In vitro sensitization of human bronchus by β₂-adrenergic agonists

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Faisy, Christophe, Emmanuel Naline, Jean-Luc Diehl, Xavier Emonds-Alt, Thierry Chinet, and Charles Advenier. In vitro sensitization of human bronchus by β₂-adrenergic agonists. Am J Physiol Lung Cell Mol Physiol 283: L1033–L1042, 2002.—Incubation of human distal bronchi from 48 patients for 15 h with 10⁻⁷ M fenoterol induced sensitization characterized by an increase in maximal contraction to endothelin-1 (ET-1) and acetylcholine (ACh). Incubation of human bronchi with 10⁻⁶, 3 × 10⁻⁶, and 10⁻⁵ M forskolin (an adenylyl cyclase activator) reproduced sensitization to ET-1 and ACh. The sensitizing effect of fenoterol was inhibited by coincubation with gliotoxine (a nuclear factor-κB inhibitor), dexamethasone, indomethacin (a cyclooxygenase inhibitor), GR-32191 (a TP prostanoid receptor antagonist), MK-476 (a cysteinyl leukotriene type 1 receptor antagonist), SR-140333 (a 10⁻⁶ B2 receptor antagonist), SR-42801 (neurokinin types 1, 2, and 3 tachykinin receptor antagonists) with or without HOE-140 (a bradykinin B2 receptor antagonist), SB-203580 (an inhibitor of the 38-kDa mitogen-activated protein kinase, p38MAPK), or calphostin C (a protein kinase C blocker). Our results suggest that chronic exposure to fenoterol induces proinflammatory effects mediated by nuclear factor-κB and pathways involving leukotrienes, prostanoids, bradykinin, tachykinins, protein kinase C, and p38MAPK, leading to the regulation of smooth muscle contraction to ET-1 and ACh.

β₂-agonists; airway sensitization; airway smooth muscle; endothelin-1; asthma

THE FATAL ACUTE ASTHMA CASES attributable to fenoterol abuse in the 1980s in New Zealand started a controversy concerning the potential worsening of the bronchial hyperresponsiveness by the β₂-adrenergic receptor agonists (7, 14, 42). Studies in animals and humans showed that chronic exposure to fenoterol or salbutamol induces a nonspecific bronchial sensitization, whereas the relaxant effects of these β₂-agonists on the airway smooth muscle are not decreased (11, 59, 60). The bronchial sensitization induced by fenoterol is similar to the sensitization provoked by ovalbumin in sensitized guinea pigs (60). Chronic administration of salbutamol at low doses to guinea pigs increases airway reactivity to histamine and methacholine (11). In humans, long-term use of salbutamol increases the bronchial hyperresponsiveness to histamine but does not cause subsensitization of β₂-adrenoceptors to salbutamol (59). In a bovine tracheal model, Katsunuma and colleagues (36) showed that prolonged incubation with fenoterol induced an increased contractile responsiveness to neurokinin A (NKA). In 1995, Peters and colleagues (47) suggested that the continuous activation of the intracellular signal transduction caused by the β₂-adrenoceptor stimulation could induce a proinflammatory process mediated by nuclear transcription factors in rat lung. A recent study in our institution showed that the transcription factor nuclear factor-κB (NF-κB) is involved in fenoterol-induced hyperresponsiveness to NKA in guinea pig isolated trachea (52).

Endothelin-1 (ET-1) is a 21-amino acid peptide recently implicated in chronic inflammatory airway diseases such as asthma and chronic obstructive pulmonary disease (25, 26, 41, 46). ET-1 is synthesized and metabolized in lung, and ET-1 receptors (ETA and ETB) are widely distributed in airway cells (21, 26, 41). ET-1 is one of the most potent contractile agents of human airway smooth muscle and can induce airway inflammation, airway hyperresponsiveness, and airway remodeling in animals and humans (25, 26, 27, 41), suggesting that ET-1 could be a major component of asthma pathophysiology (10, 22, 26, 27). The purpose of this study was to determine the sensitizing effect of fenoterol on the contraction to ET-1 of human bronchi and to investigate the role of inflammatory mediators and signal transduction pathways involved in airway sensitization to ET-1 induced by β₂-adrenoceptor agonists.

