Electromechanical coupling of ferret airway smooth muscle in response to leukotriene C_4

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MURLAS, CHRISTOPHER G., AND CRAIG A. DOUPNIK. *Electromechanical coupling of ferret airway smooth muscle in response to leukotriene C_4.* J. Appl. Physiol. 66(6): 2533–2538, 1989.—We investigated the possible electrophysiological basis for the slow, prolonged force generation by airway smooth muscle (ASM) produced by leukotriene C_4 (LTC_4). Preparations of ASM were made from ferret trachea and placed in tissue microchambers for study. Some of these preparations were arranged so that force transducers and intracellular microelectrodes (with tip resistances of 30–80 MΩ) could be used to measure isometric force and cell membrane potential (E_m) simultaneously from ASM cells stimulated by LTC_4. We found that ferret tracheal muscle was relatively sensitive to LTC_4 and that this sensitivity was not significantly affected by atropine (1 μM), phentolamine (1 μM), propranolol (3 μM), and pyrilamine (1 μM). In a 1 nM solution of LTC_4, E_m was −54.0 ± 1.2 mV from 18 impalements (n) from 6 animals (N) compared with a base-line value of −61.6 ± 0.8 mV (n/N = 29/8, $P <$ 0.0005). This change did not lead to force generation, however. Higher concentrations of LTC_4 led to progressive decreases in E_m to which force generation was closely coupled. Concentrations ≥70 nM led to phasic oscillations in E_m of 0.6–0.8 Hz and 1.7 mV in amplitude, which were abolished by 10 μM verapamil, although the base-line E_m was unaffected by this concentration. Although 300 nM LTE_4 by itself caused only a small depolarization of ferret trachealis, it substantially antagonized the electromechanical responsiveness of this smooth muscle to LTC_4. We conclude that ferret ASM is relatively sensitive to LTC_4 and that there is an electrical basis for the slow, prolonged force generation caused by this mediator.

asthma; bronchomotor tone; bronchospasm; electromechanical activity; excitation-concentration coupling; slow-reacting substance of anaphylaxis; smooth muscle electrophysiology; trachealis muscle

UNTIL THE LATE 1970s, our understanding of the cell physiology underlying bronchoconstriction induced by slow-reacting substance of anaphylaxis (SRS-A) was impeded by our lack of knowledge concerning its chemical composition. It was clear from studies in vitro, however, that SRS-A-induced airway muscle contraction was primarily dependent on extracellular Ca^{2+} (11). We now know that the constituents of SRS-A include the sulfidopeptide leukotrienes (LT) C_4, D_4, and E_4. Recent studies in vivo have demonstrated that LTC_4 may cause quite marked airway obstruction in humans as well as in other species such as the guinea pig (8). The potency in vitro of this mediator on central airway smooth muscle in humans (6), guinea pigs (7, 8, 13, 17), and ferrets (25) has been shown. In the ferret, for example, LTC_4 has been shown to be threefold more potent than carbachol on tracheal smooth muscle (25).

The mechanisms by which LTC_4 cause airway smooth muscle contraction are very unclear at present. Past studies in vitro have demonstrated LTC_4-induced airway muscle contraction to be relative slow in onset and in reaching maximal force (23). It is also known from previous studies that airway muscle contractions induced by LTC_4 are primarily dependent on extracellular Ca^{2+} influx (13, 23). Studies to date, however, have shown a variable effect of some of the Ca^{2+} channel antagonists on LTC_4-induced contraction (13).

The present electrophysiological study was designed to investigate the possible electrical effects of LTC_4 on ferret airway smooth muscle responsiveness. Such electrical effects could, in part, be mediated via voltage-dependent Ca^{2+} channels. In some experiments, the electromechanical effects of various concentrations of LTC_4 were studied with the use of preparations where isometric tension and membrane potential of single airway muscle cells could be measured simultaneously in the presence or absence of verapamil. To assess whether certain LTC_4 effects were mediated by its potential biometabolites, LTD_4 and LTE_4, additional experiments were done with preparations that were treated with LTE_4 or L-serine borate, an inhibitor of γ-glutamyltranspeptidase (27).

