

# Late asthmatic reaction decreased after pretreatment with salbutamol and formoterol, a new long-acting $\beta_2$ -agonist

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*The inhibitory effect of salbutamol and formoterol, a new long-acting  $\beta_2$ -agonist for inhalation, on the late asthmatic reaction (LAR), was studied in 12 patients with allergic asthma. After a single-blind, placebo-treatment control, equipotent bronchodilating doses of inhaled salbutamol (500  $\mu$ g) and formoterol (30  $\mu$ g) were administered 30 minutes before bronchial allergen challenge in a double-blind randomized design. The early asthmatic reaction was completely inhibited by both drugs ( $p < 0.01$ ) but not by placebo. The LAR was also significantly inhibited by both drugs ( $p < 0.01$ ); formoterol was only slightly, but significantly, more effective than salbutamol ( $p = 0.04$ ). In contrast to some earlier studies, the present study indicates an inhibitory effect of  $\beta_2$ -agonists on the LAR. (J ALLERGY CLIN IMMUNOL 1992;89:844-9.)*

**Key words:** Allergic asthma, bronchial allergen challenge,  $\beta_2$ -agonist, early asthmatic reaction, late asthmatic reaction

Three types of bronchial obstructive reactions may occur after allergen exposure: an isolated EAR within minutes, an isolated LAR within hours, and a dual response that is comprised of both the early and late components.<sup>1-5</sup> The prevalence of LAR after EAR varies, according to different studies, between 18% to 84%.<sup>6-10</sup> The EAR is completely inhibited by  $\beta_2$ -agonists when these are administered before a bronchial allergen challenge.<sup>1, 11, 12</sup> The EAR is also inhibited by sodium cromoglycate,<sup>1, 11, 12</sup> but only by corticosteroids after several days of treatment.<sup>13</sup> Among the antiasthmatic drugs studied in their effect on the LAR, sodium cromoglycate and inhaled corticosteroids are the most effective, whereas drugs, such as  $\beta_2$ -agonists, have been reported to exert no or only little effect.<sup>1, 11, 12</sup> The reason may be a too short duration of action of the  $\beta_2$ -agonists used at the present time, a lack of antiallergic (prophylactic) effect, or too low concentration of the drug at the tissue level. With previous negative results, it was of interest to study

## Abbreviations used

|       |                           |
|-------|---------------------------|
| EAR:  | Early asthmatic reaction  |
| LAR:  | Late asthmatic reaction   |
| PEFR: | Peak expiratory flow rate |
| SPT:  | Skin prick test           |
| DSCG: | Disodium cromoglycate     |

the effect of a  $\beta_2$ -agonist with longer duration of action. Thus, formoterol, a new long-acting  $\beta_2$ -agonist for inhalation with a bronchodilating effect lasting at least 10 to 12 hours,<sup>14, 15</sup> was selected as the study drug and analyzed for the effect of EAR and LAR.

## MATERIAL AND METHODS (Table I)

Twelve adult female patients, mean age, 30; range, 20 to 52 years, were selected. The inclusion criteria were a history of asthma, a positive SPT, and a positive bronchial allergen challenge to the relevant allergen. The SPT reactions were graded in accordance with the recommendations of the Nordic Society for Allergy Research in a plus system with 10 mg/ml of histamine hydrochloride as a reference.<sup>16</sup> The allergens used were cat ( $n = 6$ ), horse ( $n = 3$ ), dog ( $n = 1$ ), and cow dander ( $n = 1$ ), and birch pollen ( $n = 1$ ) purchased from the Allergological Laboratories (ALK) in Copenhagen, Denmark. The same individual batch was used. For a positive LAR, the PEFR should have fallen by  $>15\%$  4 to 10 hours after challenge at a prestudy titration day. None of the patients had recently been exposed to the

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## DESIGN:

