretreatment schedule was based on previous findings that terbutaline is more effective as an inhibitory drug when administered i.d. 2 or 5 min before rather than concomitantly with allergen in atopic subjects (26). Because salmeterol is slower to reach equilibrium in isolated airway preparations than the short-acting β_2 -agonists salbutamol and fenoterol (8), we extended the pretreatment interval for salmeterol (and terbutaline) in our skin model to 15 min. PBS injected i.d. 2 or 15 min before anti-IgE at each injection level served as control. Comparison of WFRs at drug-pretreated sites with responses at control sites was performed at each injection level in each subject. We also tested 30- and 60-min pretreatment with fixed doses of salmeterol (10^{-6} M) and terbutaline (10^{-5} M) in five subjects.

Using the experimental model described above, we examined the duration of drug action by injecting coded solutions of salmeterol (10^{-8} and 10^{-6} M), terbutaline (10^{-7} and 10^{-5} M), or vehicle at each level between 8 and 10 a.m. in approximate ID₃₀ and ID₆₀ doses, i.e., concentrations that produced 30% and 60% inhibition, respectively, of the WFRs to anti-IgE in the previous dose-response experiments. All injection sites were carefully outlined with a marking pen to enable later i.d. challenge with anti-IgE (titer 1 : 10 000) after time intervals of 15 min and 8, 24, and 48 h.

Late-phase response to high-dose anti-IgE. The volar aspects of the forearms (midway between the wrist and the elbow) were treated with coded solutions of salmeterol (10^{-6} M), terbutaline (10^{-5} M), or diluent according to the two designs described below. In both series of experiments, the choice of concentrations of the drugs was based on the dose-response experiments on inhibition of WFRs induced by low dose anti-IgE. In one set of experiments, the respective drug or diluent was injected (20 μ l) 15 min before challenge with anti-IgE (titer 1 : 100, 40 μ l). In another series of experiments, salmeterol, terbutaline, or vehicle was injected (40 μ l) into the wheal 30 min after chal-

lenge with anti-IgE (titer 1 : 100, 40 μ l). Each subject received all treatments (crossover design) with a ≥ 10 -day interval between testing. In the latter experiments, anti-IgE injection sites were marked to avoid the skin being in an LCR refractory state at the second occasion (27).

Registration of WFR and LCR. The WFRs (at 10 min) and the subsequent delayed edema/induration (LCR, at 1–24 h) were delineated on a transparent plastic sheet at indicated time points. LCRs were recorded by the test subjects themselves after thorough instructions. The areas (cm²) of the skin responses were determined by computer-assisted planimetry. Total LCR was estimated by adding the areas for all LCR time-points from 1 to 24 h.

Statistical analysis

Skin responses to anti-IgE and histamine were compared at drug-treated sites with those of simultaneously injected control sites by the Wilcoxon signed ranks test for paired observations. All values are expressed as mean $\pm$ SE, and *n* represents number of subjects per group.

Results

Methodological aspects

Anti-IgE (titer 1 : 33 000–1 : 1000) dose-dependently produced WFRs in all subjects (not shown), with titer 1 : 10 000, resulting in a flare reaction similar to that induced by 4 nmol histamine. The magnitude of the WFRs (10 min) to anti-IgE (titer 1 : 10 000) at control sites was 16.6 ± 1.4 cm² (flare) and 0.81 ± 0.05 cm² (wheal) (*n* = 10). The corresponding responses to histamine (4 nmol) amounted to 14.5 ± 0.85 cm² (flare) and 0.75 ± 0.06 cm² (wheal). The mean coefficients of variation (CV mean), calculated as previously described (28), for WFR (10 min) to anti-IgE (titer 1 : 10 000) injected at the same occasion at two injection levels on the back were 10% (wheal) and 5% (flare) (*n* = 10). The CV means for 10-min responses to anti-IgE titer 1 : 10 000 at five injection levels (28) over a 1–24-h period were 20% (wheal) and 21% (flare) (*n* = 7). The CV means for WFRs (10 min) and total LCR (1–24 h) to anti-IgE titer 1 : 100 injected on the volar aspects of forearms in the same subjects on two occasions separated by a ≥ 10 -day interval, were 14% (wheal), 8% (flare), and 30% (total 1–24 h LCR) (*n* = 9). Cutaneous responses to normal goat-IgG (titer 1 : 100) were indistinguishable from the minor reaction induced by vehicle (PBS or ethanol).