METHODS

Human bronchial tissue preparations. Bronchial tissues were surgically removed from 54 patients with lung cancer (45 men and 9 women, 62 ± 10 yr of age); all patients were
smokers or ex-smokers. Just after resection, segments of human bronchi (1–3 mm ID) were taken as far as possible from the malignant lesion. They were placed in oxygenated Krebs-Henseleit solution composed of (in mM) 119 NaCl, 4.7 KCl, 2.5 CaCl₂, 1.2 KH₂PO₄, 25 NaHCO₃, and 11.7 glucose. After removal of adhering lung parenchyma and connective tissues, rings of the same bronchi were prepared (5–7 mm long, 1–3 mm ID) and divided into two paired groups: one (control) group was placed in Krebs-Henseleit solution at room temperature (21°C) for 15 h; the other (pretreated) group was treated with 10⁻⁷ and 10⁻⁶ M fenoterol, 10⁻⁷ and 10⁻⁶ M formoterol, 10⁻⁸ M salbutamol, 10⁻⁶ M salmeterol, or a cAMP activator, i.e., forskolin (10⁻⁶, 3 × 10⁻⁶, and 10⁻⁵ M), for 15 h at room temperature. Incubation time and temperature were chosen in agreement with the work of Katsunuma and colleagues (36) and our previous work (52).

Fenoterol, formoterol, salbutamol, salmeterol, and forskolin concentrations were chosen according to Wang and colleagues (60), Katsunuma and colleagues, and our previous work (52). After removal of adhering lung parenchyma and connective tissues, rings of the same bronchi were prepared (5–7 mm long, 1–3 mm ID) and divided into two paired groups: one (control) group was placed in Krebs-Henseleit solution at room temperature (21°C) for 15 h; the other (pretreated) group was treated with 10⁻⁷ and 10⁻⁶ M fenoterol, 10⁻⁷ and 10⁻⁶ M formoterol, 10⁻⁸ M salbutamol, 10⁻⁶ M salmeterol, or a cAMP activator, i.e., forskolin (10⁻⁶, 3 × 10⁻⁶, and 10⁻⁵ M), for 15 h at room temperature. Incubation time and temperature were chosen in agreement with the work of Katsunuma and colleagues (36) and our previous work (52).

Expression and analysis of data. Contractile responses were expressed in tension (g) compared with the basal tone recorded before the start of the concentration-response curve. Values are means ± SE. The data are expressed in terms of E_max for efficacy and −log EC₅₀ (pD₂) for potency. E_max represents the maximal contraction induced by ET-1 and ACh and is expressed in tension compared with the basal tone. ΔE_max represents the difference between E_max obtained with the pretreated bronchi and E_max obtained with the paired control human bronchi. ΔE_max was expressed in grams compared with basal tone. −log EC₅₀ values were derived graphically from the log-log concentration-effect curves and defined as the negative logarithm of the drug concentration that caused 50% of maximal effect of ET-1 (10⁻⁷ M). Δ(−log EC₅₀) represents the difference between −log EC₅₀ obtained with the pretreated bronchi and −log EC₅₀ obtained with the paired control human bronchi. Bronchi with E_max of ET-1 <0.7 g were excluded from analysis, because we considered that a low level of E_max is a reliable marker of dysfunction of contractility. Statistical analysis of the results was performed using Student’s t-test (2-tailed, for paired samples). P < 0.05 was considered significant.