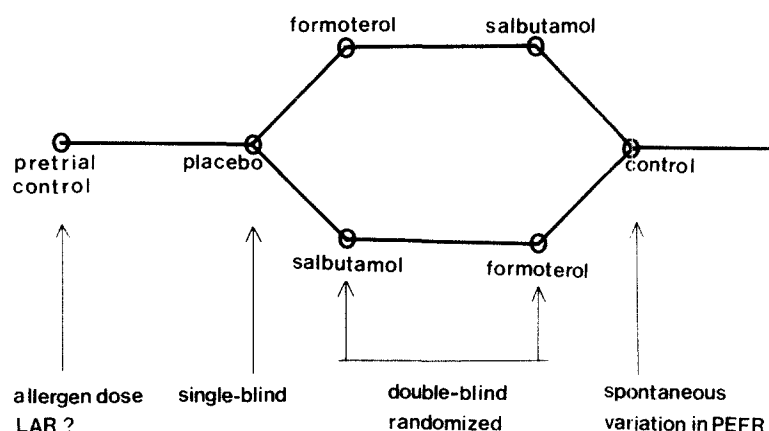


FIG. 1. Study design.

TABLE I

| Pt      | Age (yr) | Sex | FEV <sub>1</sub> (% pred) | PEFR (% pred) | SPT   | RAST (class) | Allergen | Dose (SQU/ml)   | Treatment |
|---------|----------|-----|---------------------------|---------------|-------|--------------|----------|-----------------|-----------|
| A-L. N. | 20       | F   | 113                       | 105           | ++++  | 2            | Cat      | 64 + 125        | S         |
| G-B. L. | 27       | F   | 103                       | 93            | +++   | 2            | Cat      | 125 + 250       | S         |
| G. W.   | 30       | F   | 122                       | 110           | ++++  | 2            | Dog      | 16,000 + 32,000 | S, A      |
| S. R.   | 24       | F   | 95                        | 105           | ++++  | 1            | Cow      | 1:500 wt/vol*   | S         |
| C. L.   | 26       | F   | 104                       | 89            | +++   | 1            | Horse    | 1,000 + 2,000   | S         |
| G. B.   | 36       | F   | 81                        | 76            | ++    | 0            | Cat      | 32,000          | S, D      |
| U. L.   | 22       | F   | 90                        | 105           | +++   | 3            | Cat      | 4,000 + 8,000   | S, D      |
| M. C.   | 33       | F   | 86                        | 80            | +++++ | 4            | Horse    | 125             | S, T, B   |
| I-B. K. | 52       | F   | 92                        | 95            | +++   | 3            | Cat      | 16,000          | S, A      |
| L. W.   | 24       | F   | 73                        | 84            | +++   | 3            | Cat      | 4,000 + 8,000   | S         |
| K. F.   | 28       | F   | 101                       | 98            | +++   | 2            | Horse    | 500 + 1,000     | S, T      |
| I-L. O. | 38       | F   | 76                        | 67            | +++++ | 3            | Birch    | 4,000           | S, A      |

Pt, Patient; SQU/ml, standardized quality units per milliliter; D, inhaled DSCG when it was required; S, inhaled salbutamol when it was required; A, antihistamine orally when it was required; T, theophylline orally on a regular basis; B, terbutaline orally on a regular basis.

No oral medication at least 12 hours before challenge or on challenge day. No inhaled medication at least 6 hours before challenge or on challenge day.

\*This extract in weight per volume.

relevant allergens, and none of the patients had had an airway infection within 4 weeks before the challenges. All patients had mild asthma with FEV<sub>1</sub> >70% of the predicted value, and they only occasionally used inhaled  $\beta_2$ -agonists (duration, 4 to 6 hours), DSCG, and oral antihistamines (duration, 12 hours). Two patients, however, were also taking oral bronchodilators, one patient, theophylline (duration, 12 hours), and one patient, theophylline plus a  $\beta_2$ -agonist (duration, 12 hours). Inhaled  $\beta_2$ -agonists and DSCG were withdrawn at least 6 hours before challenge. Oral  $\beta_2$ -agonists, antihistamines, and theophyllines were withdrawn at least 12 hours before the challenge. None of the patients used inhaled or oral corticosteroids. The study was approved

by the Ethics Committee of the University of Göteborg and the National Board of Health and Welfare.