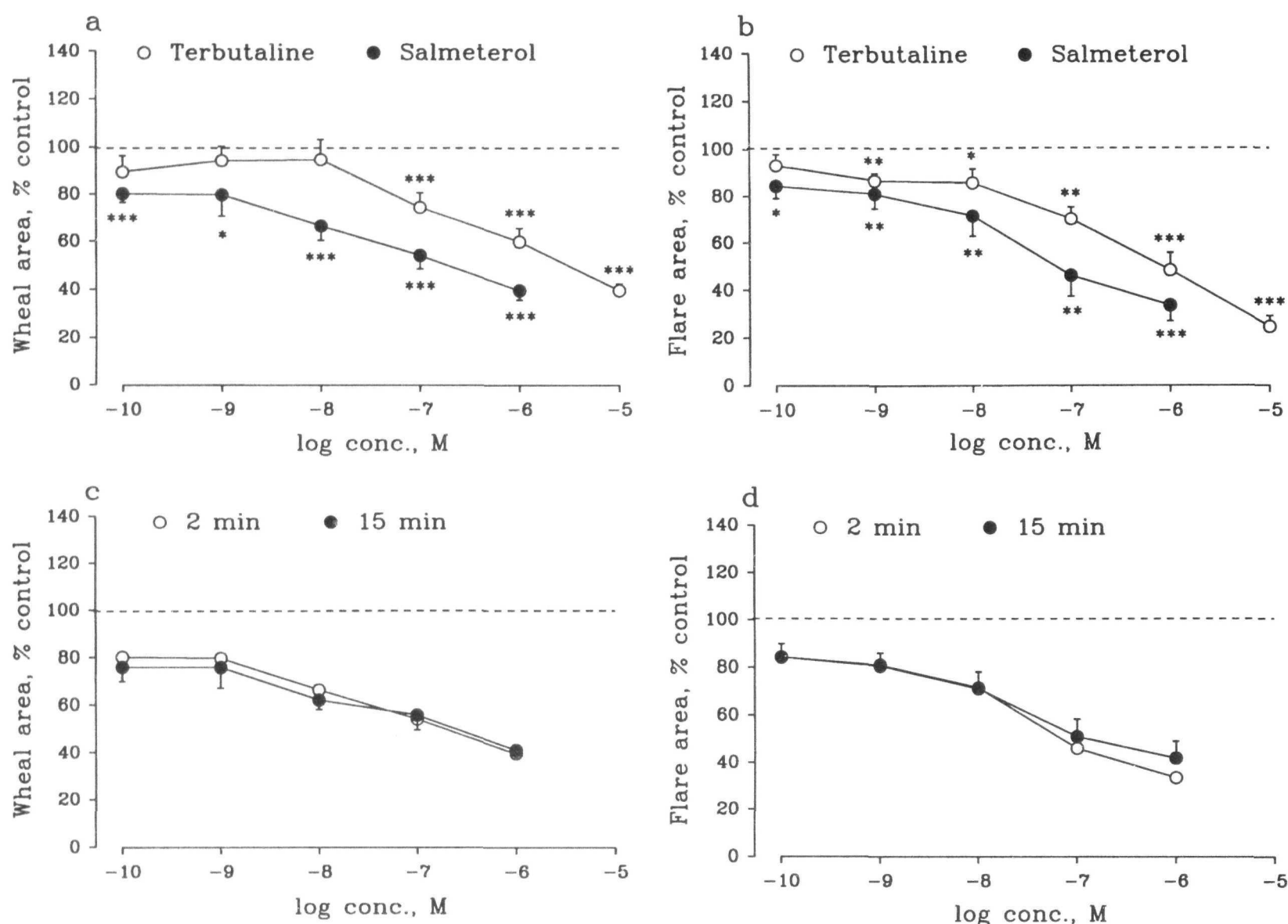


Fig. 1. Intradermal pretreatment of multiple sites on subject's back with different doses of salmeterol (10^{-10} – 10^{-6} M) or terbutaline (10^{-10} – 10^{-5} M) 2 min before induction of wheal (a) and flare (b) by i.d. challenge with anti-IgE (titer 1 : 10 000, $n = 10$). Panels c and d show comparison between pretreatment with salmeterol (10^{-10} – 10^{-6} M) for 2 and 15 min before challenge with anti-IgE ($n = 9$). WFR measured at 10 min after anti-IgE. Effects of drugs are expressed as percentage of control response to anti-IgE. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Mean $\pm$ SE.