METHODS

Tracheae were obtained from adult male ferrets ~1.5 kg in weight that had been killed by intraperitoneal pentobarbital sodium. The tracheae were quickly excised, cleaned of blood, serosal connective tissue, and fat, and placed in a modified Krebs-buffered physiological salt solution that was continuously gassed with 95% O_2-5% CO_2 and had a pH of 7.40 at 37°C. This solution was of the following composition (in mM): 137.0 Na^+, 5.9 K^+, 2.5 Ca^{2+}, 1.1 Mg^{2+}, 124.1 Cl^-, 24.9 HCO_3^-, 1.2 $H_2PO_4^-$, and 9.6 glucose.

Measurement of isometric concentration of tracheal muscle to LTC_4 stimulation. Rings ~4–5 mm long were cut from each trachea. The rings were placed in 7-ml chambers filled with continuously gassed Krebs solution. Temperature was maintained at 37°C throughout the experiments, and pH was monitored at regular intervals. Each ring was connected to the chamber base and to a tension transducer (model FT.03, Grass Instruments, Quincy, MA). The transducer was mounted so that pas-

sive tension, and therefore muscle fiber length, could be progressively increased. Signals from the force transducers were displayed on a strip chart recorder (model 8000, Gould Instrument, Cleveland OH). All rings were equilibrated at a resting tension of 10 mN for at least 60 min during which time they were washed with fresh Krebs solution every 20 min. Half the ring preparations were then exposed to 1 μM atropine, 1 μM phentolamine, 3 μM propranolol, and 1 μM pyrilamine for 30 min. Thereafter, all rings were stimulated by increasing, cumulative concentrations of LTC_4. The contractions elicited were expressed as percent of the response to the maximal concentration of LTC_4 used. Dose-response curves in the presence or absence of antagonists were then constructed.

Simultaneous measurements of membrane potential and isometric tension at rest or during stimulation by LTC_4. A small (1 × 3 × 0.5 mm) section of trachealis muscle from the posterior wall of the upper cervical trachea was dissected free from its cartilaginous attachments (19) after the mucosa was first removed. With the aid of a dissecting microscope, the muscle layer of the airway wall was exposed by cutting away adherent submucosal connective tissue to facilitate impalement by microelectrodes. This muscle strip with its luminal side up was pinned to the floor of a 2-ml Sylgard-coated bathing chamber that was continuously perfused at 2–3 ml/min with gassed Krebs solution that was continuously monitored and remained at 37°C throughout the individual experiments. Each muscle strip was mounted horizontally in the chamber, securing one end to a fixed point and the other to the lever arm of an isometric force transducer (Grass FT.03). This transducer was mounted to a micromanipulator that enabled the length of each preparation to be determined after a resting tension was imposed on the muscle.

Membrane potentials of airway muscle cells were measured using borosilicate glass intracellular microelectrodes. These were filled with 3 M KCl and had tip resistances of 30–80 MΩ. The microelectrode was connected to the input probe of an electrometer (model KS-700, W. P. Instruments, New Haven, CT). A return path, referenced to ground, was provided by a Ag-Ag_1 reference electrode contacting a 3 M KCl-agar bridge that was placed in the bathing chamber. Signals from the force transducer and electrometer were displayed on a dual-beam storage oscilloscope (model 5113, Tektronix Instruments, Beaverton, OR) and also recorded simultaneously on a FM tape recorder (model B, Vetter, Rebersburg, PA) for data storage and retrieval. Oscilloscope tracings were photographed with a Textronix oscilloscope camera (model C-5C).