RESULTS

Sample. Bronchi of 48 from 54 patients (89%) yielded an E_max of ET-1 ≥0.7 g. Incubation of these 48 bronchi for 15 h at 21°C with fenoterol (10⁻⁷ M) significantly increased their maximal contraction to ET-1 (Fig. 1) and ACh (E_max of ACh = 2.66 ± 0.18 and 1.99 ± 0.15 g in the presence and absence of fenoterol, respectively, n = 48, P < 0.01). Incubation of human bronchi with fenoterol did not change significantly the potency of ET-1 (−log EC₅₀ = 8.52 ± 0.05 and 8.41 ± 0.06 in the presence and absence of fenoterol, respectively, n = 48, not significant; Fig. 1). Because sensitization of human bronchi by fenoterol was characterized by an increase in maximal contraction to ET-1, we investigated the human bronchi with ΔE_max of ET-1 >0 after 15 h of fenoterol exposure. Among the 48 bronchi, 38 (79%)...
fenoterol. Values are means ± SE (n = 48). *P < 0.01; **P < 0.001 vs. control.

Table 1. Effect of incubation of human bronchi with β2-adrenoceptor agonists or forskolin on potency of ET-1 and ACh

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>n</th>
<th>Δ(log EC50) ET-1, log unit</th>
<th>ΔEmax, g ET-1</th>
<th>ΔEmax, g ACh</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fenoterol</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10⁻⁷ M</td>
<td>38</td>
<td>0.12 ± 0.07</td>
<td>+0.88 ± 0.09†</td>
<td>+1.06 ± 0.13‡</td>
</tr>
<tr>
<td>10⁻⁶ M</td>
<td>6</td>
<td>0.17 ± 0.11</td>
<td>+0.91 ± 0.19†</td>
<td>+1.11 ± 0.24‡</td>
</tr>
<tr>
<td>Formoterol</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10⁻⁸ M</td>
<td>6</td>
<td>0.36 ± 0.22</td>
<td>+1.09 ± 0.22†</td>
<td>+1.11 ± 0.22†</td>
</tr>
<tr>
<td>10⁻⁷ M</td>
<td>8</td>
<td>0.08 ± 0.21</td>
<td>+1.11 ± 0.32*</td>
<td>+1.13 ± 0.43*</td>
</tr>
<tr>
<td>10⁻⁸ M</td>
<td>8</td>
<td>0.12 ± 0.19</td>
<td>+0.55 ± 0.11†</td>
<td>+0.70 ± 0.16*</td>
</tr>
<tr>
<td>Salmeterol (10⁻⁶ M)</td>
<td>7</td>
<td>0.01 ± 0.14</td>
<td>+0.73 ± 0.25*</td>
<td>+0.90 ± 0.35*</td>
</tr>
<tr>
<td>Forskolin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10⁻⁶ M</td>
<td>8</td>
<td>0.49 ± 0.16</td>
<td>+0.16 ± 0.31</td>
<td>+0.34 ± 0.50</td>
</tr>
<tr>
<td>3 × 10⁻⁶ M</td>
<td>8</td>
<td>0.42 ± 0.12</td>
<td>+1.06 ± 0.38*</td>
<td>+0.98 ± 0.47</td>
</tr>
<tr>
<td>10⁻⁵ M</td>
<td>8</td>
<td>0.42 ± 0.10</td>
<td>+1.54 ± 0.58*</td>
<td>+1.94 ± 0.73*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, number of bronchi. Bronchi were pretreated for 15 h at 21°C. Δ(log EC50), difference between −log EC50 (potency) obtained with pretreated bronchi and −log EC50 obtained with paired control human bronchi; ΔEmax, difference between maximal contraction (maximal efficacy) to endothelin-1 (ET-1) and to acetylcholine (ACh) in pretreated bronchi and in paired control; *P < 0.05; †P < 0.01; ‡P < 0.001 vs. control.
Table 2. Effect of incubation of human bronchi with anti-inflammatory drugs, proinflammatory mediator receptor antagonists, or NO synthase inhibitor on maximal contraction to ET-1 and ACh in absence of fenoterol