Dose-response relationships and duration of action of salmeterol and terbutaline

Salmeterol (10^{-5} M, $n = 6$) and terbutaline (10^{-4} M, $n = 9$) (15-min pretreatment) reduced the histamine (4 nmol)-induced wheal reaction by $44.4 \pm 4.9\%$ and $41.5 \pm 7.8\%$, respectively ($P < 0.05$), and the flare response by $19.0 \pm 7.1\%$ and $15.5 \pm 8.8\%$, respectively ($P < 0.05$). Pretreatment of the skin with terbutaline (10^{-10} – 10^{-5} M) or salmeterol (10^{-10} – 10^{-6} M) 2 min before challenge produced a dose-dependent inhibition of the WFRs induced by anti-IgE (titer 1 : 10 000) (Fig. 1a, b), salmeterol being approximately 10–100 times more potent on a molar basis (ID_{30} and ID_{60} doses of the drugs, which produced $\approx 30\%$ and 60% inhibition, respectively, were selected for the subsequent experiments). Extension of the pretreatment interval for salmeterol (10^{-10} – 10^{-6} M) from 2 to 15 min did not significantly change the dose-response curve with regard to inhibition of WFRs

(Fig. 1c, d). In a separate series of experiments with fixed doses of salmeterol (10^{-6} M) and terbutaline (10^{-5} M), it was found that further extension of the pretreatment interval to 30 or 60 min was no more effective than 2 or 15 min (Fig. 2). The lack of statistical significance with 30-min pretreatment was probably due to the relatively small number of observations in this group ($n = 4$) (Fig. 2). With regard to wheal or flare, the responses to the different concentrations of terbutaline or salmeterol *per se* were indistinguishable from those caused by vehicle (not shown).

The duration of inhibition produced by terbutaline (10^{-7} and 10^{-5} M) and salmeterol (10^{-8} and 10^{-6} M) on the anti-IgE-induced WFRs is shown in Fig. 3. Considered together, both concentrations of terbutaline and salmeterol showed inhibitory activity for up to 24 h, salmeterol being significantly more effective at 24 h, particularly at the higher concentration. At 48 h, there was a trend for better inhibitory activity by salmeterol.

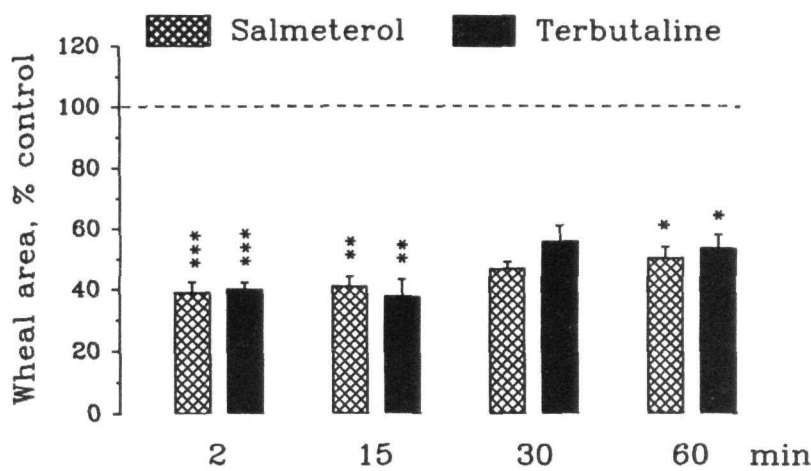


Fig. 2. Intradermal pretreatment of multiple sites on subject's back with salmeterol (10^{-6} M) or terbutaline (10^{-5} M) for indicated periods of time before i.d. challenge with anti-IgE (titer 1 : 10 000). Wheal reaction measured at 10 min. Effects of drugs are expressed as percentage of control response to anti-IgE. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Mean $\pm$ SE ($n = 4-10$).

Effect of salmeterol and terbutaline on LCR

Intradermal injection of the higher dose of anti-IgE (titer 1 : 100) induced both WFRs and late-phase reactions (Fig. 4). The LCRs were characterized by distinct, somewhat irregular, erythematous indura-

tion and edema, reaching a maximum at 4–10 h after challenge.

Pretreatment with salmeterol (10^{-6} M) 15 min before challenge had minimal effects on the WFR and the first part (1–2 h) of the following LCR to the high-dose anti-IgE, but reduced the 4–10-h LCR by approximately 30% (Fig. 5). In contrast to salmeterol, terbutaline (10^{-5} M) attenuated the WFR to anti-IgE (titer 1 : 100) by 25% (wheal) and 30% (flare) (Fig. 5). On the other hand, terbutaline was less effective than salmeterol in inhibiting the subsequent LCR (Fig. 5).