Each muscle strip was equilibrated at a resting tension of 2 mN for at least 60 min. Intracellular penetrations of individual muscle cells were determined by classical criteria (15). After control impalements were made, each muscle preparation was stimulated by progressively increasing concentrations of LTC_4 added cumulatively to 50 ml continuously gassed Krebs solution that recirculated through the bathing chamber. Impalements were made and contractions were measured after 10 min of perfusion at each LTC_4 concentration. The effects of verapamil on LTC_4-induced electromechanical activity were investigated by perfusing preparations with 10 μM and 100 μM verapamil after they had been exposed to 100 nM LTC_4. In other experiments, the effects of LTE_4 on LTC_4-induced activity were investigated by perfusing preparations for 30 min with 0.3 μM LTE_4. After control measurements of membrane potential and tone were made, these preparations were exposed to 100 nM LTC_4. Repeat measurements were made 10 min after LTC_4 perfusion.

Data and statistical analysis. At the conclusion of each experiment, the muscle strip was weighed after blotting it dry and cross-sectional area was calculated using the following formula (and assuming that tissue density equaled that of water): tissue wt (mg)/[tissue length (mm) × 1 mg/mm^3] = cross-sectional area (mm^2). Force generation was then expressed as force per cross-sectional area (mN/mm^2). Means ± SE were calculated for all responses from like groups of preparations. The unpaired t test was employed for statistical analysis. In all cases, differences were considered significant for $P < 0.05$.

Drugs and chemicals. The compounds used were obtained from the following sources: atropine, propranolol, pyrilamine, L-serine, and verapamil from Sigma Chemical, St. Louis, MO; phentolamine from Ciba Pharmaceutical, Summitt, NJ; LTC_4 and LTE_4 from Cayman Chemical, Ann Arbor, MI; sodium borate from Fisher Scientific, Springfield, NJ; and pentobarbital sodium from Butler, Columbus, OH.

RESULTS

Figure 1 shows the sensitivities of ferret tracheal smooth muscle to LTC_4 in the presence and absence of atropine, phentolamine, propranolol, and pyrilamine. Al-

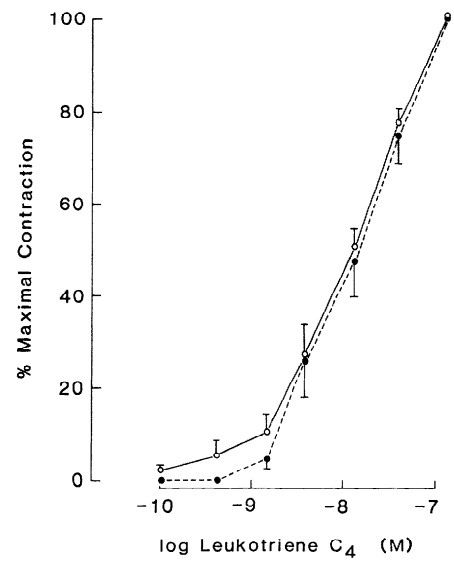

FIG. 1. Contractile responses of ferret tracheal smooth muscle to leukotriene C_4 (LTC_4) in the presence (●) and absence (○) of 1 μM atropine, 1 μM phentolamine, 3 μM propranolol, and 1 μM pyrilamine. Responses are expressed as percent of maximal contraction elicited by 100 nM LTC_4. Each symbol represents mean ± SE of 11 rings from 4 ferrets. Those without SE bars had SE less than symbol size.

though antagonist pretreatment appeared to decrease sensitivity to LTC_4 at low concentrations, this effect was not statistically significant. To inhibit possible bioconversion of LTC_4 to LTD_4 in this tissue, some preparations were pretreated with L-serine borate in a fully gassed Krebs solution having pH of 7.40 at 37°C. Concentrations of 2.5 mM or greater of L-serine borate (which have been used by other investigators) caused contractions that were sustained for 20 min or more.

