

Inhibition of anti-IgE induced skin response in normals by formoterol, a new β_2 -adrenoceptor agonist, and terbutaline

1. Dose response relation and duration of effect on the early wheal and flare response

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The intention of the present study was to compare formoterol and terbutaline regarding ability to inhibit immediate wheal and flare responses (WFR) to anti-human IgE with focus on the duration of anti-WFR action. Formoterol is a novel β_2 -adrenergic agonist with a prolonged duration of bronchodilation capacity after inhalation. The drugs injected intradermally 2 min prior to challenge with anti-IgE in volunteers produced a dose-dependent inhibition of the WFR in the range 1pg–100ng (formoterol) and 1ng–1 μ g (terbutaline). Formoterol was 70 times (flare) and 25 times (wheal) more potent (ID_{50}) than terbutaline on a weight basis. The duration of the anti-WFR action for formoterol, injected in a 25 times lower dose than terbutaline, was significantly longer, namely > 24 h versus 8 h for terbutaline. The histamine-induced wheal reaction was attenuated by 15 % and 25 % by terbutaline and formoterol, respectively. The results indicate a higher β_2 -receptor activity for formoterol with respect to inhibition of IgE-dependent mast cell mediator release in addition to an anti-leak effect exerted by both drugs. The prolonged duration of antagonistic effect by formoterol on the WFR to anti-IgE might be due to the lipophilic property of the drug, with an expected higher retention of formoterol at the target tissue compared with the more hydrophilic compound terbutaline.

Key words: anti-IgE; β -agonist; flare response; formoterol; histamine; mast cell; terbutaline; wheal response.

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CLINICAL ASPECTS

Allergen provocation in the skin of sensitized individuals induces mast cell mediator release with a resulting wheal (increased microvascular permeability) and flare (vasodilation) response (WFR). This reaction can be mimicked by injection of anti-human IgE (reversed anaphylaxis) and also induced in non-allergic subjects. When inhaled the new β_2 -agonist, formoterol, showed a higher potency and a more prolonged bronchodilation than when administered perorally. In the skin model, formoterol was about 50 times more active than terbutaline in terms of potency and produced a > 3 times longer suppression of WFR to anti-IgE. The results indicate that formoterol has a higher β_2 -receptor activity with respect to anti-allergic capacity. The prolonged duration is speculated to be due to the lipophilic property of formoterol. Formoterol appears to possess an interesting anti-allergic profile compared with terbutaline.

Formoterol fumarate (formoterol) is a novel β_2 -adrenoceptor agonist with a prolonged broncho-

dilation capacity and a high anti-allergic potency as to inhibition of histamine release from

sensitized human lung and leukocytes (9, 10, 12, 13). The β_2 -agonist, terbutaline, inhibits IgE-mediated early WFR in atopic and non-atopic skin (6, 14). Antagonism of mast cell mediator release and anti-leak effect on vessels have been proposed as a dual mechanism of inhibitory action of terbutaline and other currently available β_2 -receptor agonists (1, 3, 7, 11, 14). Studies on the receptor specificity by using other β -agonists indicated that β_2 -receptors, presumably located on the mast cells, were involved (5).

The aim of the present study was to compare the anti-allergic capacity of terbutaline with that of formoterol with focus on the duration of action.

MATERIAL AND METHODS

Antiserum and chemicals

Heterologous whole anti-human IgE was raised in rabbits against a mixture of isolated Fc fragments from the epsilon chain from a human IgE myeloma protein, and activity to serum protein other than IgE was removed by absorption with normal human serum. The antiserum was precipitated and washed with 18% Na_2SO_4 , dissolved and dialysed against sodium chloride (0.15 mol/l) to 1/5 (v/v) and the resulting solution was equivalent in gel diffusion to 20 $\mu\text{g/ml}$ of IgE (specific antibody activity). The antiserum was passed through a millipore filter (0.45 μm) and stored at +4°C. The anti-IgE and normal rabbit IgG (prepared and diluted analogously with anti-IgE) were further diluted in unbuffered saline solution containing 0.03% human serum albumin (Albumin diluent®, Pharmacia Diagnostics AB, Uppsala, Sweden). Histamine dihydrochloride and terbutaline sulphate (Bricanyl® injection solution 0.5 mg/ml, Draco, Lund, Sweden), were diluted in physiological saline on the day of each experiment, whereas formoterol fumarate in dry ampoules (kindly supplied by Ciba-Geigy Limited, Basle, Switzerland) was first dissolved in 100 μg in 10 ml sterile water to 10 $\mu\text{g/ml}$, and further diluted in physiological saline. The antisera were gener-

ously supplied by Professor S. Gunnar O. Johansson, Dept. of Clinical Immunology, Karolinska Hospital, Stockholm, Sweden.