When infiltrating the drugs in single doses 30 min after challenge with anti-IgE (1 : 100), we detected no clear pattern of inhibition (Fig. 6). The attenuation of the first 1–2-h LCR seemed somewhat more pronounced at sites treated with salmeterol (10^{-6} M), whereas the following 4–24-h LCR appeared to be more inhibited by terbutaline (10^{-5} M).

Discussion

The main objective of this study was to define the duration of action and relative potency of salmeterol in comparison with terbutaline in antagonizing

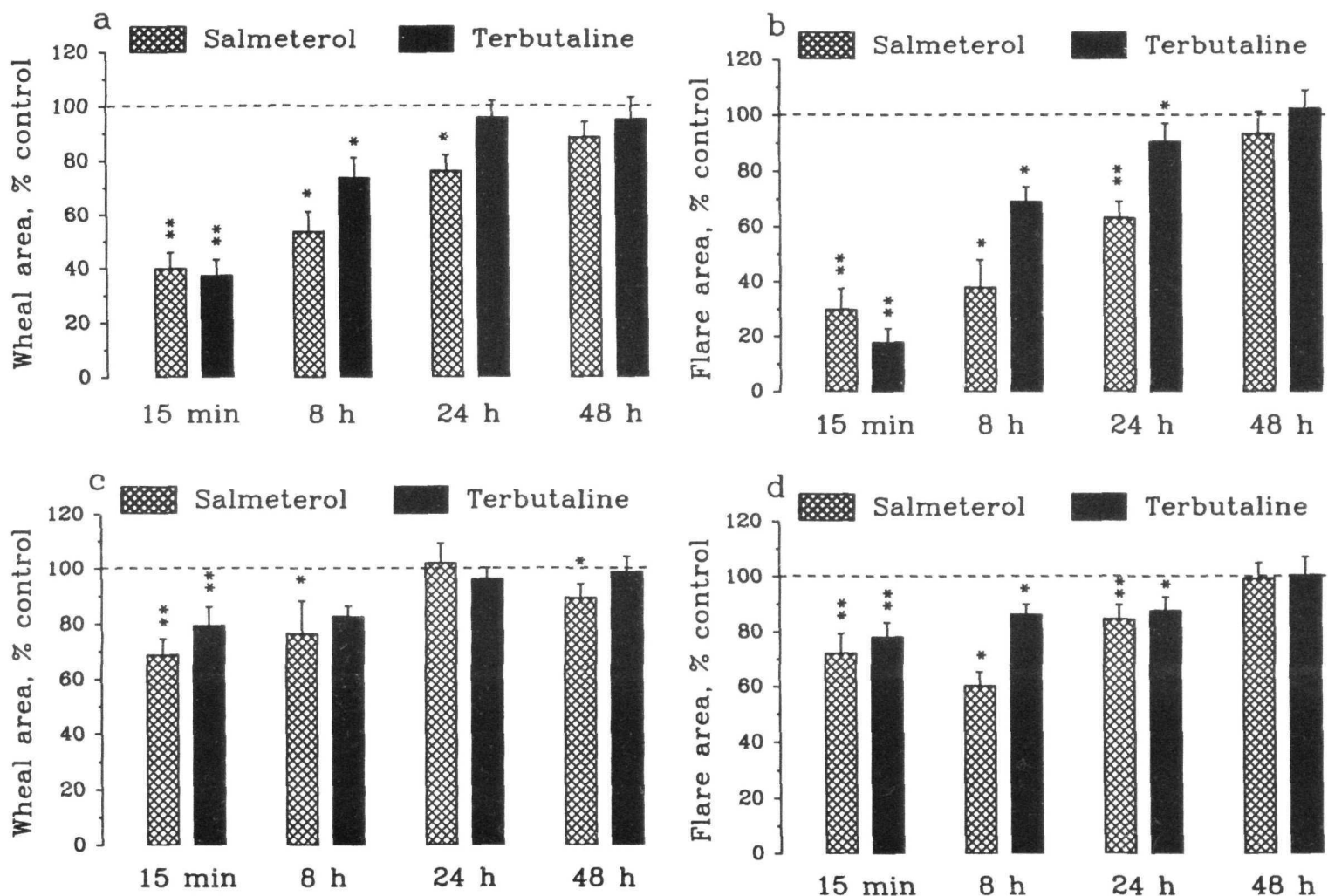


Fig. 3. Intradermal pretreatment of multiple sites on subject's back with 10^{-6} M salmeterol or 10^{-5} M terbutaline for indicated periods of time before induction of wheal (a) and flare (b) by i.d. challenge with anti-IgE (titer 1 : 10 000) (15 min, $n = 9$; 8 h, $n = 5$; 24 h, $n = 7$; 48 h, $n = 7$). Panels c and d show corresponding