The electromechanical response of airway muscle to LTC_4 is illustrated in Fig. 2. The initiation of a contractile response to this mediator was preceded in time by a depolarization that was slow in onset and slow in reaching maximal effect. The electrical behavior of the muscle in response to increasing concentrations of LTC_4 was characterized by a progressive depolarization that was closely coupled to force generation (Fig. 3). LTC_4 (100 pM) resulted in a cell membrane potential of -59.4 ± 0.8 (SE) mV [derived from 12 impalements (n) from 4 animals (N)] compared with a base-line value of -61.1 ± 0.8 mV ($n/N = 29/8$). This electrical effect was unassociated with a change in muscle tone (Fig. 3). Continuous impalements held during the transition from 100 pM to 1 nM LTC_4 showed the cell membrane depolarization to be slow, graded, and without phasic activity. 1 nM LTC_4 resulted in a membrane potential of -54.0 ± 1.2 mV ($n/N = 18/6$), which was substantially different from the base-line value ($P < 0.0005$). Concentrations of LTC_4 >1 nM uniformly resulted in substantially larger cell membrane depolarizations that were associated with force generation (Fig. 3). This force generation was characteristically slow in onset, slow in reaching a plateau, and closely followed the pattern of cell membrane depolarization produced by LTC_4.

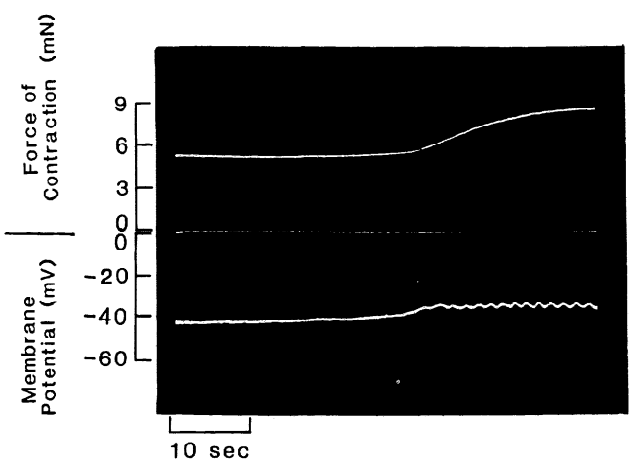

FIG. 2. Segment of oscilloscope record showing simultaneous measurements of tension (*top*) and membrane potential (*bottom*) from a single airway muscle on cell stimulation by 70 nM leukotriene C_4 (LTC_4). This concentration was added 10 min before this oscilloscope photograph was made. During this 10-min period, a slow and sustained depolarization occurred, which was coupled to a slow and sustained contraction. The beginning of this trace shows this depolarized/contracted state. Latter half of trace shows onset of a stronger force, which was associated with onset of oscillations in cell membrane potential (see text). Close electromechanical coupling was present. At ≥70 nM LTC_4, oscillations in membrane potential were provoked. These appeared to be linked to further force generation. At lesser concentrations, no such phasic electrical or mechanical activity occurred.

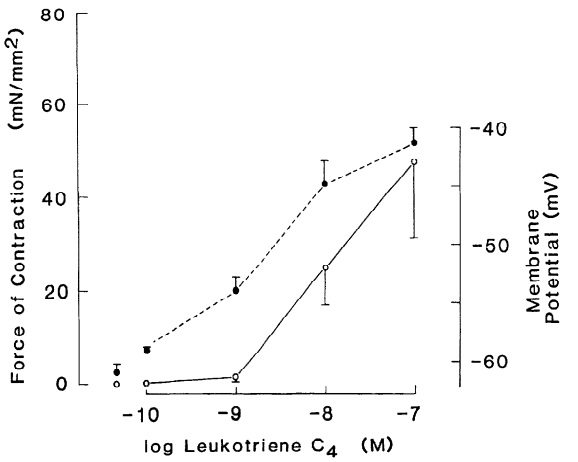

FIG. 3. Effects of increasing leukotriene C_4 (LTC_4) concentrations on simultaneously recorded membrane potential (●) and force generation (○) of ferret tracheal smooth muscle. Each symbol represents mean ± SE from 12–29 cell impalements from 4–8 animals. Those without SE bars had SE less than symbol size. First symbol pair to the *left* of the lowest LTC_4 concentration represents values obtained in the absence of LTC_4.

