Salmeterol Does Not Alter Increased Bronchial Responsiveness Caused by Organic Dust Exposure*

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Background: Exposure in a swine house induces airway inflammation and increases bronchial responsiveness to methacholine in healthy subjects.

Study objectives: The aim was to investigate whether a long-acting β2-agonist, salmeterol, alters the increased bronchial responsiveness induced in healthy subjects following exposure to organic dust in a swine barn.

Design and subjects: The study includes three separate parts. In the first part (part 1), healthy subjects inhaled salmeterol (50 μg bid, n = 8) or placebo (n = 8) over 2 weeks. In part 2, healthy subjects inhaled one single dose of salmeterol (100 μg, n = 6) or placebo (n = 6) 1 h prior to exposure in a swine barn, which was followed by a bronchial methacholine challenge. In part 3, eight healthy individuals inhaled placebo or salmeterol (100 μg), 2 h or 8 h prior to a bronchial methacholine provocation, without being exposed in the swine barn.

Results: Exposure caused an increase of bronchial responsiveness to methacholine by 3.2 doubling concentration steps (25 to 75th percentiles, 2.8 to 4.1) and 2.6 doubling concentration steps (25 to 75th percentiles, 1.4 to 3.7) in the placebo and salmeterol groups (2 weeks), respectively, with no significant differences between the groups (p = 0.3; part 1). Similar results were obtained when salmeterol was administered as a single dose (part 2) prior to exposure. However, salmeterol significantly attenuated the bronchial responsiveness to methacholine by 1.2 doubling concentration steps (0.8 to 1.7) 8 h after inhalation (part 3).

Conclusions: Salmeterol inhalation did not protect against the increased bronchial responsiveness induced in healthy subjects following exposure to organic dust when administered for 2 weeks or as a single dose prior to exposure. This lack of protection cannot be explained by homologous β2-adrenoceptor desensitization. We hypothesize that exposure to organic material may alter the airway response to β2-agonists.

Abbreviations: ANOVA = analysis of variance; IL = interleukin; PC20 = cumulative provocation concentration of methacholine causing a 20% decrease in FEV1; PEF = peak expiratory flow; TNF = tumor necrosis factor; VC = vital capacity

Key words: airway inflammation; β2-agonist; bronchial responsiveness; methacholine; organic dust; salmeterol

A few hours of exposure in a swine house induces, on average, a threefold increase in bronchial responsiveness to methacholine in healthy subjects, as has been demonstrated in a number of studies.1–4 The increase in bronchial responsiveness is accompanied by an intense airway inflammation with a massive influx of inflammatory cells and release of proinflammatory cytokines and other mediators.1,5–8 We have previously shown that increased bronchial responsiveness, observed after exposure to organic dust in a swine barn, was not influenced by pretreatment with cromoglycate, although this therapy halved the influx of neutrophils and almost totally inhibited the airway release of tumor necrosis factor.
was to find out whether inhalation of a long-acting β2-agonist would attenuate the increased response to methacholine following exposure in a swine barn. It thus seems clear that those inhaled drugs, which are frequently used in asthma due to their ability to interact with the inflammatory process and thereby decrease bronchial hyperresponsiveness, have no influence on the increase in bronchial responsiveness induced by exposure to organic dust in a swine house.

β2-Adrenoceptor agonists are functional antagonists. This means that they relax airway smooth muscle and inhibit bronchoconstriction irrespective of the nature of the bronchoconstrictor stimulus. Thus, in asthmatic subjects, β2-agonists inhibit or attenuate bronchoconstriction induced by methacholine, histamine, exercise, dry air hyperpnea, and allergen. In healthy subjects, administration of a bronchodilator causes a highly significant increase of specific airway conductance presumably by relaxation of airway smooth muscles. Although pharmacologic intervention with the inflammatory process induced by exposure in a swine barn does not influence the postexposure increase in bronchial responsiveness in healthy subjects, it seems reasonable, based on the above arguments, to assume that a β2-adrenoceptor agonist would attenuate the increased response to methacholine following exposure. Therefore, the aim of the present study was to find out whether inhalation of a long-acting β2-adrenoceptor agonist (salmeterol) alters bronchial responsiveness to methacholine in healthy subjects and whether this drug counteracts the increased bronchial responsiveness observed after exposure to organic dust in a swine barn.