effects of 10^{-8} M salmeterol or 10^{-7} M terbutaline (15 min, $n = 9$; 8 h, $n = 5$; 24 h, $n = 7$; 48 h, $n = 7$). WFR measured at 10 min. Effects of drugs are expressed as percentage of control response to anti-IgE. * $P < 0.05$; ** $P < 0.01$. Mean $\pm$ SE.

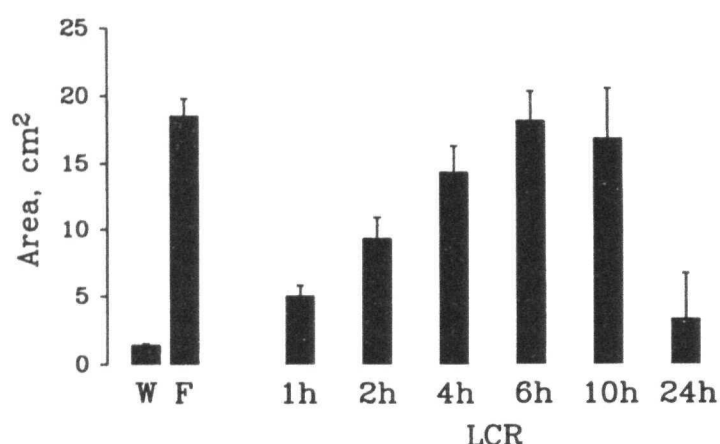


Fig. 4. Size (cm²) of WFRs (at 10 min) and LCRs after i.d. injection of anti-IgE (titer 1 : 100) on volar aspect of forearms. Mean $\pm$ SE ($n = 10$).

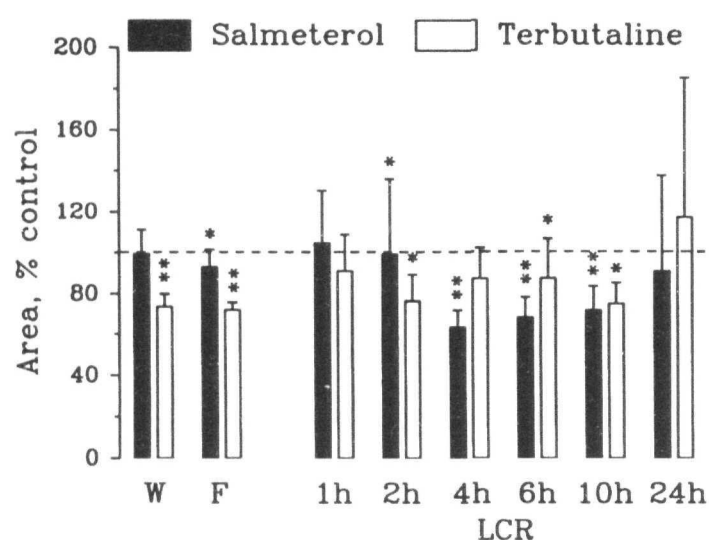


Fig. 5. Intradermal pretreatment of volar aspect of forearms with salmeterol (10^{-6} M, $n = 10$) or terbutaline (10^{-5} M, $n = 8$) 15 min before i.d. challenge with anti-IgE (titer 1 : 100). WFRs were measured at 10-min and LCRs at indicated time intervals. Effects of drugs are expressed as percentage of control response to anti-IgE. * $P < 0.05$; ** $P < 0.01$. Mean $\pm$ SE.

