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Immunology

Leukotriene E_4-induced airway hyperresponsiveness of guinea pig tracheal smooth muscle to histamine and evidence for three separate sulfidopeptide leukotriene receptors

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ABSTRACT **Bronchial hyperresponsiveness to contractile agonists and nonspecific irritants is a characteristic feature of bronchial asthma. The mechanisms causing this hyperirritability are unknown. The existence of separate receptors for leukotrienes C_4 and D_4 (LTC_4 and LTD_4) has been demonstrated previously by physiologic and radioligand binding studies. The rank order of potency of the sulfidopeptide leukotrienes for contracting tracheal spirals [leukotriene E_4 (LTE_4) > LTD_4 = LTC_4] is different from that for contracting parenchymal strips ($LTD_4 > LTE_4 > LTC_4$), thereby suggesting the existence of a separate receptor for LTE_4. We now report that LTE_4, the most stable of the leukotrienes comprising slow reacting substance of anaphylaxis, enhances the contractile response of guinea pig tracheal spirals but not of parenchymal strips to histamine in a time- and dose-dependent fashion. The ability of LTE_4 to increase histamine responsiveness occurred after removal of the free agonist and recovery of the tissues to baseline tensions and was not produced by leukotrienes C_4 and D_4, which elicited the same magnitude of contraction of tracheal smooth muscle as LTE_4. These findings suggest that LTE_4-induced airway hyperirritability is not mediated by the contractile response *per se* and may be mediated through a receptor distinct from those for leukotrienes C_4 and D_4.**

A hallmark of bronchial asthma is the elicitation of bronchoconstriction by inhalation of contractile agonists and nonspecific irritants at concentrations that are not active on the airways of normal individuals (1–3). The mechanisms responsible for this characteristic of bronchial asthma, generally termed airway hyperresponsiveness, are unknown, although such a state can be created or enhanced by respiratory infections (4) or by inhalation of allergen in asthmatic subjects (5). The observation that partially purified slow reacting substance of anaphylaxis (SRS-A), released from guinea pig lung tissue by an immediate hypersensitivity reaction, could augment the contractile response of the isolated guinea pig ileum to histamine (6) suggested that the sulfidopeptide leukotrienes now identified as constituting SRS-A, (5*S*,6*R*)-5-hydroxy-6-*S*-glutathionyl-7,9-*trans*-11,14-*cis*-icosatetraenoic acid (leukotriene C_4, LTC_4), (5*S*,6*R*)-5-hydroxy-6-*S*-cysteinylglycyl-7,9-*trans*-11,14-*cis*-icosatetraenoic acid (leukotriene D_4, LTD_4), and (5*S*,6*R*)-5-hydroxy-6-*S*-cysteinyl-7,9-*trans*-11,14-*cis*-icosatetraenoic acid (leukotriene E_4, LTE_4) (7–10), might contribute to airway hyperresponsiveness.

In normal subjects, inhaled LTC_4 and LTD_4 were 4000 and 6000 times more potent than histamine in eliciting a comparable impairment of pulmonary function, whereas in asthmatics, inhaled LTD_4 was only 200 times more potent than histamine (11–13). The finding that a group of asthmatic subjects exhibited hyperresponsiveness to inhaled histamine but not to LTD_4, as compared to normal individuals (13), suggested that LTD_4 was acting at a site in the airways distinct from histamine and might in addition be responsible for airway hyperresponsiveness to other substances. LTC_4, LTD_4, and LTE_4 are potent contractile agonists for guinea pig tracheal spirals and parenchymal strips, with a molar ratio of the amounts eliciting equivalent contractions of 1:1:0.1 and 1:0.05:0.3, respectively (14). This reversal of potency ratios for LTD_4 and LTE_4 with airway smooth muscle from the same species suggested separate receptors for each and prompted an examination of the capacity of these leukotrienes to elicit airway hyperresponsiveness of guinea pig pulmonary tissues to histamine.