**Design (Fig. 1)**

The study included 5 test days in which the main comparison (double-blind randomized) was between formoterol (long-acting) and salbutamol (short-acting). Placebo was administered on the first study day, single blindly, to confirm the adequate allergen dose. The study was completed with a control day to study spontaneous variations in PEFR (without allergen) during the corresponding time of the day.

On a special day after the double-blind study, the effect of pretreatment with inhaled adrenaline was studied in the

same patients (This study will be presented separately [Dahlöf C, Palmqvist M, Svedmyr N, et al., In manuscript.]): day 0, titration of the allergen dose producing a positive LAR; day 1, single-blind pretreatment with inhaled placebo 30 minutes before allergen challenge (five puffs via spacer); days 2 and 3, double-blind, randomized, crossover pretreatment with equipotent bronchodilating doses<sup>13</sup> of inhaled salbutamol, 500 µg (five puffs of 100 µg each), or inhaled formoterol, 30 µg (five puffs of 6 µg each) 30 minutes before challenge.

A spacer (Volumatic, Glaxco Co., Hamburg, Germany) was used to optimize the deposition of the aerosol into the airways: control day, without pretreatment and allergen challenge, the patients recorded PEFR (0 to 10 hours) during the same time period as on the preceding test days.

### Bronchial allergen challenge

The bronchial allergen challenge was conducted in a standardized method<sup>17</sup> with a Pari Inhalier Boy (Paul Ritzau Pari Wesk, Starnberg, Germany) nebulizer with an output of 0.8 ml/min (continuous nebulization) and a particle size of 0.5 to 5.5 µm. For titrating an allergen dose eliciting a positive LAR, two-fold increasing concentrations of the allergen solution were inhaled by tidal volume breathing for 2 minutes with 15-minute intervals between the doses. The FEV<sub>1</sub> was measured at 5, 10, and 15 minutes. The challenge procedure was started by ALK diluent (2 ml NaCl) followed by the lowest allergen dose, 32 standardized quality units per milliliter (ALK arbitrary unit). Provocation was stopped when the FEV<sub>1</sub> had dropped by >20% from postdiluent value. The FEV<sub>1</sub> was then measured at 20, 30, and 40 minutes and every hour up to 6 hours. The PEFR was measured 20 minutes after the last allergen dose and then every hour up to 10 hours. At this point, the patients were administered 30 mg of prednisolone orally and 0.8 mg of salbutamol by inhalation to stop the asthmatic reaction. The individual fall in PEFR after 4 to 10 hours was between 15% to 33% (mean, 21%) from baseline. On the following day (placebo), the last effective allergen dose was administered to five patients. In the other seven patients, the next doubling dose was also administered since the fall in FEV<sub>1</sub> was <20% in the early reaction. On the following 2 study days, these individual allergen doses (one or two dose steps) were constant. The changes in PEFR and FEV<sub>1</sub> was calculated from the pretreatment values (30 minutes before challenge).

### Statistics

The randomized, double-blind part of the study was analyzed as a two-period crossover trial according to the method of Hills and Armitage.<sup>18</sup> The Wilcoxon's rank-sum test was used for the comparison between sequence groups, and  $p < 0.05$  was adopted as the level of significance.

## RESULTS

### Early reaction (Fig. 2)

The mean maximum change in FEV<sub>1</sub> within 30 minutes after challenge calculated from the pretreatment

value was -35.6% (range, -27% to -59%) after placebo, +6.2% (+22% to -15%) after salbutamol, and +3.2% (+17% to -13%) after formoterol.