IgE-dependent reactions in human skin. Regarding the effects on the early WFR to anti-IgE, pretreatment with salmeterol and terbutaline (given 2 or 15 min before challenge) resulted in the same maximal inhibition (70–80%); however, salmeterol was approximately 10–100 times more potent than terbutaline. The two drugs appeared to have an equally rapid (≤ 2 min) onset of anti-inflammatory action in the skin, and extending the pretreatment interval to 30 or 60 min did not increase the effects of salmeterol. Both salmeterol and terbutaline exhibited anti-WFR activity for up to 24 h, salmeterol being significantly more potent in this regard. To provoke LCRs, we used a 100 times higher dose of anti-IgE as compared to WFR-inducing concentration, and, as described above, on the basis of the initial dose-response experiments, we selected equieffective anti-WFR doses of the drugs, i.e., a dose ratio of 1 : 10 for

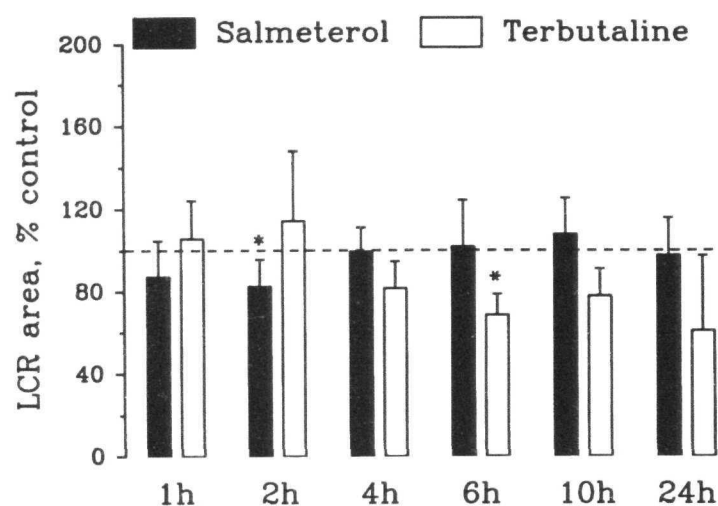


Fig. 6. Effects on LCR (in percentage of control response to anti-IgE) of salmeterol (10^{-6} M, $n = 7$) and terbutaline (10^{-5} M, $n = 7$) infiltrated into whealing 30 min after challenge with anti-IgE (titer 1 : 100) on volar aspects of forearms. * $P < 0.05$. Mean $\pm$ SE.

salmeterol : terbutaline in agreement with clinical asthma treatment. In these experiments, the LCR was moderately reduced by both drugs, with a clear tendency for a higher degree of inhibition by salmeterol. Terbutaline, but not salmeterol, inhibited the preceding WFR to the high-dose anti-IgE by $\approx 25\%$ (15-min pretreatment). When injecting the drugs in single doses into the anti-IgE-induced wheal 30 min after challenge, neither salmeterol nor terbutaline was very effective in inhibiting the LCR. If anything, terbutaline appeared slightly more efficacious at later time points in these experiments. Taken together, the results of this study show that salmeterol was clearly more potent and somewhat more long-acting than terbutaline in inhibition of anti-IgE-induced inflammation in human skin.

Our present experiments do not explain the mechanism(s) by which salmeterol and terbutaline inhibited the IgE-mediated reactions. However, the fact that histamine-induced whealing was attenuated by up to 40% by both drugs suggests direct antagonism of plasma protein extravasation, rather than inhibition of mast-cell activation, as the main mode of action. Yet, it is possible that the reduction of WFR to high-dose anti-IgE by terbutaline involved inhibition of mast-cell mediator release. This is supported by our recent skin-chamber experiments in which anti-IgE-induced histamine release into skin-chamber fluid was significantly reduced by terbutaline, but not by salmeterol (drugs given locally 30 min before and together with anti-IgE) (29). In contrast, a preincubation period of 20–30 min with salmeterol has been reported to inhibit antigen-induced mediator release from passively sensitized human lung fragments (1, 13).

We also noted that the β_2 -agonists, which are generally considered to have vasodilatory properties, slightly but significantly reduced the histamine-induced flare response. While this is in line with previous findings with β_2 -agonists in human skin (23), we can presently not explain the mode of action by salmeterol and terbutaline in this regard. However, in view of the suggestion that β_2 -agonists directly inhibit release of transmitters from both cholinergic and nonadrenergic noncholinergic nerves (30, 31), it is tempting to speculate that salmeterol and terbutaline may have attenuated the well-established neurogenic component of the histamine-induced flare response.

