Leukotriene D₄ Contractions in Human Airways are Blocked by SK&F 96365, an Inhibitor of Receptor-Mediated Calcium Entry

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ABSTRACT
In human bronchial muscle preparations, nifedipine (3 μM) significantly inhibited the histamine, ACh and KCl contractions. However, the dihydropyridine did not modify the contractile responses induced by either leukotriene D₄ (LTD₄) or anti-human IgE (α-IgE). In human airways, SK&F 96365 (30 μM and 100 μM) markedly reduced the KCl and, at the higher concentration, LTD₄ maximal contractions. In addition, when preparations were treated with nifedipine (3 μM), SK&F 96365 (100 μM) significantly blocked responses to both LTD₄ and a-IgE. This calcium chelating agent ethylene glycol-bis (β-amino-ethyl ether) N,N,N’,N’-tetraacetic acid (4 mM) also inhibited the α-IgE-induced contractions. These data demonstrate that the nifedipine-resistant component of the LTD₄ and α-IgE contractions was inhibited by SK&F 96365 and suggest that the cysteinyl-leukotriene receptor in human airways may be intimately linked with a receptor-operated calcium-entry mechanism.

In guinea pig airways, a differential sensitivity to the inhibitory effects of calcium antagonists has been reported for a variety of contractile agonists (Foster et al., 1984; Ahmed et al., 1984; Ahmed et al., 1985; Cuthbert et al., 1994). These results suggested that there was a source of calcium that was not readily affected by blocking the availability of extracellular calcium via a VOC. The mobilization of calcium from internal stores by contractile agonists was one suggestion for explaining the considerable resistance of contractions induced by some agonists to calcium antagonists such as verapamil and nifedipine (Foster et al., 1984; Ahmed et al., 1985). However, another explanation—that a ROC entry mechanism was involved—(Benham and Tsien, 1987) has also been proposed. Data from electrophysiological studies have provided some evidence for the mobilization of calcium entry via a ROC in platelets (Merritt et al., 1990) and airway smooth muscle (Murray and Kotlikoff, 1991).

Initial reports in guinea pig airways have shown that the contractions induced by LTD₄ exhibited little or no dependence on extracellular calcium when compared with data from a number of other contractile agonists (Weichman et al., 1983; Raeburn and Rodger, 1984). Cuthbert and co-workers (1994) have recently shown that in guinea pig tracheal preparations, the contractile response to LTD₄ was dependent to some extent on the extracellular calcium because nifedipine significantly reduced these responses. Thus results from functional studies in this species suggested that LTD₄ may mobilize calcium via a VOC. However, the LTD₄ contractions were to some extent resistant to high concentrations of the dihydropyridines, which suggested that the contribution of the VOC in the airways of the guinea pig may represent only one possible route for Ca²⁺ mobilization during the LTD₄ response. Unfortunately, the little information that is available on the mechanisms of calcium entry in isolated human airways (Jones et al., 1982; Drazen et al., 1983; Roberts et al., 1986; Kohrogi et al., 1985) is conflicting. Therefore, the aim of this investigation was to evaluate the LTD₄- and a-IgE-induced contractions in human airways in the presence of nifedipine and the putative VOC/ROC antagonist, SK&F 96365 (Merritt et al., 1990).

Materials and Methods
Isolated preparations. Human lung samples were obtained from 17 male and two female patients who had undergone surgery for lung carcinoma. The age was 59 ± 4 years (mean ± S.E.M.). After resection, the bronchus was dissected free from parenchymal lung tissue and placed in Tyrode’s solution at 4°C for 12 h. Experiments were performed on 180 subsegmental bronchial preparations derived from 19 lung samples (N). The bronchial ring preparations were 2 to 5 mm in internal diameter.