Materials and Methods

Subjects

All participating subjects were healthy, nonatopic nonsmokers and had no history of asthma or allergic diseases. The study involves three separate parts. Each subject participated in only one part of the study. To be considered eligible for participation, all subjects were required to have normal results of physical examination and spirometry. Absence of atopy was evaluated by a questionnaire and, in parts 2 and 3, this was confirmed by a negative skin-prick test result with extracts from 15 common aeroallergens. The studies were approved by the ethics committee at the Karolinska Institute and were conducted with the informed consent of all subjects. In part 3, only subjects in whom a cumulative provocation concentration of methacholine causing a 20% decrease in FEV1 (PC20) could be defined were included, enabling detectable decreases in bronchial responsiveness after salmeterol inhalation.

Exposure and Measurements

Parts 1 and 2 of the study include a 3-h stay in a swine barn. The exposure took place while weighing pigs in a swine-confinement building. Part 1 was performed in a swine house containing 700 to 900 pigs; since this building was later closed, part 2 had to be performed in another swine house containing 300 pigs. The participants assisted the farmer and guided the pigs through weighing boxes.

The individuals carried personal equipment to sample inhalable dust (IOM inhalable dust sampler; SKC Ltd; Blandford, UK) and respirable dust (plastic cyclone samplers; Casella London Limited; Bedford, UK), which were weighed and analyzed for endotoxin (Limulus amebocyte assay, QCL-1000; Endotoxin; BioWhittaker; Walkersville, MD).

Study Design

Part 1: Sixteen subjects (4 women; mean age, 27 years; range, 21 to 46 years) were randomized to inhale either salmeterol (50 μg bid, n = 8) or placebo (bid, n = 8) during 10 to 14 days prior to exposure in a swine house, in a single-blind manner.

Part 2: Twelve subjects (7 women; mean age, 24 years; range, 18 to 32 years) were randomized to inhale one single dose of salmeterol (100 μg, n = 6) or placebo (n = 6) 1 h prior to exposure in a swine-confinement building, in a single-blind manner. In healthy subjects, inhalation of one dose of 100 μg of salmeterol is safe from side effects.

In parts 1 and 2, exposure in the swine house started 1 h after the last dosing. A bronchial methacholine challenge was performed at least 2 to 4 weeks before the exposure and 7 h after the start of the exposure in the swine house (ie, 8 h after the last dosing).

Part 3: Eight individuals (six women; mean age, 37 years; range, 24 to 52 years) inhaled placebo or salmeterol (100 μg) 2 h or 8 h prior to a methacholine provocation without being exposed in a swine barn. The study was performed in a randomized, single-blind, cross-over design on 4 different days, separated by at least 48 h.

Symptoms

Following exposure in swine houses, symptoms (shivering, headache, malaise, muscle pain, and nausea) were assessed using a questionnaire. The symptoms were graded according a severity scale (1 = no symptoms, 5 = severe symptoms). Only scores of 4 or 5 were classified as significant. In parts 1 and 2, oral temperature was measured directly before and immediately after exposure and thereafter, with 2-h interval, over 10 h.

Lung Function Tests

Lung function (FEV1 and vital capacity [VC]) was measured using a wedge spirometer (Vitalograph; Medical Instrumentation; Buckingham, UK) according to the American Thoracic Society criteria. Local reference values were used. Peak expiratory flow (PEF) was measured with a mini-Wright peak flowmeter (Clement Clarke Ltd; London, UK) before and every hour after medication until methacholine provocation was performed. The best of three blows was registered.