Subjects

Eight female healthy subjects, 23–49 years of age (mean 36), on no medication except for occasional contraceptives, gave their informed consent to participate in the study, which was approved by the Ethics Committee at Huddinge University Hospital, Stockholm, Sweden.

Skin testing

The back of the test subject was divided into 25 sections from the upper level of the scapulae to the second lumbar vertebra giving five injection sites at each level. All injection volumes were 20 μl and administered by the same investigator (R.G.).

Determination of concentration of anti-IgE

On a *first* pretrial test anti-IgE was injected in 3.3-fold increments (v/v) and histamine in 2 μg intradermally (i.d.) delivered by an Agla micrometer syringe (Wellcome Laboratories, Beckenham, England) and the extent of the WFR was delineated at 10 min by using a pen and a transparency sheet placed over the injection site. Later, the areas were measured by planimetry and the approximate dose of anti-IgE corresponding to the ED_{60} , i.e. 60% of the maximal flare response to histamine, was determined in each individual. Normal rabbit IgG was injected i.d. in a dose corresponding to that of anti-IgE for specificity reasons. Any individual exhibiting a WFR to normal IgG that exceeded that to normal saline, was excluded from further experiments.

Dose response relation of effect of the β -agonists

On a *second* occasion skin sites were randomly treated with coded solutions of formoterol fum-

arate or terbutaline sulphate in 10-fold incremental doses i.d. by a tuberculin syringe 2 min prior to injection of the selected dose of anti-IgE delivered as above. Physiological saline was always injected before anti-IgE at each injection level (control). The treatment schedule of giving the drugs shortly prior to challenge was based on a previous finding that terbutaline proved to be more effective as an inhibitory drug when administered i.d. 2 to 5 min before rather than concomitantly with allergen (4). Comparison of WFR at drug-pretreated sites with responses at control site was performed at each injection level in each individual and dose response curves for drug effects constructed.

Duration of drug effect on WFR

On a *third* occasion, coded solutions of the drugs were injected between 8 and 10 a.m. and at 4 p.m. (16h pretreatment interval) each in approximate ID_{30} and ID_{50-70} doses, i.e. doses that produced 30 and 50–70% inhibition of the flare response to anti-IgE in the previous dose response experiment, and physiological saline (control) at each of five injection levels. The sites were carefully outlined with a marking pen to enable later i.d. challenge with the selected concentration of anti-IgE after a time interval of 2 min, 2 h, 4 h, 8 h, 16 h and 24 h, respectively, in order to determine the duration of drug effects.

The planimetric analyses from all occasions were performed by an independent investigator with no knowledge of the code of solutions.

Statistical analyses

WFR in mm^2 were compared at i.d. drug treated sites versus those of simultaneously injected control sites by the aid of Student's *t*-test for paired observations in the dose-response experiment and by analysis of variance and covariance in a split-plot model (with treatment and dose as classification variables and time as a covariate, in the duration study of drug effects). Tukey's method was used for multiple comparisons.

RESULTS

Dose-response relation of the effect of β -agonists

Pretreatment of the skin with terbutaline (1ng–1 μ g) (9.1×10^{-8} – 10^{-5} M) and formoterol (1pg–100ng) (6.2×10^{-11} – 10^{-6} M) 2 min before challenge produced a dose-dependent inhibition of the anti-IgE induced WFR (Figs. 1, 2). Formoterol was about 70 times (flare) and 25 times (wheal) more potent (ID_{40}) than terbutaline on a