ABBREVIATIONS: LTD₄, leukotriene D₄; α-IgE, anti-human IgE; EGTA, ethylene glycol-bis (β-amino-ethyl ether) N,N,N’,N’-tetraacetic acid; CysLT₁, cysteinyl-leukotriene receptor; VOC, voltage-operated channel; ROC, receptor-operated channel; AIC, (atropine, 1 μM; indomethacin, 3 μM and chlorpheniramine, 1 μM).
Agonist contractions. Bronchial ring preparations were set up in 10-ml organ baths containing Tyrode’s solution of the following composition (mM): NaCl, 139.2; KCl, 2.7; CaCl₂, 1.8; MgCl₂, 0.49; NaHCO₃, 11.9; NaH₂PO₄, 0.4 and glucose, 5.5; pH 7.4. The rings were mounted under initial loads of 2 to 3 g, maintained at 37°C and gassed with 5% CO₂/95% O₂. Changes in force were monitored on Lineis recorders using isometric force-displacement transducers (Narco F-60). The preparations were allowed to equilibrate for 90 min, and the medium was replaced every 15 min with fresh Tyrode’s solution. After the 90-min equilibration period, bronchial rings were contracted with ACh (100 µM; 2.28 ± 0.28 g; N = 19 lung samples). When the response reached a plateau, the tissues were washed every 10 min by exchanging the medium until the basal tone was re-established. The bronchial tissues were then incubated for 30 min with Tyrode’s solution or Tyrode’s solution containing the different drugs (nifedipine, SK&F 96365 or nifedipine/SK&F 96365) at different concentrations (0.01 µM–100 µM). Cumulative concentration-effect curves were then produced with ACh, histamine, KCl and LTD₄.

a-IgE-induced contractions. After the equilibration period, the bronchial tissues were contracted with ACh (100 µM). When the ACh response reached a plateau, the rings were washed until the basal tone was re-established. The preparations were then incubated for 30 min with Tyrode’s solution (control), with Tyrode’s solution containing AIC (30 min) or with Tyrode’s solution containing this drug combination and either nifedipine (3 µM) or nifedipine (3µM/SK&F 96365 (100 µM)). At the end of the incubation period, the tissues were challenged with a-IgE (1:1000). In protocols involving EGTA (4 mM), the tissues, which had been incubated with AIC, were subsequently exposed to this chelator for 5 min before a-IgE stimulation. The tissues used were not passively sensitized because the a-IgE serum was directed against the Fc fraction of the IgE (that is, the constant domains on the e chain) found on the membranes of mast cell and basophils. In one experimental protocol, the a-IgE response was monitored every 6 min until the contraction reached a plateau (30 min).

Calculations. The changes in force produced by the different agonists were determined from recordings and expressed as percent of ACh (100 µM) contractions. Because the calcium antagonists blocked the agonist maximal contractile response, no attempt was made to interpolate the effective concentration (EC₅₀) values. Results were evaluated using the ANOVA followed by Student’s t test for the individual data points.

Drugs. The drugs and their sources were as follows: acetylcholine chloride, atropine sulfate, chlorpheniramine maleate, indomethacin, histamine dihydrochloride and EGTA (Sigma Chemical Co., St. Louis, MO), sheep antiserum to human IgE (anti-IgE; Nordic Immunological Laboratories, Tilberg, Netherlands). LTD₄ was synthesized by Bayer plc (Stoke Court, UK). Nifedipine and SK&F 96365 were a gift from Dr. P.J. Gardiner (Bayer plc). The receptor antagonists and indomethacin were dissolved in dimethylsulfoxide, and subsequent dilutions were made in Tyrode’s solution. The leukotrienes were shipped on dry ice from Bayer plc in vials containing a stock solution of LTD₄ in 20% ethanol and physiological salt solution at pH 7.2.

Results

The data presented in figure 1 demonstrate the effects of nifedipine on contractions induced by several agonists in human airways. Although nifedipine (3 µM and 10 µM) significantly reduced the histamine, ACh and KCl responses, nifedipine (3 µM) did not alter the LTD₄ contractions. At a higher concentration (nifedipine, 10 µM), no effect on the maximal LTD₄ contractions was observed (control 142 ± 16% and nifedipine 147 ± 44%, N = 3).