Methacholine Provocation

Bronchial responsiveness was assessed by a methacholine challenge performed with a jet nebulizer (Sidestream; Medic-Aid; Pagham, UK) according to the method described by Sund-
Continuous breathing (2 s of inhalation and 2 s of exhalation) was performed over 1 min, starting with the diluent followed by inhalation of increasing concentrations of methacholine (0.5 to 32 mg/mL in part 2 and 0.5 to 64 mg/mL in parts 2 and 3). Each increase represented a doubling of the concentration. The time interval between two subsequent inhalations was 6 min, and the challenge was stopped when FEV₁ fell by 20% of the value obtained after inhalation of the diluent or after inhalation of the highest concentration of methacholine (32 mg/mL or 64 mg/mL). In subjects who had not attained a 20% decrease in FEV₁ when the maximum concentration was reached, but had a >15% decline, PC₂₀ was calculated by extrapolation (log-extrapolated from the last two points). For the statistical calculations using doubling concentration steps, all who had not declined 20% at the maximum concentration were assigned a maximum PC₂₀ value (65 mg/mL in part 1 and 33 mg/mL in part 2). The results were expressed as the PC₂₀, cumulative provocative dose of methacholine causing a 20% fall in FEV₁, and dose-response slope, i.e., the percentage of FEV₁ decrease as a function of the cumulated dose of methacholine calculated by linear regression.25,26

Statistical Analysis

Lung function data and body temperature are presented as mean ± SD or range, and comparisons were made using Student t test. Results of PEF and temperature measurements were calculated using analysis of variance (ANOVA). Concentration of airborne dust and endotoxin, bronchial responsiveness, exhaled nitric oxide, and results from serum analyses are presented as median (25 to 75th percentiles), and comparisons were made using Wilcoxon signed-rank sum test and Mann-Whitney U test. Comparisons of bronchial responsiveness between groups were calculated using Mann-Whitney U test (PC₂₀ doubling concentration steps) and ANOVA (log-transformed dose-response slope data); p < 0.05 was considered significant. Statistical analysis was performed using software (StatView, version 5.0.1; SAS Institute; Cary, NC). We have in previous studies1,24 shown that every single individual increases by 2.5 to 3 doubling dose steps in bronchial responsiveness following 3 h of exposure in a swine house. Since β₂-agonists are functional antagonists, we anticipated a protection in every participant. We therefore assumed that six to eight subjects would be sufficient to detect a clear effect.

RESULTS

Exposure Measurements

In part 1, median inhalable and respirable dust levels during the exposure were 23.1 mg/m³ (21.2 to 38.1 mg/m³) and 1.02 mg/m³ (0.76 to 1.27 mg/m³), respectively. The corresponding endotoxin concentrations were 665 ng/m³ (375 to 1,003 ng/m³) and 30 ng/m³ (13 to 54 ng/m³). In part 2, the inhalable and respirable dust levels during the exposure were 11.5 mg/m³ (10.4 to 18.3 mg/m³) and 0.68 mg/m³ (0.55 to 0.93 mg/m³), respectively. The corresponding endotoxin concentrations were 236 ng/m³ (123 to 281 ng/m³), and 41.1 ng/m³ (28.1 to 44.3 ng/m³). There were no significant differences in exposure between groups in either study.

Symptoms

In part 1, five participants in the placebo group and four participants in the salmeterol group experienced symptoms (grade 4 to 5) following exposure. The maximal temperature increase during the day was 1.5°C (range, 0.6 to 3.0°C) in the placebo group (p < 0.01) and 0.9°C (range, -0.2 to 1.7°C) in the salmeterol group (p < 0.001), with no significant difference between the groups (F = 1.4; p = 0.2).

In part 2, five participants in the placebo group and three participants in the salmeterol group experienced symptoms (grade 4 to 5) following exposure. The maximal temperature increase during the day was 1.1°C (range, 0.9 to 1.5°C) in the placebo group (p < 0.01) and 1.4°C (range, 1.0 to 1.6°C) in the salmeterol group (p < 0.001), with no significant difference between the groups (F = 0.8; p = 0.6).