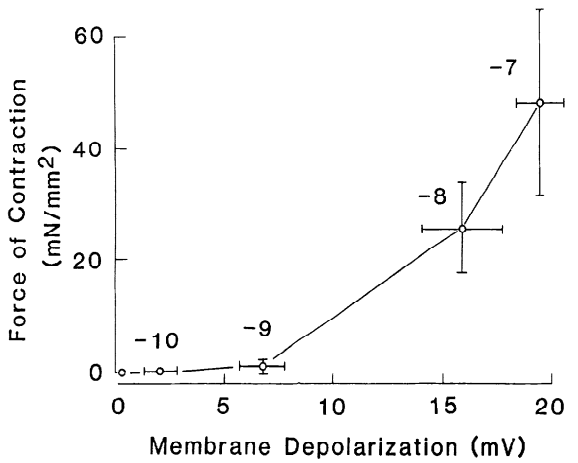

FIG. 4. Effect of various leukotriene C_4 (LTC_4) concentrations [log concn (in M) indicated by numbers over symbols] on membrane potential and force generation simultaneously measured from ferret tracheal smooth muscle. (Graph derived from data described in Fig. 3.) LTC_4 concentrations >1 nM depolarized these airway muscle preparations beyond their mechanical threshold, and the force generated was substantial.

The voltage-tension relationship of ferret tracheal muscle LTC_4 is shown in Fig. 4. Beyond the threshold for force generation, there appeared to be a direct relationship between the cell membrane depolarization produced by this mediator and the force generation that developed (Fig. 4).

Concentrations of LTC_4 ≥70 nM caused oscillations in cell membrane potential (Fig. 2), which were 1–7 mV in amplitude and 0.6–0.8 Hz in frequency. These oscillations in membrane potential were associated with phasic oscillations of equal frequency in airway muscle tone.

The effect of verapamil on LTC_4-induced electromechanical responsiveness of ferret airway muscle is shown in Fig. 5. At 10 and 100 μM, verapamil caused a 70 and 80% relaxation, respectively, of the contraction induced by 100 nM LTC_4. Both of these concentrations of verapamil effectively abolished the oscillations in membrane

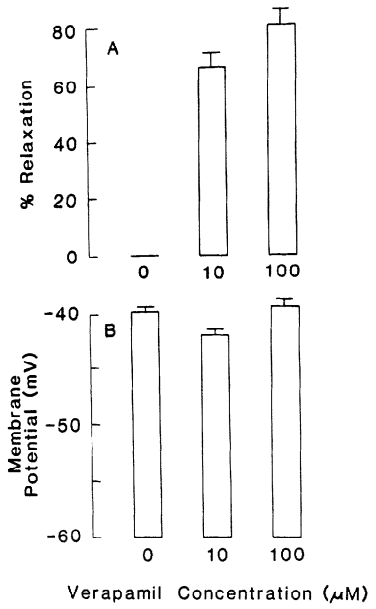

FIG. 5. Effects of 2 concentrations of verapamil on simultaneously recorded mechanical (*A*) and electrical responses (*B*) of ferret tracheal smooth muscle to 100 nM leukotriene C_4 (LTC_4). Relaxation induced by 10 μM ($N = 4$ ferrets) and 100 μM ($N = 4$) verapamil shown in *A* is expressed as percent of contractile response initiated by 100 nM LTC_4. Bars represent means ± SE from 10–12 preparations. Verapamil inhibited both membrane oscillations and force generated by LTC_4. It did not, however, significantly affect cell membrane potential.