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>n</th>
<th>∆E_{max}, g ET-1</th>
<th>∆E_{max}, g ACh</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gliotoxine (10⁻⁶ M)</td>
<td>12</td>
<td>+0.22 ± 0.26</td>
<td>+0.28 ± 0.33</td>
</tr>
<tr>
<td>Dexamethasone (10⁻⁶ M)</td>
<td>12</td>
<td>−0.06 ± 0.28</td>
<td>−0.21 ± 0.34</td>
</tr>
<tr>
<td>Indomethacin (10⁻⁶ M)</td>
<td>12</td>
<td>+0.36 ± 0.37</td>
<td>+0.25 ± 0.41</td>
</tr>
<tr>
<td>GR-32191 (10⁻⁷ M)</td>
<td>12</td>
<td>+0.25 ± 0.20</td>
<td>+0.48 ± 0.21</td>
</tr>
<tr>
<td>GR-32191 (10⁻⁶ M)</td>
<td>12</td>
<td>+0.18 ± 0.20</td>
<td>+0.08 ± 0.24</td>
</tr>
<tr>
<td>MK-476 (10⁻⁸ M)</td>
<td>12</td>
<td>−0.01 ± 0.35</td>
<td>−0.11 ± 0.40</td>
</tr>
<tr>
<td>MK-476 (10⁻⁷ M)</td>
<td>12</td>
<td>+0.54 ± 0.39</td>
<td>+0.38 ± 0.39</td>
</tr>
<tr>
<td>t-NAME (10⁻³ M)</td>
<td>12</td>
<td>+0.11 ± 0.39</td>
<td>−0.12 ± 0.49</td>
</tr>
<tr>
<td>SR-142801 (10⁻⁷ M)</td>
<td>12</td>
<td>−0.13 ± 0.30</td>
<td>−0.21 ± 0.37</td>
</tr>
<tr>
<td>SR-140333 + SR-48968 + SR-142801 (10⁻⁷ M)</td>
<td>8</td>
<td>+0.49 ± 0.37</td>
<td>+0.27 ± 0.35</td>
</tr>
<tr>
<td>SR-140333 + SR-48968 + SR-142801 + HOE-140 (10⁻⁷ M)</td>
<td>8</td>
<td>+0.33 ± 0.49</td>
<td>+0.38 ± 0.63</td>
</tr>
<tr>
<td>SB-203580 (10⁻⁷ M)</td>
<td>8</td>
<td>−0.19 ± 0.25</td>
<td>+0.46 ± 0.41</td>
</tr>
<tr>
<td>Calphostin C (10⁻⁷ M)</td>
<td>8</td>
<td>−0.04 ± 0.22</td>
<td>−0.35 ± 0.27</td>
</tr>
<tr>
<td>Calphostin C (10⁻⁷ M) + SB-203580 (3 × 10⁻⁷ M)</td>
<td>8</td>
<td>−0.13 ± 0.35</td>
<td>−0.23 ± 0.48</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, number of bronchi. Bronchi were pretreated for 15 h at 21°C. Gliotoxine, nuclear factor-κB inhibitor; GR-32191; prostanoid and prostaglandin TP receptor antagonist; MK-476, cysteinyl leukotriene receptor antagonist; t-NAME, N-nitro-l-arginine methyl ester, a nitric oxide (NO) synthase inhibitor; SR-14033, SR-48968, and SR 142801, tachykinin NK₁, NK₂, and NK₃ receptor antagonists; HOE-140, bradykinin B₂ receptor antagonist; SB-203580, 38-kDa mitogen-activated protein kinase inhibitor; calphostin C, protein kinase C blocker. No difference was statistically significant.

