Rapid reversibility of the allergen-induced pulmonary late-phase reaction by an intravenous $\beta_2$-agonist

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Peebles, R. Stokes, J r., Solbert Permutt, and Alkis Togias. Rapid reversibility of the allergen-induced pulmonary late-phase reaction by an intravenous $\beta_2$-agonist. J. Appl. Physiol. 84(5): 1500–1505, 1998.—This study was performed to determine the degree to which $\beta_2$-adrenergic receptor agonists can reverse the allergen-induced late reduction in lung function. On two occasions, seven asthmatic subjects were administered terbutaline or its vehicle by intravenous infusion 7 h after inhaled allergen, at which point the forced expiratory volume in 1 s was 57% of baseline. On another occasion, terbutaline was infused at baseline to determine maximal attainable bronchodilation. After allergen challenge, terbutaline rapidly improved lung function. At the end of terbutaline infusion, the forced expiratory volume in 1 s reached 100 ± 1.3% of baseline and 84.2 ± 4.3% of maximal attainable value, but the bronchodilating effect of the $\beta_2$-agonist did not plateau. The values for forced vital capacity were 102 ± 1.3% of baseline and 95.1 ± 3% of maximal attainable value. The kinetics of the terbutaline effect, when it was infused at baseline, were similar to those in the late phase. Because the late-phase reduction in lung function is rapidly reversible by $\beta_2$-adrenergic agonists, we conclude that it is caused mainly by bronchial smooth muscle spasm.

asthma; bronchospasm; bronchoprovocation; bronchodilator

ALLERGEN-INDUCED PULMONARY late-phase responses occur in ~40–60% of individuals with asthma who inhale a sufficient dose of antigen to cause 20% or greater immediate fall in forced expiratory volume in 1 s (FEV1) (16). The physiological mechanisms leading to increased airway obstruction during the late-phase reaction are unknown. Some investigators in the field believe that the predominant obstructive mechanism is thickening of the airway wall (secondary to edema and to cellular infiltration) and hypersecretion with plugging of mucus in the small airways. This is contrasted with the immediate obstructive reaction to allergen, which is clearly reversible by inhaled $\beta_2$-adrenergic bronchodilators (6) and is believed to derive from the smooth muscle spasmosgens (histamine, leukotrienes, prostaglandins) that mucosal mast cells release in response to the allergen-immunoglobulin E interaction taking place on their surface. The above concept of the late-phase reaction is based on the fact that, whereas anti-inflammatory agents given before allergen inhalation fully inhibit the late phase, inhaled $\beta_2$-adrenergic bronchodilators, whether administered before or at the peak of the late phase, have only a partial effect (6, 15, 19, 25).

To determine the magnitude of the component of airway obstruction in the late phase that is not due to readily reversible causes, we designed this study in which a $\beta_2$-agonist was administered intravenously and at a high dose at the time point considered to be the peak of the allergen-induced, late-phase reaction.

METHODS

Subjects. Seven allergic subjects with mild asthma [27.1 ± 2.6 (SD) yr; range 21–42 yr] were recruited to participate in the study. Each subject had previously demonstrated methacholine hyperreactivity with a concentration of methacholine that induces a 20% fall in FEV1 of <3 mg/ml [average: 0.5 ± 0.8 (SD) mg/ml]. Average baseline FEV1 expressed as percent of predicted was 83.8 ± 19.3 (SD)%, and forced vital capacity (FVC) was 98.3 ± 13.5%. All subjects had a positive epicutaneous skin test to ragweed extract, and their history pointed to worsening asthma symptoms during the ragweed pollen season. All subjects had previously performed whole lung antigen challenge in our laboratory and were known to develop pulmonary late-phase reactions to ragweed extract (Greer Laboratories, Lenoir, NC), with a nadir FEV1 at 4–8 h after whole lung antigen challenge, that was at least 15% lower than the value obtained after the preantigen inhalation of diluent. Subjects were nonsmokers; had not suffered an upper respiratory tract infection in the 4 wk before the study; and were not on oral or inhaled steroids, cromolyn, or nedocromil. They also refrained from using inhaled short-acting or oral bronchodilators for at least 8 h before testing; no subject had ever used a long-acting inhaled $\beta_2$-agonist. Informed consent was obtained from each subject, and the protocol was approved by the Johns Hopkins Bayview Medical Center Institutional Review Board.