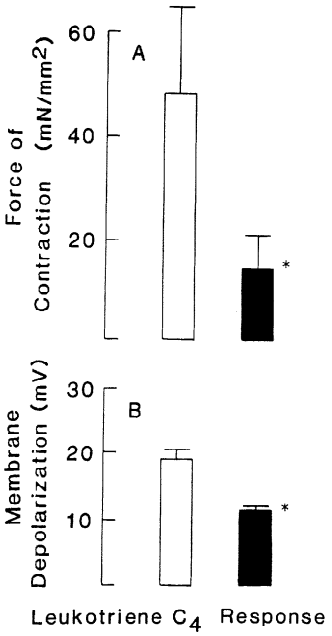

FIG. 6. Effects of 300 nM leukotriene E_4 (LTE_4, closed bars) on simultaneously recorded mechanical (*A*) and electrical responses (*B*) of ferret tracheal muscle to 100 nM LTC_4. Each bar represents mean ± SE from 13–15 cell impalements of 4 animals. * Significant differences from responses measured in the absence (open bars) of LTE_4 ($P < 0.05$). LTE_4 substantially antagonized electromechanical response of airway muscle to LTC_4.

potential and mechanical activity caused by this concentration of LTC_4, but the base-line membrane potential remained unaltered (Fig. 5).

A concentration of 300 nM LTE_4 itself produced a membrane potential of -59.7 ± 0.6 mV ($n/N = 13/4$) compared with a base-line value of -61.9 ± 0.7 mV ($n/N = 14/4$). This change was statistically insignificant and was not associated with a change in muscle tone. This concentration, however, produced a substantial antagonism of the electrical and mechanical response of ferret trachealis to 100 μM LTC_4 (Fig. 6). After a 30-min pretreatment in 300 nM LTE_4, the membrane potential in LTC_4 was -49.9 ± 0.2 mV ($n/N = 15/4$), compared with 41.2 ± 1.2 mV ($n/N = 14/5$) in the absence of LTE_4 ($P < 0.05$). A corresponding decrease in the contractile response of LTC_4 (Fig. 6*A*) was observed in the presence of LTE_4 ($P < 0.05$).

DISCUSSION

In species where LTC_4 is known to be a potent constrictor of airway smooth muscle, its cellular mechanisms of action are not known. Electromechanical effects of LTC_4 on airway muscle have been identified and characterized in this study. LTC_4 produced slow, progressive, and prolonged cell membrane depolarization. These observations and their relation to the characteristics of LTC_4-induced slow force generation in this tissue, suggest to us that bronchoconstriction produced by this mediator is, at least in part, due to its electrical effects on airway smooth muscle as is that produced by K^+ (19).

To our knowledge, this evidence is the first to identify the electrophysiological effects that this sulfidopeptide LT has on airway smooth muscle. The contribution of electromechanical coupling in LTC_4-induced airway muscle contraction is evidenced by the voltage-tension relationship we observed. It seems likely that the depolarizing effect of LTC_4 we observed on airway muscle cell membrane potential is solely mediated by this agonist. It is possible, however, that the observed depolarization could also be influenced by a LTC_4 metabolite, specifically LTD_4 and/or LTE_4. In other smooth muscle tissues, previous investigators have demonstrated that LTC_4 is metabolically converted to LTD_4 by a membrane-bound enzyme, γ-glutamyltranspeptidase (18, 20, 24) and can then be converted by LTE_4 by an aminopeptidase (24, 26). [Although LTE_4 may be a bronchoconstrictor in some species (23, 24), it appeared to antagonize rather than augment ferret trachealis responsiveness to LTC_4 in this and another study (25)]. Krilis et al. (18) found that the biometabolism of LTC_4 in ileal longitudinal smooth muscle could be inhibited by 10 mM L-serine borate, an inhibitor of γ-glutamyltranspeptidase (27). This inhibition was associated with depressed contraction to LTC_4 in vitro, suggesting that biometabolism of LTC_4 to LTD_4 (which is more potent) occurs in that tissue. In this regard, it is interesting that Snyder and Krell (25) recently reported ferret tracheal muscle pretreated with 45 mM L-serine borate appeared to be more sensitive to LTC_4. We found in this study that concentrations of L-serine borate ≥ 2.5 mM resulted in ferret tracheal muscle contractions that were sustained. Such a complicating mechanical effect could influence smooth muscle responsiveness to LTC_4 independent of transpeptidase inhibition by L-serine borate, and thus, interpretation of these experiments is problematic.