SK&F 96365 (30 µM and 100 µM) significantly reduced the maximal contractions induced by KCl and, at the higher concentration, by LTD₄ in human bronchial preparations (fig. 2). However, the maximal histamine contractions were not significantly altered by SK&F 96365 at 100 µM (control 137 ± 25% and SK&F 96365 96 ± 16%, N = 3). In contrast, the maximal ACh contractions (148 ± 10%) were significantly reduced (92 ± 23%; N = 5; P < .05) by SK&F 96365.
(100 μM). In bronchial preparations treated with nifedipine (3 μM), SK&F 96365 (30 μM and 100 μM) also blocked the LTD₄ contractions in human airways (fig. 3).

When human airways were pretreated with AIC, the contractions produced by a-IgE were not significantly increased compared with the response in control tissues (table 1). However, a significant reduction in the anti-IgE-induced contraction of human airways was observed in tissues treated for 5 min with EGTA (4 mM). This reduced response after EGTA treatment was observed in the absence and presence of AIC (table 1).

Nifedipine (3 μM) in AIC-treated tissues did not alter the anti-IgE-induced contraction. However, SK&F 96365 (100 μM) in the presence of nifedipine (3 μM) significantly reduced responses to anti-IgE in human airways in the presence of AIC (fig. 4).

Discussion

In human airways (present report), nifedipine (3 μM), which significantly reduced the histamine, ACh and KCl contractions, failed to block the LTD₄ and anti-IgE-induced responses. However, treatment of human airways with SK&F 96365 reduced the maximal response obtained with LTD₄ and a-IgE. Because contractions produced by a-IgE in human airways (Bjorck and Dahlén, 1993; Gorenne et al., 1994) are due to the effects of endogenously released leukotrienes, these results suggest that stimulation of the CysLT₁ receptor may be associated with the mobilization of calcium via a ROC in human airways.

In guinea pig tracheal preparations, verapamil and nitrendipine markedly inhibited the contractions induced by barium chloride, whereas the responses to other agonists (histamine, carbachol) were not affected (Duncan and Douglas, 1985). Recently, Cuthbert and co-workers (1994) demonstrated that in physiological medium (calcium present), nifedipine (1 μM and 10 μM) significantly inhibited the LTD₄ contractions and did not alter the ACh responses. These latter results are similar to those results previously reported in guinea pig tracheal preparations for LTD₄ (Raeburn and Roger, 1984). In addition, Raeburn and Roger (1984) clearly showed that LTD₄ did not stimulate calcium uptake in guinea pig airway smooth muscle preparations and suggested that the mobilization of Ca²⁺ during LTD₄ contraction may involve internal Ca²⁺ pools.

The LTD₄ contractions of human airways have also been reported to rely preferentially on intracellular calcium, whereas methacholine contractions rely on the extracellular source of this ion (Roberts et al., 1986). However, these latter results in human airways are not consistent with an earlier observation by Henderson and co-workers (1983), who demonstrated that histamine, ACh and antigen-induced contractions were reduced by nifedipine, which suggests that a voltage-dependent Ca²⁺ channel entry mechanism may be involved. Other investigators (Black et al., 1986; Roberts et al., 1986; Raeburn et al., 1986) using human airways in calcium-depleted medium reported a significant reduced response to a variety of contractile agonists. The data (present report) support these latter observations; nifedipine (3 μM) significantly inhibited ACh, histamine and KCl contractions in human airways but did not block LTD₄ and anti-IgE-induced responses.

Although the presence of VOC in human airway smooth muscle cells (Marthan et al., 1989) has been demonstrated, an exploration of the ROC in airway muscle function has

### Table 1

**Effects of EGTA on anti-IgE-induced contractions in human airways in absence or presence of AIC**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Response to ACh (g)</th>
<th>Effects of Drugs on Basal Tone (g)</th>
<th>Emax anti-IgE (1/1000) (%ACh)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.33 ± 0.42</td>
<td>-0.04 ± 0.03</td>
<td>63 ± 13</td>
</tr>
<tr>
<td>EGTA</td>
<td>2.04 ± 0.20</td>
<td>-0.30 ± 0.05*</td>
<td>26 ± 13*</td>
</tr>
<tr>
<td>AIC</td>
<td>1.86 ± 0.32</td>
<td>-0.47 ± 0.09</td>
<td>99 ± 19</td>
</tr>
<tr>
<td>AIC and EGTA</td>
<td>1.88 ± 0.41</td>
<td>-0.45 ± 0.10</td>
<td>41 ± 12*</td>
</tr>
</tbody>
</table>