Study protocol. In designing this study, we considered two important issues. The first issue is the deposition of an aerosol in an obstructed airway tree. Under such circumstances, the aerosol will be deposited more centrally than required to affect the small airways and to attain maximal bronchodilatory response (5, 13). For this reason, we decided to deliver the $\beta_2$-agonist intravenously. We chose terbutaline because of the clinical experience with intravenous administration of this agent in the obstetrics field (11, 12). The second issue is the fact that even mild asthmatic subjects are, to some extent, obstructed at baseline. Given that the nature of baseline obstruction (airway smooth muscle constriction vs. edema and/or cellular infiltration) is unknown, we felt that we first had to establish the maximal bronchodilation that can be attained at baseline and to compare it with the maximal pulmonary function attained with the administration of $\beta_2$-agonist at the peak of the allergen-induced, late-phase reaction. For this reason, our study was designed to involve two phases. In the first phase, to determine the maximal attainable bronchodilation, terbutaline was administered intravenously at baseline. In the second phase, terbutaline was delivered intravenously at the presumed peak of the allergen-induced, late-phase reaction and was compared with its vehicle in a randomized crossover design.
The study consisted of three single-day visits. The first and second visits took place on consecutive days, whereas the second and third visits were separated by at least 2 wk. Baseline FEV₁ was >60% of the predicted value on all three visits. On the first visit (phase of the study), subjects came to the laboratory at 3 PM, and baseline pulmonary function testing was performed. An intravenous catheter was placed in each subject’s arm at 3:45 PM. Terbutaline was then administered by an intravenous loading dose of 200 µg over a 10-min interval; immediately thereafter, a constant infusion rate of 10 µg/min of terbutaline [60 ml/h of 0.1 mg/ml terbutaline in 5% dextrose in water (D₅W)] was started. The infusion rate was increased by increments of 5 µg/min every 10 min to a final dose of 25 µg/min. The final dose was maintained for 15 min before the infusion was terminated. Conventional forced expiratory spirometry was performed after the 200-µg bolus infusion and every 5 min thereafter. Throughout the terbutaline infusion, subjects were under continuous electrocardiographic monitoring, and blood pressure and pulse rate measurements were performed at 5-min intervals. A pilot study showed that this dosing strategy was effective in achieving a bronchodilation plateau, which we designated as the maximal effect of terbutaline in reversing airway obstruction.

On the second and third visits (phase II of the study), subjects came to the laboratory at 8 AM. Spirometry was first performed to ensure that FEV₁ was >60% of the predicted value. Subjects then underwent an allergen-inhalation challenge. This involved inhalation of phosphate-buffered saline (diluent challenge) followed by half-log increasing concentrations of ragweed extract. The initial concentration of ragweed was determined by a previously performed intradermal skin test titteration as the concentration that resulted in a wheal of at least 10-mm mean diameter. The inhaled ragweed for occlusion was terminated when FEV₁ declined by at least 20% from the values obtained after saline inhalation. By interpolation of the dose-response curve of the antigen challenge, the cumulative provocative dose of ragweed that caused a 20% decline in FEV₁ was calculated in breath units (1 breath unit = 1 breath of a 1 protein nitrogen unit/ml solution).

Subjects remained in the laboratory for 7 h for hourly pulmonary function tests. An intravenous catheter was placed into an antecubital vein at around 3:45 PM (7 h after the end of the allergen-inhalation challenge).

During phase II, subjects were randomized to receive either terbutaline, identical to the above-detailed protocol for visit 1, or a vehicle infusion. It is important to note that the time the infusion of terbutaline or vehicle was started, ~3:45 PM, was the same for all three study visits (phase I and phase II). Four subjects received terbutaline infusion on visit 2, and three received terbutaline infusion on visit 3. On the day vehicle was administered, the total volume infused was the same as in the other two visits in which terbutaline was used. Also, the rates of infusion and the time frames for rate increase were identical in the terbutaline and the D₅W visits. Pulmonary function measurements during the intravenous infusion on visits 2 and 3 were performed in a manner identical to the first visit.