The mean change in PEFR at 20 minutes, calculated from the pretreatment value, was -29.4% (range, -10% to -61%) after placebo, +7.2% (+28% to -3%) after salbutamol, and +4.9% (+17% to -5%) after formoterol. Compared to pretreatment PEFR values, the mean values at this point were significantly different. The FEV<sub>1</sub> values were, except after formoterol treatment, also significantly different from pretreatment values. The mean fall in FEV<sub>1</sub> and PEFR was significantly smaller after the two active drugs compared with these values after placebo treatment ( $p < 0.01$ ). There was no significant difference between the two drugs.

### Late reaction (Fig. 2)

The mean maximum change in PEFR 4 to 10 hours after challenge calculated from the pretreatment value was -15.7% (range, -4% to -36%) for placebo, -7.0% (+2% to -31%) for salbutamol, and -2.2% (+6% to -24%) for formoterol. The individual results are presented in Table II.

The mean maximum fall after pretreatment with formoterol was not significantly different from the pretreatment value, but after pretreatment with placebo and salbutamol, the corresponding fall in PEFR was significant.

The mean maximum fall in PEFR (4 to 10 hours) was significantly smaller after formoterol than after salbutamol treatment ( $p = 0.04$ ).

### Control day (Fig. 2)

On the control day, the mean maximum changes in PEFR was -1% (range, +5% to -4%). The intraindividual difference at each time was not significant.

## DISCUSSION

The bronchial allergen challenge test is a clinical model for studying allergic asthma. The EAR, which reaches its maximum within 30 minutes, is believed to be a direct effect of the action of mast cell mediators.<sup>19</sup> The LAR, recurring after some hours, is believed to be due to an inflammatory reaction induced by the mediators generated in the early reaction.<sup>20-22</sup> The bronchial obstruction during the late phase cannot be fully reversed by  $\beta_2$ -agonists, indicating the presence of edema of the bronchial mucosa.<sup>23</sup> Several studies have supported the hypothesis of a significant inflammatory component during the LAR, which has made the LAR interesting as a model for the development of chronic asthma.<sup>24-26</sup>

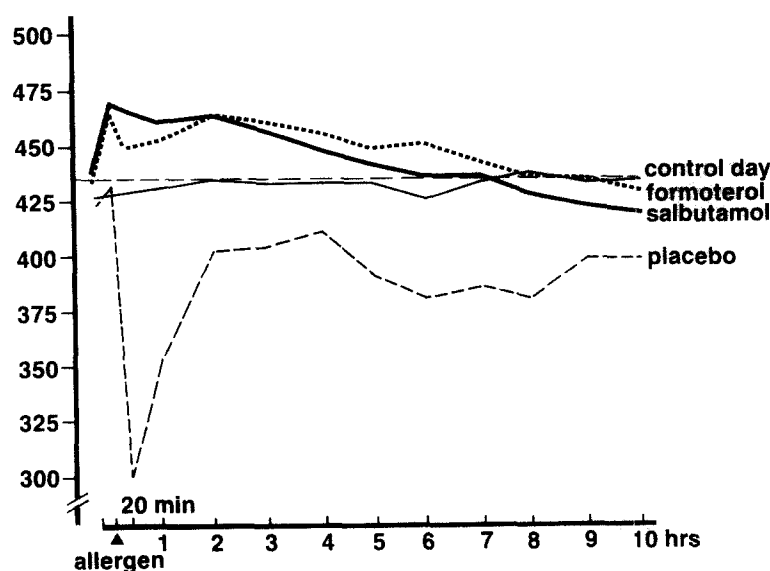


FIG. 2. PEFR (mean), immediate and late phase, after allergen challenge and pretreatment with formoterol, salbutamol, and placebo, respectively, and on control day (without medication or allergen challenge).