Fig. 2. Effect of coincubation for 15 h at 21°C with gliotoxine (a nuclear factor-κB inhibitor), dexamethasone, indomethacin (a cyclooxygenase inhibitor), GR-32191 (a prostanoid and prostaglandin TP receptor antagonist), MK-476 (a cysteinyl leukotriene receptor antagonist), nitro-l-arginine methyl ester (t-NAME, a nitric oxide synthase inhibitor), and SR-142801 (a tachykinin NK₂ receptor antagonist) on E_{max} of ET-1 and ACh in the presence of 10⁻⁷ M fenoterol. Values are means ± SE (n = 12). *P < 0.05; **P < 0.01; ***P < 0.001 vs. control.

Fig. 3. Effect of coincubation for 15 h at 21°C with SR-140333 + SR-48968 + SR-142801 (tachykinin NK₁, NK₂, and NK₃ receptor antagonists) and SR-140333 + SR-48968 + SR-142801 + HOE-140 (a bradykinin B₂ receptor antagonist) on E_{max} of ET-1 and ACh in the presence of 10⁻⁷ M fenoterol. Values are means ± SE (n = 8). *P < 0.05; **P < 0.01 vs. control.
with L-NAME (10⁻³ M) increased significantly the maximal contraction to ET-1 and ACh (Table 4). Incubation for 45 min at 37°C with indomethacin, GR-32191, MK-476, or combinations of the tachykinin NK₁ + NK₂ + NK₃ receptor antagonists did not alter the maximal contraction of the human bronchi (Table 4). Addition of HOE-140, a bradykinin B₂ receptor antagonist, to the tachykinin NK₁ + NK₂ + NK₃ receptor antagonists did not modify the Eₘₐₓ of ET-1 and ACh of the bronchi (Table 4). When the same paired bronchi were sensitized to ET-1 and ACh by fenoterol for 15 h at 21°C (control bars, Fig. 5), incubation for 45 min at 37°C with indomethacin, GR-32191, MK-476, and L-NAME did not affect the sensitizing effect induced by fenoterol (Fig. 5). In contrast, incubation for 45 min at 37°C with SR-140333 + SR-48968 + SR-142801 or SR-140333 + SR-48968 + SR-142801 + HOE-140 significantly decreased the rise of the maximal response elicited by fenoterol (Fig. 6). Addition of HOE-140 to SR-140333 + SR-48968 + SR-142801 did not significantly increase the inhibition of SR-140333 + SR-48968 + SR-142801 on the sensitizing effect induced by fenoterol.

DISCUSSION

In this study, we observed an in vitro sensitization to ET-1 and ACh of human bronchi by fenoterol. This sensitization is not specific to fenoterol, inasmuch as we found the same phenomenon with formoterol, salbutamol, and salmeterol. We then investigated the transduction pathways involved in sensitization of human bronchi by β₂-adrenoceptor agonists and showed...
that the nuclear transcription factor NF-κB and p38MAPK play a pivotal role in this event. Furthermore, several inflammatory processes appear to be involved in the sensitization of human bronchi by fenoterol.

Sensitization of human bronchi is not limited to fenoterol but is also observed with several β2-adrenoceptor agonists in a range of concentrations known to cause submaximal relaxation (45). We found that prolonged exposure of human bronchi to fenoterol affects maximal efficacy of ET-1 but not its potency. Our results are in agreement with the work reported by Wang and colleagues (60), who showed that chronic fenoterol exposure increased maximal airway response to ACh but not ACh EC_{50} in guinea pigs. Potency of an agonist depends in part on the affinity of receptors for binding the agonist and in part on the efficiency with which agonist-receptor interaction is coupled to response. Maximal efficacy of an agonist is determined by the characteristics of the receptor-effector system involved. In this way, our results suggest that chronic exposure to β2-adrenoceptor agonists involves changes in the contractile proteins of human bronchi but does not alter affinity of the receptors for ET-1. In addition, forskolin, a cAMP activator, increased the maximal response to ET-1 and ACh of human bronchi in a concentration-dependent manner. This suggests that prolonged activation of the cAMP-protein kinase A (PKA) system could cause sensitization. Indeed, prolonged activation of cAMP-PKA may induce stimulation of proinflammatory nuclear transcription factors, such as NF-κB or AP-1 (1, 33), and may enhance the expression of several types of receptors, such as bradykinin B_1 and B_2 and NK_1 and NK_2 receptors implied in nonspecific airway hyperresponsiveness in animals and humans (23, 32, 36, 52). However, short-term activation of the cAMP-PKA system may decrease the activity of proinflammatory enzymes such as constitutive phospholipase A_2 (cPLA_2), COX-2, 5-lipoxygenase, and MAPK (38, 54, 57). Eickelberg and colleagues (16) showed that incubation with salmeterol or salbutamol induced, probably via calmodulin stimulation, a ligand-independent activation of the glucocorticoid receptor in cultured human lung fibroblasts and vascular smooth muscle cells. Our results conflict with these findings, but it is not well known whether inflammatory processes may regulate activation of the glucocorticoid receptor in human bronchus. In asthma, various studies underlined that long-acting β2-adrenoceptor agonists exhibit very small, if any, anti-inflammatory effects when given alone (37).