Previous experiments with formoterol, a lipophilic drug with high affinity and efficacy at β -adrenoceptors (7), showed that formoterol (2-min pretreatment) was 25–70 times more potent than terbutaline in inhibition of anti-IgE-induced wheal and flare (28). In parallel with salmeterol, the duration of inhibition of WFRs produced by formoterol was 24 h. Formoterol (10^{-7} – 10^{-5} M) given 2 min before anti-IgE reduced the WFR by 25–40% and the following 1–24-h LCR up to 50%, formoterol being clearly superior to terbutaline in this respect. Moreover, when formoterol was injected in single dose into the wheal 30 min after challenge, the anti-IgE-induced LCR was reduced by 50% (20). While these findings suggest that formoterol may be somewhat more effective than salmeterol in inhibiting later stages of IgE-mediated inflammation in human skin, it is important to emphasize that potential differences between the two drugs must be confirmed by direct comparison in the same study.

β_2 -Agonists, particularly long-acting ones, reduce leukocyte accumulation in various animal models (3–6). Thus, since IgE-mediated LCRs in human skin are characterized by local infiltration of inflammatory cells (22, 32), it is tempting to speculate that the observed anti-LCR effects by salmeterol and formoterol may be related to inhibition of leukocyte recruitment. However, such an effect by the long-acting β_2 -agonists remains to be documented in man. Indeed, we recently failed to detect inhibition by salmeterol of anti-IgE-induced leukocyte accumulation in skin chambers (29). Moreover, bronchoalveolar-lavage or sputum-sampling studies provide no evidence that salmeterol or formoterol inhibits recruitment of inflammatory cells in asthmatics (12, 14–16), in whom both salmeterol and formoterol are more efficacious in reducing late airway responses to antigen inhalation than shorter-acting β -agonists (9, 10, 12, 33). However, the number of studies in man is too limited to exclude the possibility that long-acting β_2 -agonists exert clinically significant anti-inflammatory actions.

Acknowledgments

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References

1. Butchers PR, Vardey CJ, Johnson M. Salmeterol: a potent and long-acting inhibitor of inflammatory mediator release from human lung. *Br J Pharmacol* 1991;104:672-6.
2. Erjefält I, Persson CGA. Pharmacologic control of plasma exudation into tracheobronchial airways. *Am Rev Respir Dis* 1991;143:1008-14.
3. Teixeira MM, Williams TJ, Hellewell PG. Anti-inflammatory effects of a short-acting and a long-acting β_2 -adrenoceptor agonist in guinea pig skin. *Eur J Pharmacol* 1995;272:185-93.
4. Whelan CJ, Johnson M, Vardey CJ. Comparison of the anti-inflammatory properties of formoterol, salbutamol and salmeterol in guinea-pig skin and lung. *Br J Pharmacol* 1993;110:613-18.
5. Bowden JJ, Sulakvelidze I, McDonald DM. Inhibition of neutrophil and eosinophil adhesion to venules of rat trachea by β_2 -adrenergic agonist formoterol. *J Appl Physiol* 1994;77:397-405.
6. Whelan CJ, Johnson M. Inhibition by salmeterol of increased vascular permeability and granulocyte accumulation in guinea-pig lung and skin. *Br J Pharmacol* 1992;105:831-8.
7. Anderson GP. Pharmacology of formoterol: an innovative bronchodilator. New drugs for asthma therapy. *Agents Actions* 1991;34:97-115.
8. Johnson M, Butchers PR, Coleman RA, et al. The pharmacology of salmeterol. *Life Sci* 1993;52:2131-43.
9. Palmqvist M, Balder B, Löwhagen O, Melander B, Svedmyr N, Wåhlander L. Late asthmatic reaction decreased after pretreatment with salbutamol and formoterol, a new long-acting β_2 -agonist. *J Allergy Clin Immunol* 1992;89:844-9.
10. Twentyman OP, Finnerty JP, Harris A, Palmer J, Holgate ST. Protection against allergen-induced asthma by salmeterol. *Lancet* 1990;336:1338-42.
11. Twentyman OP, Finnerty JP, Holgate ST. The inhibitory effect of nebulized albuterol on the early and late asthmatic reactions and increase in airway responsiveness provoked by inhaled allergen in asthma. *Am Rev Respir Dis* 1991;144:782-7.
12. Wong BJ, Dolovich J, Ramsdale EH, et al. Formoterol compared with beclomethasone and placebo in allergen-induced asthmatic responses. *Am Rev Respir Dis* 1992;146:1156-60.
13. Nials AT, Ball DI, Butchers PR, et al. Formoterol on airway smooth muscle and human lung mast cells: a comparison with salbutamol and salmeterol. *Eur J Pharmacol* 1994;251:127-35.
14. Gardiner PV, Ward C, Booth H, Allison A, Hendrick DJ, Walters EH. Effect of eight weeks of treatment with salmeterol on bronchoalveolar lavage inflammatory indices in asthmatics. *Am J Respir Crit Care Med* 1994;150:1006-11.
15. Gratziau C, Roberts JA, Walls A, Holgate ST, Howarth P. Lymphocyte population in BAL of asthmatics after salmeterol treatment [Abstract]. *Eur Respir J* 1992; Suppl 15:207s.