MATERIALS AND METHODS

Tracheal spirals and parenchymal strips obtained from male guinea pigs (300- to 400-g body weight) were prepared *in vitro* for recording of isometric contractions after a stable baseline tension had been established as described (15). The tissues were exposed to logarithmically increasing concentrations of histamine (0.1–100 μM) (Sigma) to construct a cumulative histamine concentration-effect relationship for each tissue preparation. The initial contraction elicited by 100 μM histamine was assigned a value of 100, and all subsequent contractile responses to any agonist in that tissue were expressed as a percentage of this reference contraction. After completion of the initial histamine dose–response curve, the tissues were washed with oxygenated Tyrode's solution at 15-min intervals for 1 hr and were used either to establish a cumulative dose–response curve for a sulfidopeptide leukotriene or to assess responsiveness to histamine after exposure of the tissues to a defined dose of sulfidopeptide leukotriene.

Synthetic leukotrienes, prepared by total chemical synthesis (16–18), were diluted in Tyrode's buffer and added to organ baths to establish cumulative dose–response curves within the range of 0.02–100 nM for LTE_4 and LTD_4 and 0.06–80 nM for LTC_4. Alternatively, a defined concentration (0–80 nM) of synthetic leukotriene was added to each organ bath for 5–20 min, and the tissues were washed with 750 ml of buffer over 2 hr, during which time the tension of each fell to that recorded before leukotriene exposure; a second histamine concentration-effect relationship was then established on each tissue. The concentration of histamine that elicited a 50% maximum contraction (EC_{50}) was interpolated from the second histamine dose–response curve for each tissue, and the geometric means of these EC_{50} values were computed (19).

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Abbreviations: SRS-A, slow reacting substance of anaphylaxis; LTC_4, leukotriene C_4; LTD_4, leukotriene D_4; LTE_4, leukotriene E_4.

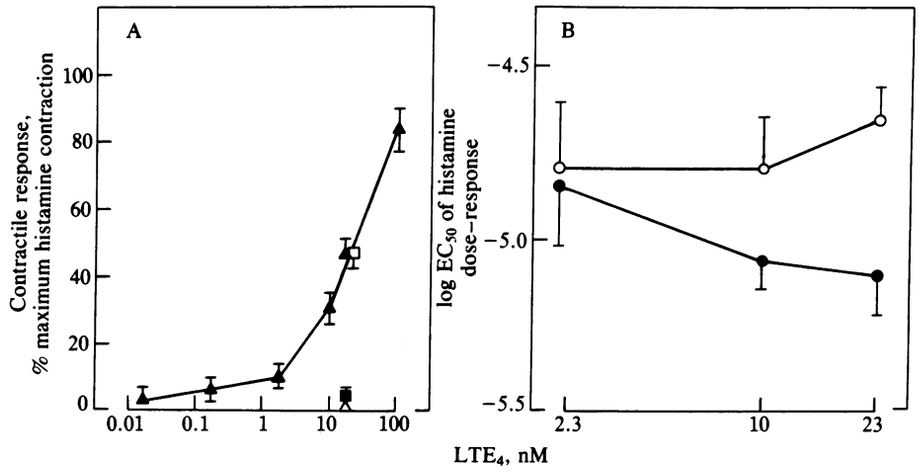

FIG. 1. (*A*) The effect of increasing LTE_4 concentrations on the contractile responses of guinea pig tracheal smooth muscle (mean ± SEM, $n = 7$) (▲), and the action of indomethacin (Indo; 1 μg/ml, $n = 4$) (□) and of FPL55712 at 1 μg/ml ($n = 6$) (■) and at 10 μg/ml ($n = 5$) (△) on the contraction elicited by 23 nM LTE_4. (*B*) log EC_{50} of histamine dose–response effect in LTE_4-treated (●) and control (○) tracheal tissues as a function of the concentration of LTE_4 used to pretreat tracheal spirals. The results at 2.3 nM, 10 nM, and 23 nM LTE_4 are the mean ± SEM of 4, 6, and 8 tissues, respectively; significance at those concentrations was evaluated as no significance, $P < 0.05$, and $P < 0.01$, respectively.