Katsunuma and colleagues (36) showed that dexamethasone and cycloheximide (a protein synthesis blocker) inhibited the increased bovine tracheal smooth muscle contractile response to NKA induced by fenoterol. Saulnier and colleagues (52) abolished the fenoterol-induced tracheal sensitization in guinea pigs with two transcription factor NF-κB inhibitors (gliotoxine and pyrrolidine dithiocarbamate). In agreement with these authors, we found that gliotoxine (an NF-κB inhibitor) and dexamethasone (an NF-κB and AP-1 inhibitor) abolished the sensitization induced by fenoterol in human bronchi. These results underline the pivotal role played by NF-κB in the process of sensitization. NF-κB is involved in the expression of proinflammatory molecules and mediators (cPLA_2, COX-2, prostanooids, and leukotrienes) implicated in cellular events in asthma (2, 4, 6, 13). Effects of β2-adrenoceptor agonists on NF-κB pathways are not well known in humans. Korn and colleagues (39) recently showed that expression of interleukin-8, a proinflammatory cytokine stimulated in part by NF-κB, was markedly increased by formoterol (10^{-10} M) in cultured human bronchial epithelial cells. In contrast, Wilson and colleagues (62) observed a reduction of NF-κB expression in mucosal eosinophils and epithelial cells in bronchial biopsies from 10 atopic asthmatic patients after 8 wk of treatment with formoterol. In this study, formoterol did not reduce the immunoreactivity for adhesion molecules and proinflammatory cytokines stimulated by NF-κB, in contrast to glucocorticosteroid treatment. We found that indomethacin, GR-32191, and MK-476 significantly decreased or abolished the sensitization induced by fenoterol, suggesting that prolonged activation of the cAMP-PKA system by fenoterol may induce an enzymatic inflammatory process (cPLA_2 and COX-2) mediated by NF-κB.

We established that a mixture of tachykinin NK_1, NK_2, and NK_3 receptor antagonists decreased the sensitization elicited by fenoterol. In contrast to results obtained previously in the guinea pig trachea by Saulnier and colleagues (52), we found that the NK_3 receptor antagonist SR-142801, when used alone, did not significantly reduce the fenoterol-induced sensitization. NK_3 receptors seem to be involved in airway sensitization in guinea pigs (43), but its role is not well known in humans. However, SR-142801 has been shown to inhibit interleukin-1β-induced hyperresponsiveness to [Sar^3, Met(O)_{2}]^{11} substance P (4) and nerve growth factor in human bronchi (19). Also, our results suggest that the tachykinin NK_1 and NK_2 receptor agonists are involved in the mechanisms of sensitiza-

**Fig. 6. Effect of incubation for 45 min at 37°C with SR-140333 + SR-48968 + SR-142801 and SR-140333 + SR-48968 + SR-142801 + HOE-140 on E_{max} of ET-1 and ACh after sensitization of human bronchi by 10^{-7} M fenoterol for 15 at 21°C (control). Values are means ± SE (n = 7). ∗P < 0.05; ∗∗P < 0.01 vs. control.**
tion of human bronchi by fenoterol. This is in agreement with the recent works reported by Katsunuma and colleagues (35, 36), who found an increase of NK2 receptor expression in bovine tracheal smooth muscle after treatment with fenoterol.