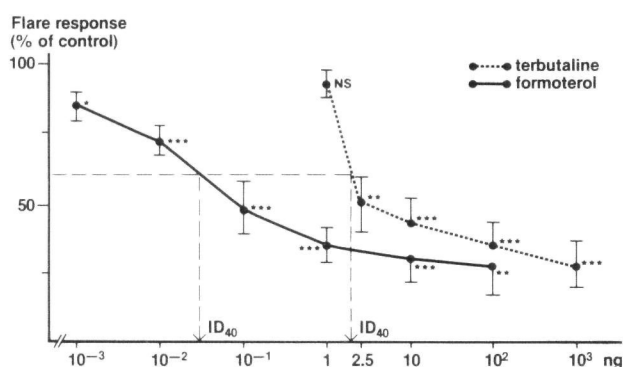


Fig. 1. Dose-response relations for the effect of terbutaline (1ng–1 μ g) and formoterol (1pg–100ng) on anti-IgE induced flare response. The compounds were injected intradermally 2 min prior to anti-IgE challenge. Results are expressed as percent of control (mean $\pm$ SEM, $n = 8$, formoterol 100ng only injected in $n = 4$ subjects). Formoterol was 70 times (ID_{40}) more potent on a weight basis than terbutaline. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, NS = non-significant. *P*-values denote comparison of drug with control.

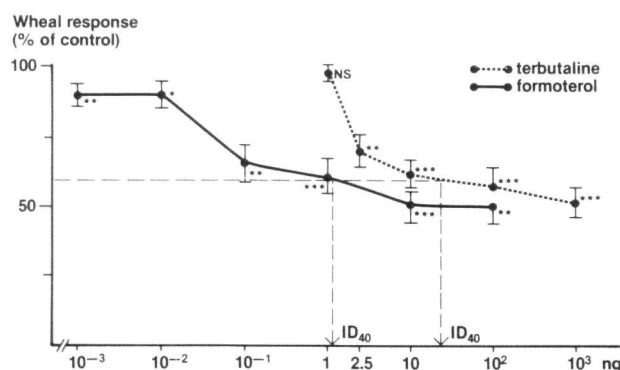


Fig. 2. Dose-response relations for the effect of terbutaline (1ng–1 μ g) and formoterol (1pg–100ng) on anti-IgE induced wheal response. The compounds were injected intradermally 2 min prior to anti-IgE challenge. Results are expressed as percent of control (mean $\pm$ SEM, $n = 8$, formoterol 100ng only injected in $n = 4$ subjects). Formoterol was 25 times more potent on a weight basis than terbutaline. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, NS = non-significant. *P*-values denote comparison of drug with control.

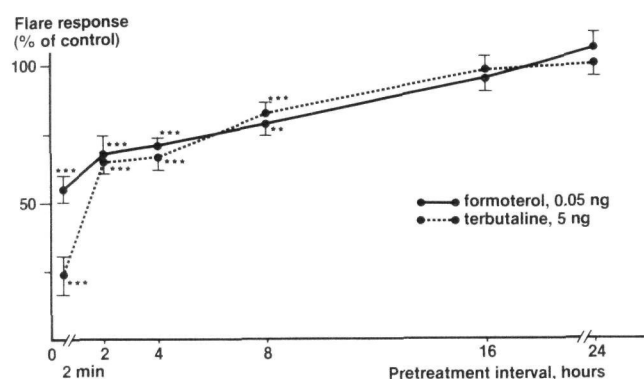


Fig. 3. The duration of inhibition produced by formoterol (0.05 ng) and terbutaline (5 ng) on anti-IgE induced flare response. Results are expressed as percent of control (mean±SEM, $n = 8$). The compounds were injected intradermally at various skin sites, which were later challenged with anti-IgE at the defined time intervals. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. P -values denote comparison of drug with control.

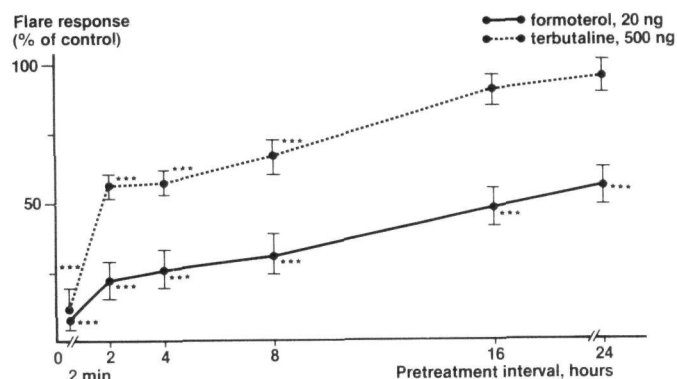


Fig. 5. The duration of inhibition produced by formoterol, (20ng) and terbutaline (0.5μg) on anti-IgE induced flare response. Results are expressed as percent of control (mean±SEM, $n = 8$). The compounds were injected intradermally at various skin sites, which were later challenged with anti-IgE at the defined time intervals. *** $P < 0.001$. P -value denotes comparison of drug with control.