RESULTS

The contractile activity of LTE_4 for tracheal spirals is shown in Fig. 1*A*. The concentrations of LTE_4, interpolated from the dose–response curve, giving rise to contractions equal to 10%, 30%, and 50% of the maximum histamine contraction were 2.3 nM, 10 nM, and 23 nM, respectively. When tracheal spirals were pretreated with these concentrations of LTE_4 for 15 min (the average time taken for the LTE_4-induced contraction to reach a plateau), washed, and permitted to return to baseline tensions, there was an augmentation of contractile responses to histamine that was related to the initial dose of LTE_4. The log EC_{50} values of the second histamine dose–response curve in tissues treated with 2.3 nM, 10 nM, and 23 nM LTE_4 were −4.85 ± 0.20 (mean ± SEM, $n = 4$), −5.05 ± 0.09 ($n = 6$), and −5.10 ± 0.11 ($n = 8$), respectively. The differences between LTE_4-treated and control tissues were significant at 10 nM and 23 nM LTE_4 (Fig. 1*B*). The ability of LTE_4 to enhance subsequent responses to histamine was not observed at 5 or 20 min of prior contact between leukotriene and tracheal spirals and was maximal at 10–15 min of prior contact time (Fig. 2).

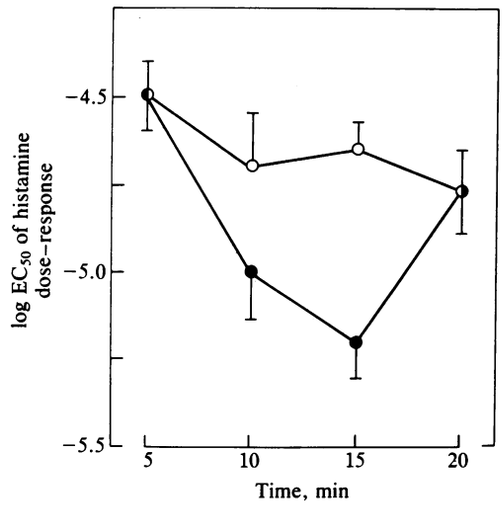

FIG. 2. log EC_{50} of histamine dose–response effect in LTE_4-treated (●) and control (○) tracheal tissues as a function of the duration of LTE_4 pretreatment. Each point represents the mean ± SEM of four tissues.

The concentrations of LTE_4 giving rise to contractions of parenchymal strips equal to 10%, 30%, and 50% of the maximum histamine contraction were 0.02 nM, 0.2 nM, and 10 nM, respectively (Fig. 3*A*), compatible with the previous findings that LTE_4 is a preferential peripheral contractile agonist but is less selective than LTC_4 (18). When parenchymal strips were treated with 0.02–10 nM LTE_4 for 15 min, there was no significant difference between the log EC_{50} values for LTE_4-treated and control tissues (Fig. 3*B*). Tracheal and parenchymal tissues pretreated with higher concentrations of LTE_4 did not relax to pre-leukotriene baseline tensions, even with extensive washing, and thus were not used for further analysis.