Leukotrienes could amplify neurogenic inflammation by increasing release of the tachykinins from the C-fibers of the nonadrenergic noncholinergic system in asthma (28). We found that the bradykinin B2 receptor antagonist HOE-140 did not significantly enhance the inhibition of the fenoterol-induced sensitization by the tachykinin NK1, NK2, and NK3 receptor antagonists. Ricciardolo and colleagues (49) showed that a combination of the NK1 and NK2 tachykinin receptor antagonists abolished the increased bronchoconstriction produced by NKA and inhibited partially the contractile response induced by bradykinin in ovalbumin-sensitized guinea pigs, whereas HOE-140 had no effect on the increase in bronchoconstriction produced by NKA, suggesting that bradykinin induces the release of tachykinins from sensory nerves in guinea pig airways. Moreover, bradykinin may stimulate the MAPK pathways via the activation of the protein Rho GTPase, PKC, and NF-κB in human lung (29). Also, our study highlights the role of NF-κB, leukotrienes, prostanoids, tachykinin NK1 and NK2 receptor agonists, and bradykinin in the mechanisms of sensitization of human bronchi by fenoterol (Fig. 7).

Our results also show that SB-203580, a p38MAPK inhibitor, at a concentration that did not inhibit COX-1 or COX-2 activity and thromboxane synthesis (Table 3), abolishes the sensitization elicited by fenoterol. Recent studies underline the role of the MAPK in the intracellular processes of airway smooth muscle proliferation and sensitization (2, 30, 32, 46, 58, 61). Three distinct MAPK pathways have been identified in mammals: 1) p42/44MAPK, 2) stress-activated protein kinase (SAPK) or c-Jun NH2-terminal kinase (JNK), and 3) p38MAPK (15). MAPKs are involved in multiple proinflammatory mechanisms, implying humoral and neurogenic mediators. Expression of NF-κB, cPLA2, COX-2, tachykinins, bradykinin, ETB, and muscarinic M3 receptors is upregulated by MAPK in airway smooth muscle cells (12, 23, 32, 53). Moreover, MAPK increases the Gt protein activity, which results in a functional uncoupling between Gt protein and β2-adrenoceptor (2, 32, 37). In addition, MAPK enhances the myosin light chain kinase activity and the heavy chain of myosin expression (32) and may increase smooth muscle contraction probably via h-caldesmon phosphorylation and actin-F remodeling (30). Interestingly, prostanoids such as thromboxane A2 stimulate the

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**Fig. 7. Proposed mechanisms underlying sensitization of human airway smooth muscle induced by fenoterol.**

NF-κB, nuclear factor-κB; cPLA2, constitutive phospholipase A2; COX, cyclooxygenase; 5-LPO, 5-lipoxygenase; TxA2, thromboxane A2; MAPK, mitogen-activated protein kinase; PKA, protein kinase A.
MAPK by coupling the TP receptor with Go_α (activation of PKC) or G_β/γ proteins (2, 32, 34), and leukotrienes increase the MAPK expression in humans (48, 49). These data suggest that MAPK pathways could amplify the inflammatory processes induced by NF-κB and could sensitize the airway smooth muscle after prolonged exposure to fenoterol (Fig. 7).