Lung Function

In all participating subjects (n = 36), FEV₁ was 100.4 ± 10.2% of predicted value, VC was 95.6 ± 11.1% of predicted value, and FEV₁/VC ratio was 84.6 ± 5.8%. In parts 1 and 2, exposure in the swine house caused a slight decrease of VC and FEV₁ in all subjects, without significant differences between the groups (Table 1).

In part 2, PEF significantly fell in all individuals after exposure. The maximal PEF reduction compared to the premedication value 1 h prior to exposure was 68 ± 42 L/min in the placebo group (p < 0.05) and 58 ± 33 L/min (p < 0.01) in the salmeterol group (F = 2.0; p = 0.08 between the groups; Fig 1).

In part 3, VC (percentage of predicted) was 98.4 ± 10.1% and 99.6 ± 10.7% 2 h and 8 h after inhalation of placebo and 98.6 ± 10.1% and 99.2 ± 9.2% 2 h and 8 h after inhalation of salmeterol, respectively (not significant). FEV₁ (percentage of predicted) was 104.0 ± 11.9% and 105.1 ± 11.7% 2 h and 8 h after placebo and 106.8 ± 11.6% and 107.7 ± 11.7% 2 h and 8 h after inhalation of salmeterol, respectively (p < 0.05 between treatments 2 h and 8 h after dosing). The maximum PEF difference, representing the change between premedication value and the maximal PEF value obtained 8 h after medication, was 6.1 ± 7.4% following placebo inhalation and 9.1 ± 4.8% following salmeterol inhalation (F = 0.04, p = 1.0).

Bronchial Responsiveness

Pre-exposure PC₂₀ was 5.4 mg (3.4 to 12.8 mg) in the female subjects (n = 17) and 9.5 mg (3.8 to 26.4 mg) in the male subjects (n = 19). These values are within the normal range according to our own
Table 1—VC and FEV<sub>1</sub> in Part 1 of the Study (Medication for 2 wk) and Part 2 (a Single Dose) Presented as Percentage of Predicted Values or as Postexposure Difference, Measured 7 h After the Start of the 3-h Exposure in a Swine House and Expressed as Percentage of Pretrial Values<sup>*</sup>

<table>
<thead>
<tr>
<th>Variables</th>
<th>Pretrial VC, % Predicted</th>
<th>After Exposure, VC Difference, %</th>
<th>Pretrial FEV&lt;sub&gt;1&lt;/sub&gt;, % Predicted</th>
<th>After Exposure, FEV&lt;sub&gt;1&lt;/sub&gt; Difference, %</th>
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</thead>
<tbody>
<tr>
<td>Study part 1 (n = 8)</td>
<td></td>
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<tr>
<td>Placebo</td>
<td>89.1 ± 10.0</td>
<td>-3.6 ± 2.9†</td>
<td>95.1 ± 7.3</td>
<td>-7.8 ± 4.1‡</td>
</tr>
<tr>
<td>Salmeterol</td>
<td>100.4 ± 12.3</td>
<td>-4.0 ± 5.4</td>
<td>104.0 ± 8.2</td>
<td>-5.4 ± 5.1§</td>
</tr>
<tr>
<td>Between groups</td>
<td>p = 0.6</td>
<td></td>
<td></td>
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<tr>
<td>Study part 2 (n = 6)</td>
<td></td>
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<tr>
<td>Placebo</td>
<td>95.4 ± 9.0</td>
<td>-3.2 ± 2.8§</td>
<td>100.4 ± 7.2</td>
<td>-5.9 ± 4.3§</td>
</tr>
<tr>
<td>Salmeterol</td>
<td>93.1 ± 11.5</td>
<td>-2.9 ± 4.4</td>
<td>96.3 ± 13.7</td>
<td>-3.3 ± 3.6</td>
</tr>
<tr>
<td>Between groups</td>
<td>p = 0.7</td>
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</table>

*Data are presented as mean ± SD. The change in VC and FEV<sub>1</sub> did not differ between the salmeterol and placebo groups.  
†p < 0.01 compared to pre-exposure values.  
‡p < 0.001 compared to pre-exposure values.  
§p < 0.05 compared to pre-exposure values.

reference material: 3.8 mg (2.1 to 9.5 mg) in women (n = 101), and 7.5 mg (2.7 to 26.9 mg) in men (n = 102). One female and one male subject in the present study had a PC<sub>20</sub> value below the tenth percentile of our reference material.