Data analysis. To analyze the results, we employed nonparametric statistics because of the small number of subjects involved in the study. Friedman ANOVA was used to compare baseline pulmonary function values among the three visits. We also used Friedman ANOVA to compare the postinfusion final time point among the three study visits. If ANOVA yielded statistically significant results, the various time points were compared by using the Wilcoxon matched pairs, signed-ranks test as a post hoc approach. To compare the 11 time points before and during the course of the terbutaline or placebo infusions, we used the Wilcoxon test. ANOVA results could not be computed in these cases because of the relatively small sample size (7 subjects, 11 repeated measures). Results are presented in the text, Table 1, and Figs. 1–4 as means ± SE, unless otherwise indicated. Two-tailed P values ≤ 0.05 were considered statistically significant.

RESULTS

Baseline pulmonary function measurements on all three visits are shown in Table 1. Friedman ANOVA yielded a significant difference in baseline FEV₁ among the three visits. Post hoc analysis indicated that the difference was significant between visit 1 and the placebo visit, but not between the terbutaline visit and placebo visit, or between visit 1 and the terbutaline visit. The ragweed-challenge provocative dose that caused 20% decline in FEV₁ is also shown in Table 1. This value was not significantly different between the two phase II visits, indicating that no confounding factor with respect to the amount of antigen delivered was introduced during phase I.

The response to whole lung antigen challenge as measured by FEV₁ is shown in Fig. 1. As a group, subjects experienced a classic late-phase reaction to inhaled ragweed challenge. All subjects on the day of terbutaline infusion, and six of the seven subjects on the day of placebo infusion, fulfilled the conventional criteria for a late-phase reaction (FEV₁ < 85% of the control saline challenge at any single point between hours 4 and 7). No statistically significant differences between the two phase II visits were found in the early reaction (represented by the measurement that followed the highest dose of allergen) or in any of the seven hourly time points that followed. Therefore, pulmonary function at the beginning of terbutaline or placebo infusion was not different between the two allergen challenge visits.

Figure 2 shows FEV₁ values at baseline, before drug infusion, and during the course of the infusion, on all three study visits. During visit 3 (phase I), terbutaline led to significant improvement in pulmonary function. Every measurement that followed the 200-µg bolus infusion was significantly higher than the preinfusion value (P < 0.03 in every case). We also found significant differences between the postbolus infusion and the 30-min time point (P = 0.02), as well as between the 30-min time point and the 60-min time point (P = 0.03). Table 1. Baseline FEV₁ and FVC on the three study visits and log of ragweed PD₂₀ on the 2 allergen-inhalation challenge days
and the 40-min time points ($P = 0.05$). There was no difference between 40 and 45 min ($P = 0.31$), indicating that a plateau in bronchodilation was attained. Also, when $FEV_1$ values at the end of the terbutaline infusion were compared with the predicted ones, no significant difference was noted ($P = 0.5$). Before the beginning of the terbutaline infusion, $FEV_1$ was lower than predicted, although not at a statistically significant level ($P = 0.06$). Still, two of the seven subjects had $<80\%$ predicted $FEV_1$ after the termination of the terbutaline infusion, suggesting the presence of either a bronchodilator-resistant obstructive component or a relative insensitivity to $\beta$-agonists.

On the day $D_5W$ was infused 7 h after allergen challenge, we found no significant change in $FEV_1$ over the preinfusion value ($P > 0.2$ at all time points). In contrast, on the day terbutaline was infused 7 h after allergen challenge, every measurement following the bolus infusion yielded a value that was significantly higher than preinfusion ($P < 0.02$ in every case). Approximately 50\% of the total terbutaline-induced improvement in pulmonary function was obtained after the bolus administration; however, improvement in $FEV_1$ continued to occur throughout the terbutaline infusion. By the end of the infusion (45 min), the baseline (preantigen challenge) values of that day were reached (100.6 $\pm$ 2.4\% baseline). $FEV_1$ was significantly higher than the respective 45-min time point of the vehicle-infusion protocol ($P < 0.02$) but was 84.2 $\pm$ 4.3\% of the maximal bronchodilation attained on visit 1 ($P < 0.02$). However, a plateau in bronchodilation was not attained on the day terbutaline was infused at the peak of the late-phase reaction: each of the last three measurements, i.e., 35, 40, and 45 min, yielded values that were significantly higher than the respective preceding time point ($P < 0.03$ in every case). This clearly indicates that the maximal bronchodilatory effect of terbutaline was not reached by the end of the postallergen infusion protocol.