Values are means ± S.E.M. obtained from seven lung samples. * Values different from appropriate control data (P < .05).
received less attention, because appropriate antagonists have not been available. Merritt and co-workers (1990) provided the initial observations on a putative inhibitor (SK&F 96365) for the calcium entry during agonist-receptor interactions. This compound has recently been reported to block effectively the nifedipine-resistant contractions of ACh and LTD4 in guinea pig airways (Cutbert et al., 1994). These results from guinea pig airways, together with those derived from human airways (present report), suggest that certain agonists and their specific receptors may be more closely associated than others with a ROC type of calcium mobilization. The observation that SK&F 96365 (100 µM) had no significant effect on histamine responses although it significantly inhibited KCl, ACh and LTD4 contractions suggests that this compound may have both VOC and ROC inhibitory activity. However, the compound is less effective than nifedipine as a VOC antagonist. The observation that SK&F activity. However, the compound is less effective than nifedipine as a VOC antagonist. The observation that SK&F 96365 (100 µM) had no significant effect on histamine responses although it significantly inhibited KCl, ACh and LTD4 contractions suggests that this compound may have both VOC and ROC inhibitory activity. However, the compound is less effective than nifedipine as a VOC antagonist. The observation that SK&F 96365 (100 µM) had no significant effect on histamine responses although it significantly inhibited KCl, ACh and LTD4 contractions suggests that this compound may have both VOC and ROC inhibitory activity. However, the compound is less effective than nifedipine as a VOC antagonist. The observation that SK&F 96365 (100 µM) caused a marked reduction in LTD4 contractions, as compared with the data obtained with histamine, provides further indirect evidence that certain receptors may be intimately linked to a ROC mechanism in human airways.

Henderson and co-workers (1983) showed that human airways, after passive sensitization, contracted when challenged with a specific allergen. This contraction was reduced, though only marginally, by treatment of the tissues with nifedipine (100 µM). Previous reports have shown that under specific experimental conditions, the major mediators involved in antigen-induced contraction in human airways are cysteinyl-leukotrienes (Bjorck and Dahlén, 1993; Gorenne et al., 1994). In human airways (present report), the contractions induced by LTD4 and a-IgE are apparently independent of any mobilization of calcium via a VOC, because nifedipine did not affect either response. These results support the work of Bourdillat and co-workers (1987), who showed that LTD4 contractions in human bronchial preparations were resistant to treatment with the VOC inhibitor diltiazem.

In the presence of both antagonists (nifedipine and SK&F 96365), the contractions induced by LTD4 and a-IgE were markedly inhibited. These results suggest that LTD4 and a-IgE induced contraction by activating an SK&F 96365-sensitive/nifedipine-insensitive calcium-entry mechanism. Whether this calcium mobilization system that is activated by LTD4 is identical to a receptor-mediated calcium channel entry mechanism in human airways is presently not known. However, these observations are similar to data reported by Soergel and co-workers (1992), who demonstrated that maitotoxin activates phosphoinositide breakdown in C6 glioma cells and is inhibited by SK&F 96365 but not by nifedipine. In contrast, these authors also showed that in RIN cells, the effects of endothelin, which stimulated a specific receptor and activated phosphoinositide breakdown, were not inhibited by SK&F 96365. Such observations lend support to the initial studies in human platelets (Merritt et al., 1990), rat pancreatic acini (Busik et al., 1993) and PC12 cells (Fasolato et al., 1990), which suggested that SK&F 96365 was an inhibitor of a receptor-mediated calcium-entry channel.

The inhibition of LTD4 and a-IgE contractions by SK&F 96365 provides indirect evidence that a ROC mechanism may be present and functional in human airway muscle. Because human airways contain a single cysteinyl-leukotriene receptor responsible for contraction (CysLT1; Buckner et al., 1986; Labat et al., 1992), these data suggest that the CysLT1 receptor may be intimately linked to ROC and that LTD4 may be a potent activator of this channel.

References

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