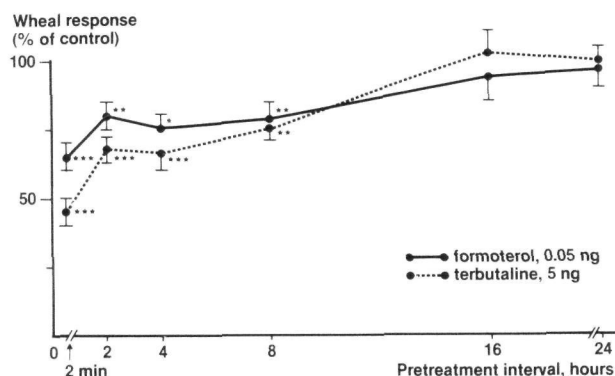


Fig. 4. The duration of inhibition produced by formoterol (0.05 ng) and terbutaline (5ng) on anti-IgE induced wheal response. Results are expressed as percent of control (mean±SEM, $n = 8$). The compounds were injected intradermally at various skin sites, which were later challenged with anti-IgE at the defined time intervals. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. P -values denote comparison of drug with control.

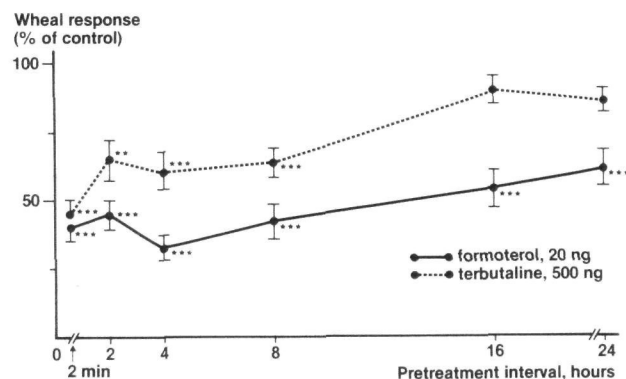


Fig. 6. The duration of inhibition produced by formoterol (20ng) and terbutaline (0.5μg) on anti-IgE induced wheal response. Results are expressed as % of control (mean±SEM, $n = 8$). The compounds were injected intradermally at various skin sites, which were later challenged with anti-IgE at the defined time intervals. ** $P < 0.01$, *** $P < 0.001$. P -values denote comparison of drug with control.

weight basis ($P < 0.001$) as well as in molar concentrations.

Duration of drug effect on WFR to anti-IgE

The duration of inhibition produced by terbutaline and formoterol on the anti-IgE induced WFR is demonstrated in Figs. 3–6.

In a low dose range (Figs. 3, 4) formoterol (0.05 ng) was inferior to terbutaline (5 ng) at 2 min (WFR) and 2 h (wheal) pretreatment intervals ($P < 0.0001$). In spite of our effort to find a standard 50% inhibition dose for the

duration study, the inhibition of the flare response after terbutaline injection was 75% initially. This may be due to the individual variation, since another group of subjects was used in this test. Despite the choice of a 100 times lower dose of formoterol producing an ID_{30-40} compared with ID_{50-75} for terbutaline at the 2 min pretreatment interval, the drugs were equipotent, when given 4–24 h before challenge, with a significant inhibition up to 8 h for both drugs. In a high-dose range (ID_{60} – ID_{80} doses chosen from Fig. 1) (Figs. 5, 6) formoterol (20 ng) was superior to terbutaline (0.5 μg) in the pretreatment interval 2 h and onwards ($P < 0.0001$).

Formoterol in this dose, exhibited a prolonged duration of action with > 50 % inhibition when injected 24 h before challenge versus 8 h for terbutaline, given in a 25 times higher dose than formoterol.

Finally, when comparing all the doses of the drugs involved (Figs. 3–6), the high dose (20 ng) of formoterol was superior to the others as to duration of anti-WFR effect from 2 h and onwards ($P < 0.0001$).