Part 1: After 2 weeks of placebo or salmeterol inhalations (50 µg bid), bronchial responsiveness to methacholine increased in both groups following swine house exposure. In the placebo group, PC<sub>20</sub> fell from 4.68 mg/mL (3.71 to 12.85 mg/mL) before exposure to 0.83 mg/mL (0.33 to 1.08 mg/mL) after exposure (p = 0.01), representing an increase in bronchial responsiveness by 3.2 doubling concentration steps (2.8 to 4.1 doubling concentration steps).

In the salmeterol group, PC<sub>20</sub> fell from 14.81 mg/mL (5.27 to 24.37 mg/mL) before exposure to 1.46 mg/mL (0.78 to 4.20 mg/mL) after exposure (p = 0.02) representing an increase in bronchial responsiveness by 2.6 doubling concentration steps (1.4 to 3.7 doubling concentration steps). There was no significant difference between the groups (p = 0.3; Fig 2). The methacholine dose-response slope increased in the placebo group (p = 0.01) and in the salmeterol group (p = 0.07), with no significant difference between the groups (F = 2.2; p = 0.2; Fig 2).

Part 2: Bronchial responsiveness to methacholine increased in all subjects after exposure in a swine barn after one single dose of inhaled placebo or salmeterol (100 µg). In the placebo group, PC<sub>20</sub> fell from 8.75 mg/mL (3.95 to 16.00 mg/mL) before exposure to 0.85 mg/mL (0.31 to 1.57 mg/mL) after exposure (p = 0.03) and in the salmeterol group from >32 mg/mL (6.70 to >32 mg/mL) before to 4.37 mg/mL (1.38 to 24.25 mg/mL) after exposure (p = 0.04). Thus, the bronchial responsiveness to methacholine increased by 3.3 doubling concentration steps (2.9 to 4.4 doubling concentration steps) in the placebo group and by >1.7 doubling concentration steps (0.4 to 2.4 doubling concentration steps) in the salmeterol group. Exact calculations are not possible in the salmeterol group since pre-exposure PC<sub>20</sub> was >32 mg/mL in three of the subjects. There was no significant difference between the groups (p = 0.1; Fig 3). The methacholine dose-response slope increased from 5.0%/mg (2.3 to 9.4%/mg) to 69.0%/mg (34.9 to 286.0%/mg) in the placebo group (p = 0.03) and from 1.0%/mg (0.5 to 6.5%/mg) to 13.9%/mg (1.6 to 32.9%/mg) in the salmeterol group (p = 0.03), with no difference between the groups (F = 2.5; p = 0.1).

![Figure 1](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/22032/ on 06/22/2017)

Figure 1. PEF before and after exposure in a swine barn in healthy subjects who inhaled one dose of placebo (n = 6) or one dose of salmeterol (100 µg, n = 6) 1 h prior to exposure (part 2). Results are presented as mean and SEM. *p < 0.05 and **p < 0.01 compared with pre-exposure values. There is no significant difference between the group (F = 2.0; p = 0.08). 2w = 2 weeks.
Part 3: Bronchial responsiveness to methacholine was attenuated by 2.1 doubling concentration steps (1.2 to 2.8 doubling concentration steps) after inhalation of one dose of salmeterol (100 µg) compared with inhalation of placebo, when methacholine challenge was performed 2 h after inhalation. Eight hours after inhalation, the corresponding value was 1.2 doubling concentration steps (0.8 to 1.7 doubling concentration steps) [Fig 4]. The PC₂₀ was 15.6 mg/mL (7.51 to 25.12 mg/mL) 2 h after salmeterol inhalation and 3.69 mg/mL (2.39 to 4.69 mg/mL) 2 h after placebo inhalation (p = 0.01). At 8 h, PC₂₀ was 8.72 mg/mL (5.60 to 11.74 mg/mL) after salmeterol inhalation and 3.75 mg/mL (2.40 to 5.34 mg/mL) after placebo inhalation (p = 0.01). The dose-response slope was lower after inhalation of salmeterol (3.7%/mg [2.1 to 6.5%/mg] at 2 h and 4.1%/mg [3.3 to 9.4%/mg] at 8 h) than after placebo (13.5%/mg [10.4 to 23.8%/mg] at 2 h and 13.3%/mg [9.9 to 23.0%/mg at 8 h], p = 0.02 and p = 0.04, respectively).