PKC is a cyclic nucleotide-independent protein kinase implicated in regulation of airway smooth muscle tone (61). PKC-ζ enhances the activity of the protein Raf-1 and NF-κB, which activate the p^38MAPK and SAPK/JNK pathways (12). Recent publications showed that bradykinin and thromboxane A_2 activate MAPK pathways via PKC-dependent G_α_1 protein coupling in human cells (20, 29). In addition, PKC may increase the contractility of the airway smooth muscle by inhibiting caldesmon (via the MAPK pathways) and calponin (directly), which are involved in modulation of the actin-myosin interaction (30, 61). We show here that blockage of PKC by calphostin C effectively inhibits fenoterol-induced sensitization. Thus our results suggest that PKC plays a major role in the intracellular mechanisms leading to fenoterol-induced sensitization of human airway smooth muscle (Fig. 7).

We also investigated the mechanisms involved in the increase of the contractility to ET-1 and ACh of human bronchi after sensitization by fenoterol in a protocol where drugs were added after incubation with fenoterol but 45 min before addition of ET-1 or ACh at 37°C for contraction. Our results showed that neither prostanoids nor leukotrienes were involved in this mechanism. In contrast, the NK_1, NK_2, and NK_3 receptors appeared to be implicated in the increase of contractility after sensitization by fenoterol as well as bradykinin, which tended to potentiate, but not significantly, inhibition of the fenoterol-induced sensitization by the NK_1, NK_2, and NK_3 receptor antagonists. In the absence of incubation with fenoterol, we found that tachykinins and bradykinin were not involved in the process of contraction to ET-1 and ACh. Additional studies are needed to clarify the role of tachykinins and bradykinin in the contraction mechanisms after sensitization by fenoterol in human bronchi.

Studies in animal and human airways have shown that the epithelial ETA receptor may mediate NO production (via the constitutive NOS) and prostaglandin E_2 production (via the epithelial COX-2) (3, 8, 31, 44). Epithelial NO and prostaglandin E_2 are relaxant for contraction mechanisms after sensitization by fenoterol in the epithelium of the human airway smooth muscle. In a recent study, Naline and colleagues (44) found that NO is the major determinant of the epithelial regulation of the human airway smooth muscle contraction to ET-1. Our results are in agreement with these authors, because we showed that L-NAME, but not indomethacin and GR-32191, enhanced the contractility to ET-1. The epithelial regulation of the contractility to ACh was also mediated by NO. On the contrary, after sensitization by fenoterol, L-NAME failed to enhance the maximal contractility to ET-1 and ACh. We suggest that chronic exposure to fenoterol induces a disruption of the epithelial regulation of the airway smooth muscle contractility to ET-1 and ACh. Further investigations are needed to confirm and elucidate this mechanism.

Our study has several limitations. First, we studied bronchi obtained from nonhealthy subjects, who were all previous smokers. ΔE_max was increased by fenoterol exposure in only 38 of 48 bronchi. β_2-Adrenoceptor polymorphisms inducing variable response to β_2-agonists may constitute a possible explanation of this fickle sensitizing effect (55). For instance, the Gly^16 β_2-adrenoceptor polymorphism could be associated with asthma severity (37). However, in patients treated chronically with salmeterol, exacerbations are not correlated with the Gly^16 polymorphism (56). The β_2-agonist concentrations that we used to sensitize the bronchi may not be consistent with the in vivo concentrations obtained by actual treatment using β_2-agonists. In a randomized study comparing clinical efficacy of nebulized vs. intravenous salbutamol in severe acute asthma, Salmeron and colleagues (51) found plasma concentrations of salbutamol on the order of 10^-5 M. In healthy volunteers, plasma formoterol concentration reached 10^-9 M after inhalation of a single dose of 120 μg of formoterol fumarate (40). Therapeutic plasma concentration of fenoterol of ~10^-8 M was recommended (18), but plasma concentration associated with serious toxicity is not known.

In summary, our study demonstrates the proinflammatory effects of chronic exposure to β_2-agonists in human bronchus. Our results suggest that these proinflammatory effects are mediated by NF-κB and lead to sensitization of airway smooth muscle. MAPK, PKC, and tachykinins seem to play a major role in the sensitization of the human bronchus after chronic exposure to β_2-adrenergic agents.

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REFERENCES