Effect on WFR to histamine

The higher doses of terbutaline and formoterol, both injected 2 min before challenge, attenuated the whealing to histamine (2 µg) by 15 % ($P < 0.05$) and 25 %, respectively ($P < 0.01$) without influence on the flare response, as evaluated in six individuals (not shown).

Methodological aspects

The magnitude (mm^2 , mean $\pm$ SEM) of the WFR at control skin sites was 1430 ± 75 (flare), corresponding to ED_{60} for histamine (2 µg) elicited flare response, and 80 ± 5 (whealing). The concentration of anti-IgE varied between 1/10,000 to 1/3300 v/v due to varying sensitivity of the subjects. The mean coefficient of variation, calculated as previously described (7), for responses to anti-IgE injected simultaneously at two injection levels was 14 % (wheal response) and 11 % (flare response).

Corresponding data at six injection levels (3) over a 1–24 h period were 16 % and 17 %, respectively.

Normal rabbit IgG and the drugs injected *per se* in the selected dose range caused no reaction in excess to that of saline, thereby permitting evaluation of anti-allergic and anti-histaminic properties of the agents.

DISCUSSION

We demonstrated that formoterol was approximately 50 times more potent than terbutaline on a weight basis as WFR inhibitor when injecting

the drugs intradermally shortly before anti-IgE challenge in normal skin.

Secondly, formoterol compared with terbutaline had a more than three times longer duration of antagonistic action on the WFR to anti-IgE, when studying a pretreatment interval of 24 h.

The challenge with anti-IgE has been found particularly useful, since it can be performed also in the skin of non-atopics, thereby avoiding the possible influence on the results of disease state and previous or concurrent therapy (7). The WFR to anti-IgE are analogously highly reproducible in both atopic and non-atopic skin, as documented in previous (6, 7) and the present experiments, and permit evaluation of drug effects. We have previously compared atopics with normals and found equivalent responsiveness as to the potency of terbutaline, (i.d. injected) to inhibit WFR to anti-IgE in these two groups of subjects (6).

Since, in the dose range used, terbutaline and formoterol lacked discernible vascular effects *per se*, the influence of the drugs on the skin reactions is interpreted to mainly reflect inhibition of mediator release from skin mast cells. Such a protection of the mast cells was indicated in biopsy studies in sensitized individuals when skin sites allergen-challenged in the presence of terbutaline were compared with conditions at non-protected control sites (2, 14). Mast cell bound β_2 -receptors appear to be involved, and the inhibition of allergen-induced WFR by the drug was abolished by addition of propranolol (5).

In addition, β -agonists appear to carry an anti-leak action. Thus, in analogy with previously presented data on various β_2 -receptor selective agonists (1, 7), terbutaline analogously with formoterol, slightly suppressed histamine-induced whealing in the present experiments. This anti-permeability action adds to the suppressive action on mast cell release, i.e. a dual anti-allergic action that β_2 -receptor selective agonists have in common (11).

Formoterol fumarate is a new catecholamine analogue which was 15 times more potent than salbutamol when inhaled by asthmatics (9). The duration of bronchodilation was rather long,

being clinically relevant for at least 8 h. (9). The prolonged duration of formoterol was not seen after peroral treatment of the drug (9). An *in vitro* study in guinea pigs (8) showed the formoterol-induced relaxation of carbachol-contracted trachea strip preparations to be more resistant to washing than salbutamol and fenoterol, both with a shorter duration of bronchodilation in man. A high lipophilicity of formoterol compared with the other two more hydrophilic β₂-adrenergic substances and a retention by the tissue *in vitro* of formoterol were suggested as the cause of the long effect duration. This hypothesis may also apply to our present findings in the skin model. Here, we also reproduced our data previously shown in atopics of the anti-WFR action of terbutaline limited to 8 h after allergen challenge.

It can be summarized that formoterol, in comparison with terbutaline, appears to offer an additional potency and a prolonged duration regarding antagonism of IgE-dependent mast cell mediator release in man. These properties could be potentially useful in the clinic, for example when treating atopic dermatitis or other skin afflictions and allergic asthma. Our data harmonize with those from studies on the potency and duration of bronchodilation capacity of formoterol in asthmatics.

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