Discussion

In the present study, we have shown that 2 weeks of treatment or one single dose of the long-acting β₂-agonist salmeterol failed to influence the increase in bronchial responsiveness induced by exposure in a swine barn. We also found that a single dose of salmeterol inhibits methacholine-induced bronchoconstriction in unexposed healthy subjects. We are
not aware of any other situation in which a \( \beta_2 \)-agonist fails to attenuate bronchial responsiveness to a bronchoconstrictor stimulus.

Regular inhalation of a \( \beta_2 \)-agonist induces tachyphylaxis and loss of the bronchoprotective effect against bronchoconstrictor stimuli. Thus, tolerance to the protective effect of \( \beta_2 \)-agonists against bronchoconstrictor stimuli such as methacholine, exercise, and allergen has been demonstrated in a number of studies. There are data to support that tolerance to the bronchoprotective effect of salmeterol against methacholine induced bronchoconstriction occurs within 12 h after the start of twice-daily treatment. Thus, in the present study, it was reasonable to assume that the lack of protective effect after 2 weeks of salmeterol inhalations against increased methacholine responsiveness induced by organic dust exposure was, at least in part, due to the development of \( \beta \)-adrenoceptor tachyphylaxis. However, we found that neither one single dose nor 2 weeks of treatment attenuated the increased bronchial responsiveness following exposure. Furthermore, the almost identical effect of regular dosing and a single dose of salmeterol makes it unlikely that the lack of effect after 2 weeks of treatment was a consequence of homologous \( \beta_2 \)-adrenoceptor down-regulation.

A single dose of salmeterol offers a protective effect against methacholine-induced airway obstruction in asthmatic subjects and the decrease in responsiveness, assessed either as cumulative provocative dose of methacholine causing a 20% fall in
FEV<sub>1</sub> or PC<sub>20</sub>, has in previous studies varied between 2.4 doubling doses and 3.8 doubling doses. In a study of asthmatic children, a maximum protective effect was found 1 h after inhalation (3.8 doubling doses) followed by a gradual decrease, to 2.0 doubling doses at 12 h. As we were not aware of any studies showing that salmeterol actually attenuates bronchial responsiveness to methacholine in healthy subjects, we performed a control experiment (part 3). As expected, we found that a single dose (100 μg) of inhaled salmeterol offered a good protection against methacholine-induced bronchoconstriction in healthy subjects and that PC<sub>20</sub> in all subjects substantially increased 2 h and 8 h after salmeterol inhalations compared to placebo. These findings implicate that the exposure in a swine barn has altered the airway response to salmeterol.

Taken together, one dose of salmeterol offered a protection against methacholine-induced bronchoconstriction in all unexposed subjects with a median increase of approximately 1.2 doubling concentration steps, 8 h after inhalation in healthy subjects. In the present study, exposure in a swine barn caused an increase in bronchial responsiveness by > 1.7 doubling concentration steps in the subjects who inhaled one dose of salmeterol prior to exposure. However,
in the subjects inhaling placebo before exposure, the bronchial responsiveness increased 3.3 concentration steps. This finding does not exclude a small protective effect of one dose of salmeterol. However, such an interpretation is contradicted by the finding that the methacholine dose-response slope, which is possible to calculate in all subjects (also in those with no defined pre-exposure PC20), showed no differences between the placebo and salmeterol groups.