In our experience, LTC_4-induced airway muscle con-

traction did not appear to be indirectly mediated via muscarinic, α-adrenergic, β-adrenergic, or histaminic receptor stimulation. In ferret tracheal muscle, other investigators have demonstrated that LTC_4 stimulation does not result in the synthesis of cyclooxygenase products that could be spasmogenic (25). This may contrast with its effect on lung parenchymal cells in some species such as the guinea pig (7, 21). In human lung parenchyma, LTC_4 does not appear to stimulate the synthesis of cyclooxygenase products (2). Thus the available evidence suggests that the electromechanical response of airway muscle induced by LTC_4 may be the result of its direct effect on the cell membrane rather than via an intermediary. Furthermore, this effect does not appear to undergo tachyphylaxis rapidly in that the membrane depolarization and force generation that were observed remained stable over 10 min or more at each concentration tested.

The oscillations in membrane potential induced by LTC_4 that were observed to occur at ~ -40 mV further illustrates the important electrophysiological consequences that LTC_4 may have on airway smooth muscle. At least in ferret tracheal muscle, these oscillations may be caused by other stimuli as well that produce membrane depolarization to about -35 to -40 mV (4). We found that these electrical oscillations were abolished by verapamil although base-line membrane potential itself was unaffected. This observation suggests to us that the upstroke of these oscillations in membrane potential are dependent on voltage-sensitive Ca^{2+} channels as are tetraethylammonium-induced action potentials in airway smooth muscle in our experience (22). Previous investigators have reported that certain bronchoconstrictors also produce cell membrane oscillations similar to those seen here whereas other bronchoconstrictors do not (1, 5, 9, 14, 16, 18a). Such studies suggest that activation of voltage-dependent Ca^{2+} channels in airway muscle may be dependent on both the degree of depolarization induced and on the particular bronchoconstrictor tested. These oscillations in membrane potential may represent an unstable or excited electromechanical state that could further enhance the responsiveness of airway muscle to other stimuli.

It is clear from this study that LTC_4-induced airway muscle contraction is not mediated solely by its electrical effects. If this were the case, one might expect that the contractile response could be entirely antagonized by a voltage-dependent Ca^{2+} channel antagonist such as verapamil. It is not, although a substantial component is in our own and in other's experience (13). This evidence suggests that voltage-independent, possibly receptor-operated Ca^{2+} channels are also involved in the mechanical response of airway muscle to LTC_4 stimulation as they appear to be on muscarinic stimulation (5). Complementary information to this point is provided by a recent study published by Israel et al. (12). These investigators identified a so-called "D receptor" that, when stimulated by LTC_4, resulted in airway muscle contraction that was unaffected by calcium channel blockers, and thus, was presumably independent of membrane potential-related, extracellular Ca^{2+} entry. In contradistinction to this LTC_4 receptor subtype, Israel et al. identified a "C receptor" whose activation appeared to be linked to membrane calcium channel activation. Concerning the events involved in the contractile response of ileal smooth muscle to LTD_4, other investigators have speculated that the roles played by release of cellular Ca^{2+} stores and mechanisms independent of extracellular Ca^{2+} entry may be minor (10).

From our study, it appears that airway muscle contraction on LTC_4 stimulation derives, at least in part, from its electrical consequences on the cell membrane. Our evidence suggests that the electromechanical effects of LTC_4 on airway muscle may result from membrane depolarization and an increase in the number of voltage- and time-dependent Ca^{2+} channels available for activation and extracellular Ca^{2+} influx. Based on the available evidence, however, it is difficult to determine the primary effect of LTC_4 because the initial depolarization caused by LTC_4 may induce secondary changes such as the activation of voltage-dependent Ca^{2+} channels. Future investigations employing single airway smooth muscle cells and patch clamp electrophysiological techniques (3) will facilitate the study of possible primary effects of LTC_4 on cell membrane conductances for Ca^{2+}, Na^+, and/or K^+ independent of its effects on resting membrane potential.

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