Figure 3 depicts FVC values at baseline, before and during terbutaline or $D_5W$ infusion, on all 3 study visits. During visit 1 (phase I) terbutaline was infused immediately after initial pulmonary function determination, whereas during other 2 visits (phase II) either terbutaline or $D_5W$ (placebo) was infused 7 h after termination of an antigen-inhalation challenge (preinfusion). BSL, baseline $FEV_1$ before initiation of antigen challenge. Thick vertical arrow indicates bolus infusion of 200 $\mu$g terbutaline (or equivalent volume of $D_5W$); horizontal arrows indicate duration of infusion for each of the increasing concentrations of terbutaline (or the equivalent $D_5W$). *$P < 0.05$ against preceding $FEV_1$ measurement; ¥$P = 0.02$ against equivalent time point of antigen challenge/terbutaline infusion visit; †$P = 0.02$ against equivalent time point of antigen challenge/$D_5W$ infusion visit.
recorded on visit 1 (95.1 ± 3%, P = 0.25) or from the predicted values (P = 0.9). When the two phase I visits were considered, FVC at the end of the terbutaline infusion was significantly higher than that at the end of vehicle infusion (P = 0.02).

To obtain some indication whether the terbutaline-induced bronchodilation on visit 1 and on the allergen-challenge visit was driven by the same pharmacological mechanism, we examined the kinetics of both responses. Figure 4 depicts the terbutaline effect on FEV\textsubscript{1} as a percentage of the maximal effect of each visit. The values for each time point were derived by using the following formula: \((\text{FEV}_1 \text{ at each time point} - \text{FEV}_1 \text{ at the preinfusion time point})/(\text{maximal FEV}_1 \text{ during terbutaline infusion for that visit} - \text{FEV}_1 \text{ at the preinfusion time point})\). As can be seen, the kinetic curves for both terbutaline infusions on visit 1 and after inhaled ragweed challenge are superimposed; no statistically significant differences between the two protocols were detected at any point.

DISCUSSION

Our study demonstrated that the late reduction in lung function caused by allergen-inhalation challenge in asthmatic subjects is rapidly and almost completely reversible by an intravenous \(\beta_2\)-adrenoreceptor agonist.

Since the late 1970s, several studies have demonstrated that administration of inhaled, short-acting \(\beta\)-adrenergic agonists before allergen-inhalation challenge inhibited the acute obstructive response but failed to affect the late phase (6, 10, 21). Although one can admit that this was not surprising given the short duration of action, this finding was used as one of the observations to support the hypothesis that airway obstruction in the late phase is mainly due to mechanisms other than smooth muscle spasm. The concept was further supported by the fact that the late-phase reaction is highly inhibitable by inhaled or oral corticosteroids and, to a significant degree, by cromolyn and nedocromil, agents that have anti-inflammatory but no bronchodilator properties (4, 6, 10, 18). Furthermore, inflammatory cell infiltration has been demonstrated in airway biopsy samples and bronchoalveolar lavage fluids obtained during the late-phase reaction (1, 2, 7, 9).

In a recent study (24), albuterol was given before antigen challenge and protected against the late-phase reaction. The authors argued that this effect may have been the result of anti-inflammatory properties, which could include inhibition of mast-cell mediator release, prevention of bronchial wall edema, and inhibition of mucous production. Such properties of \(\beta\)-agonists have been observed in variable degrees in in vitro systems.
and in animal models (3, 17). In another recent study, salmeterol, a long-acting β-agonist, was found to inhibit both the early- and the late-phase reactions as well as the inhaled antigen-induced increase in bronchial reactivity in subjects with asthma (23). Because of its long duration of action, salmeterol may inhibit the late-phase airflow limitation through a direct effect on smooth muscle. However, the authors raised several arguments to support the concept that their observation was primarily the result of anti-inflammatory activity (23), yet such activity has been questioned by other in vivo work in humans (8). These studies have shown that β-agonists can prevent the late-phase response but provided no evidence that they can reverse it.

In 1990, Van Bever et al. (25) used a more appropriate protocol by delivering an inhaled β-agonist (feno-terol) to reverse an already established late-phase pulmonary reaction. In this study, 57.7% of the late-phase reaction was reversed by the β-agonist. The authors concluded that smooth muscle contraction played a significant role in the antigen-induced, late-phase airway obstruction (25). In a retrospective evaluation of late asthmatic reactions to occupational sensitizers, Malo and colleagues (15) also reported that, in 66% of patients with late reduction in lung function in whom 200 µg of albuterol were administered by a metered-dose inhaler 7–8 h after the end of the inhalation challenge, lung function returned to >90% of baseline. The magnitude of the β-agonist-induced reversal of late airway obstruction is much more dramatic in our study, making the conclusion that a rapidly reversible obstructive component dominates the pulmonary late-phase reaction more definitive. This may be the result of some design elements that we decided to incorporate into our protocol.