The explanation to the failure of a β2-agonist, being a functional antagonist, to protect against dust-induced increased bronchial responsiveness is not clear. There is a possibility that proinflammatory cytokines, released in response to agents present in the swine house environment, have direct effects on airway smooth-muscle cells that lead to β2-adrenergic receptor desensitization and reduced ability of the cells to relax when stimulated by β2-agonists. Previous studies support that IL-1β and TNF-α may induce a heterologous desensitization of β2-adrenoceptors leading to decreased responsiveness of the β2-adrenoceptor. The precise mechanism underlying this hyporesponsiveness is not fully explored. Wills-Karp et al showed that IL-1β and TNF-α reduce the β2-adrenoceptor mediated relaxation in guinea pig trachea in an antigen model, and Hakonarson et al have shown that IL-1 decreases the β2-adrenergic relaxation in atopic asthmatic sensitized airway smooth muscle. There is a hypothesis that IL-1β and TNF-α synergize to induce cyclo-oxygenase-2 expression leading to enhanced production of prostaglandin-E2. This in turn, may increase intracellular cyclic adenosine monophosphate formation leading to phosphorylation of the β2-adrenergic receptor by activated protein kinase A, and consequently a functional desensitization of the response to β2-adrenoceptor stimulation. Thus, IL-1β and, to a lesser extent, TNF-α may be in part responsible for reduced relaxant responses to β2-adrenoceptor agonists in asthmatic patients and in animal models of asthma.

We know from previous studies in vivo (BAL performed 24 h after exposure) and in vitro (studies on alveolar macrophages and epithelial cells) that exposure to organic dust from swine houses induces production and release of a number of proinflammatory cytokines, including IL-1β and TNF-α. Therefore, it is likely that postexposure formation and release of TNF-α and IL-1β from cells in the lower airways may have influenced the β2-adrenoceptors on airway smooth-muscle cells. A high number of airborne bacteria and fungi (ranging from 10^4 to 10^6 cfu/m3 of air) as well as microbial products are found in swine barns. Inhalation of bacterial endotoxin induces a local and systemic inflammatory response in humans and endotoxin is a potent inducer of macrophage activation, resulting in the production of proinflammatory cytokines such as TNF-α and IL-1. Acute exacerbations of asthma are often induced by viral or bacterial respiratory infections, and during asthma exacerbations the bronchodilator potency of inhaled β2-agonists is often impaired. Many patients report that inhaled β2-agonists “stop working” during asthma worsening due to a respiratory infection. Reddel et al have shown that PEF variation is strikingly different during asthma exacerbations compared with poor asthma control and that the fall in PEF during clinical respiratory infections does not adequately reverse either spontaneously or after treatment with bronchodilators. These results suggest abnormality of β2-adrenoceptor function during acute exacerbations of asthma due to infections. The acute inflammatory airway reaction to exposure in a swine barn thus has many features in common with an acute exacerbation of asthma, and the mechanism behind the failure of the β2-agonist to counteract bronchoconstriction in these two situations may be similar.

It could be argued that the increased bronchial responsiveness to methacholine following exposure in a swine barn is due to mucosal swelling. We know from previous studies that arachidonic acid metabolites are produced in the airway reaction to swine dust exposure, and it could be assumed that these mediators would contribute to airway edema. However, attempts to block the increase in bronchial responsiveness following exposure by the administration of zileuton, a leukotriene synthesis inhibitor, failed. In addition, if the increased methacholine responsiveness is due to mucosal swelling, β2-adrenoceptor agonists would still have a protective effect by the relaxation of bronchial smooth muscle. In conclusion, we have demonstrated that salmeterol attenuates the bronchial response to methacholine but fails to influence the increased bronchial responsiveness induced by exposure in a swine barn in healthy subjects.

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