16. Howarth PH. The investigation of the non-bronchodilator properties of asthma therapies *in vivo* [Abstract]. *Eur Respir J* 1991; Suppl 14:399S.
17. Kay AB. Asthma and inflammation. *J Allergy Clin Immunol* 1991;87:893-910.
18. Montefort S, Holgate ST, Howarth PH. Review. Leukocyte-endothelial adhesion molecules and their role in bronchial asthma and allergic rhinitis. *Eur Respir J* 1993;6:1044-54.
19. Svensjö E. The hamster cheek pouch as a model in microcirculation research. *Eur Respir J* 1990; 3 Suppl 12:595s-601s.
20. Grönneberg R, Zetterström O. Inhibitory effects of formoterol and terbutaline on the development of late phase skin reactions. *Clin Exp Allergy* 1992;21:257-63.
21. Weiss ME, Trent P, Fisher R, Norman PS, Waterbury WE, Adkinson NF Jr. Rabbit F(ab')₂ antihuman IgE is a universal skin test reagent in the evaluation of skin mast cell degranulation *in vivo*. *J Allergy Clin Immunol* 1989;83:1040-8.
22. Gleich GJ. The late phase of the immunoglobulin E-mediated reaction: a link between anaphylaxis and common allergic disease? *J Allergy Clin Immunol* 1982;70:160-9.
23. Grönneberg R, Zetterström O. Inhibition of anti-IgE induced skin response in normals by formoterol, a new β_2 -adrenoceptor agonist, and terbutaline. II. Effect on the late phase reaction. *Allergy* 1990;45:340-6.
24. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976;72:248-54.
25. Raud J, Halldén M, Roquet A, et al. Anti-IgE-induced leukocyte recruitment, mediator release, and albumin extravasation in skin-chamber fluid from healthy and atopic subjects. *J Allergy Clin Immunol* 1996;97:1151-63.
26. Grönneberg R, Strandberg K, Hägermark Ö. Effect of terbutaline, a β_2 -adrenergic receptor stimulating compound, on cutaneous responses to histamine, allergen, compound 48/80 and trypsin. *Allergy* 1979;34:303-9.
27. Shaikh W, Umemoto L, Poothullil J, Hargreave FE, Dolovich J. Relative refractory state for late cutaneous allergic responses. *J Allergy Clin Immunol* 1977;60:242-6.
28. Grönneberg R, Zetterström O. Inhibition of anti-IgE induced skin response in normals by formoterol, a new β_2 -adrenoceptor agonist, and terbutaline. I. Dose response relation and duration of effect on the early wheal and flare response. *Allergy* 1990;45:334-9.
29. Grönneberg R, van Hage-Hamsten M, Halldén G, Hed J, Raud J. Effects of salmeterol and terbutaline on IgE-mediated dermal reactions and inflammatory events in skin chambers in atopics. *Allergy* 1996;51:640-6.
30. Verleden GM, Belvisi MG, Rabe KF, Miura M, Barnes PJ. β_2 -Adrenoceptor agonists inhibit NANC neural bronchoconstrictor responses *in vitro*. *J Appl Physiol* 1993;74:1195-9.
31. Wessler I, Reinheimer T, Brunn G, Anderson GP, MacLagan J, Racké K. β -Adrenoceptors mediate inhibition of [³H]-acetylcholine release from the isolated rat and guinea-pig trachea: role of the airway mucosa and prostaglandins. *Br J Pharmacol* 1994;113:1221-30.
32. Zweiman B. Mediators of allergic inflammation in the skin. *Clin Allergy* 1988;18:419-33.
33. Pedersen B, Dahl R, Larsen BB, Venge P. The effect of salmeterol on the early- and late-phase reaction to bronchial allergen and postchallenge variation in bronchial reactivity, blood eosinophils, serum eosinophil cationic protein, and serum eosinophil protein X. *Allergy* 1993;48:377-82.

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