We chose the intravenous route for drug administration because of the variable deposition of inhaled medications during severe bronchoconstriction (5). This approach makes us more confident that the β-agonist was equally distributed in the bronchial tissue of all study participants. Of course, systemic administration of an agent has the potential for inducing additional effects that may confound the conclusion that the smooth muscle relaxant property of terbutaline is the sole mechanism by which reversal of the late-phase reaction was achieved. However, such alleged effects could still occur if the agent were to be delivered by inhalation.

The control experiment we incorporated was to administer the intravenous β-agonist at baseline, under an infusion protocol identical to that of the late-phase reaction. We reasoned that, by comparing the maximal bronchodilation attained with terbutaline infusion during the late-phase reaction to that attained with terbutaline infusion at baseline, we could accurately measure the true reversibility of the antigen-induced late airway obstruction. Such experiment was not incorporated into the aforementioned study by Van Bever et al. (25).

When we compared the endpoint of terbutaline infusion on visit 1 with that on the day when inhaled ragweed was administered, we found a difference of ~16 and 5% for FEV1 and FVC, respectively (Figs. 2 and 3). This was statistically significant for FEV1 and could be interpreted as indicative of a terbutaline-resistant component of the late-phase airway obstruction. However, the fact that we did not observe a plateau in FEV1 with terbutaline infusion during the late phase suggests that maximal bronchodilation was not reached and that, had we continued the infusion of terbutaline, complete reversal of airway obstruction may have been achieved. Because of concerns about potential drug toxicity, however, we had designed the study to be completed after 15 min of 25 µg/min terbutaline infusion. Indeed, at the time the infusion was terminated, over one-half of our subjects had pulse rates over 120 beats/min and all but one complained of tremulousness. Despite this argument, we cannot exclude the possibility that a small β-agonist-resistant component may contribute to late-phase physiology. This may be due to airway swelling and mucous plugging or to reduced efficiency of β-agonist-receptor coupling that could occur as a result of antigen-induced inflammation. The apparently better effect of terbutaline on FVC compared with FEV1 may indicate that early closure of the small airways during the late-phase reaction is more reversible by β-agonists compared with large airway obstruction.

It could also be argued that the mode of action of terbutaline, when administered under baseline conditions, may differ from its mode of action when it is administered in the midst of an ongoing allergic inflammatory reaction. To address this possibility in an indirect fashion, we compared the kinetics of the terbutaline effect both on visit 1 and on the late-phase visit (Fig. 4). With the kinetics being identical, we believe that terbutaline resulted in bronchodilation, through the same mechanism of action, on both study visits.

The results of this study are consistent with the interpretation that spasmogens of bronchial smooth muscle are the predominant mediators of both the early and late responses to antigen challenge. In this respect, it is worth noting that, in a recently reported study, the allergen-induced, late-phase reaction in patients with asthma was very significantly inhibited by the concurrent administration of an antihistamine and a leukotriene-receptor antagonist (20). Both histamine and sulfidopeptide leukotrienes have been found in increased concentrations in the bronchoalveolar lavage fluids of subjects undergoing antigen provocation and

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1 It is possible that terbutaline may have exerted some of its effect by reversing tissue edema. To address this possibility, our volunteers were given allergen intradermally, and the skin late-phase reaction was monitored in the course of both the terbutaline and D5W infusions. We were only able to eliciting skin late-phase reactions in three subjects, and this did not allow us to perform analysis with enough power to detect statistical differences between the two arms. In the subjects in whom late-phase skin reactions were elicited, the size of skin induration did not change after the terbutaline, relative to the D5W infusion.
should be considered prime candidates for the generation of the late-phase reaction (14, 22). It is important to note that these autacoids not only produce smooth muscle contraction but can also increase vascular permeability, cause edema, and increase mucus production. These events may also contribute to the airway obstruction seen after allergen challenge. However, the magnitude of this contribution may not be as significant as that of smooth muscle constriction.

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