TABLE II. Maximal percent change in PEFR from baseline 4 to 10 hours after allergen challenge (LAR) on titration day and after pretreatment with inhaled placebo, salbutamol, and formoterol

| Pt No. | Titration | Placebo | Salbutamol | Formoterol |
|--------|-----------|---------|------------|------------|
| 1      | -23       | -20     | -2         | +2         |
| 2      | -15       | -4      | +2         | +2         |
| 3      | -15       | -10     | -2         | -1         |
| 4      | -18       | -4      | 0          | +6         |
| 5      | -16       | -9      | -1         | +5         |
| 6      | -27       | -11     | -3         | -7         |
| 7      | -18       | -6      | 0          | 0          |
| 8      | -33       | -30     | -31        | -24        |
| 9      | -16       | -21     | -11        | -8         |
| 10     | -30       | -36     | -17        | 0          |
| 11     | -15       | -11     | -8         | +4         |
| 12     | -27       | -25     | -12        | -7         |

Pt, Patient.

In this study, the new long-acting  $\beta_2$ -agonist, formoterol, was compared with the more short-acting salbutamol.<sup>27</sup> Salbutamol, as well as terbutaline, has been reported to be ineffective against the LAR.<sup>11, 12</sup> The present study demonstrated, however, an inhibitory effect on the LAR by both active drugs. Formoterol was somewhat more effective than salbutamol ( $p = 0.04$ ). The effect by formoterol on the LAR might be explained by its long bronchodilating duration. However, the effect of salbutamol can hardly be explained by its bronchodilating effect that, at the time for the LAR, is negligible. Since the effect by

salbutamol appeared to be significant up to more than 10 hours, it is more likely explained by some antiallergic or anti-inflammatory mechanism. An inhibition of mediator release has also been demonstrated for  $\beta_2$ -agonists in in vitro and in vivo models.<sup>28-30</sup> These results are supported by later studies on the effect of inhaled salbutamol<sup>31</sup> and salmeterol<sup>32</sup> on late-phase reactions.

Since the placebo-treatment day was not randomized, the placebo values have not been included in the statistical analysis because of the risk of period effects. However, during the randomized period (days 2 and

3), there was no indication of a period effect, strongly indicating that the differences between placebo and the two active drugs are relevant.

There may be several reasons why this study on  $\beta_2$ -agonists, in contrast to some earlier studies, demonstrated a positive result: different observation times, different study designs, different statistical methods,<sup>11</sup> and different doses and modes of administration of the drugs.<sup>11, 12</sup> In subjects with asthma with a varying degree of bronchial obstruction, the pre- and post-treatment values may differ considerably, especially when bronchodilators are being studied. To be able to compare different studies of LAR, the importance of deciding a uniform baseline value has been demonstrated.<sup>33</sup> In the present study, we selected the pre-treatment value as the most basal and stable baseline value.

An LAR can be mixed with a spontaneous decrease in the patient's pulmonary function during the day as a consequence of lack or withdrawal of antiasthmatic medication. For this reason, PEFR was also recorded on a separate control day when patients refrained from using any medication. There were no signs of spontaneous deteriorations during the day.

There appears to be a general agreement in the literature that the LAR should reach its maximum 4 to 8 hours after allergen exposure.

However, there is no general agreement concerning the beginning and end of the reaction, the type of pulmonary function test to be used, and the degree of changes. To be considered a significant LAR in this study, a fall in PEFR of >15% 4 to 10 hours after challenge was required. The 15% limit is chosen mainly because of practical reasons. In the pretrial, in which 25 patients were tested, most of the tested patients demonstrated a varying degree of change in their PEFR, from 0% to 45% fall in PEFR, demonstrating that there is a sliding scale from no reaction to a strong LAR.<sup>34</sup> For a more precise clinical definition, we therefore suggest that an LAR is defined as a bronchial obstruction 4 to 10 hours after allergen exposure that significantly exceeds the patient's spontaneous change in pulmonary function.

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