Because the receptor for LTC_4 appears to be distinct from that for LTD_4 (15, 20–23), LTE_4 was compared to LTC_4 and LTD_4 in terms of ability to elicit hyperresponsiveness to histamine. The concentrations of LTC_4 and LTD_4 giving rise to a tracheal contraction equal to 50% of the maximum histamine contraction were 80 nM and 40 nM for LTC_4 and LTD_4, respectively, indicating that LTE_4 is approximately 4-fold more active than LTC_4 and 2-fold more active than LTD_4 in contracting tracheal smooth muscle. Because the greatest enhancement of histamine responses by LTE_4 (23 nM) was observed after a concentration that gave a contraction equal to 50% of the maximum histamine contraction, concentrations of LTC_4 (80 nM) and LTD_4 (40 nM) eliciting similar contractile reactions were used to pretreat tracheal tissues for 15 min. The log EC_{50} of the second histamine dose–response curve in the tissues treated with 23 nM LTE_4 was −5.10 ± 0.11 (mean ± SEM, $n = 8$), which was significantly different from the −4.66 ± 0.10 (mean ± SEM, $n = 8$) of the control tissues ($P < 0.01$) (Fig. 4). In contrast, there was no significant difference between the log EC_{50} values from the second histamine dose–response curve of the tissues treated with 80 nM LTC_4 (−4.85 ± 0.19, mean ± SEM, $n = 7$) and their controls (−4.62 ± 0.12, $n = 7$) or of the tissues treated with 40 nM LTD_4 (−4.77 ± 0.12, $n = 7$) and their controls (−4.65 ± 0.03, $n = 7$).

Pretreatment of tracheal spirals with 1 and 10 μg of FPL55712 (Fisons, Loughborough, U.K.) per ml for 30 min completely inhibited the contraction elicited by 23 nM LTE_4 (Fig. 1). In contrast, the LTE_4-induced augmentation of the response to histamine persisted in the presence of FPL55712

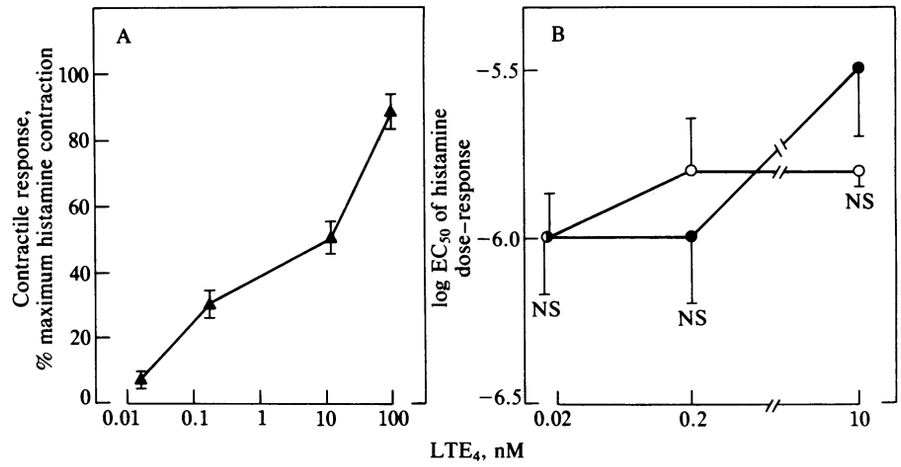

FIG. 3. (*A*) Effect of increasing LTE_4 concentrations on the contractile response of guinea pig parenchymal strips (mean ± SEM, n = 13). (*B*) log EC_{50} of histamine dose–response effect in LTE_4-treated (●) and control (○) parenchymal strips as a function of the concentration of LTE_4 used to pretreat tissues (mean ± SEM, n = 6). NS, not significant.

at 1 μg/ml but was completely inhibited by this drug at 10 μg/ml (Fig. 4). Pretreatment of tracheal spirals with 1 μg/ml indomethacin (Sigma) for 30 min did not suppress the contraction elicited by 23 nM LTE_4 (Fig. 1) but completely inhibited the augmentation of the response to histamine (Fig. 4).

DISCUSSION

The capacity of LTE_4 to enhance the responsiveness to histamine of tracheal smooth muscle was notably persistent, since the tissues were washed extensively and rested for 2 hr before the assessment of the augmented histamine response. The ability of LTE_4 to augment responsiveness to histamine may be particularly pertinent *in vivo*, since there is bioconversion of LTC_4 and LTD_4 to LTE_4 during the contractile response with isolated muscle (24) and because LTE_4 is the most biologically stable of the SRS-A leukotrienes in plasma or in the presence of activated cells (25, 26). LTE_4-induced hyperresponsiveness to histamine was unrelated to the contractile response alone because LTC_4 and LTD_4 did not elicit

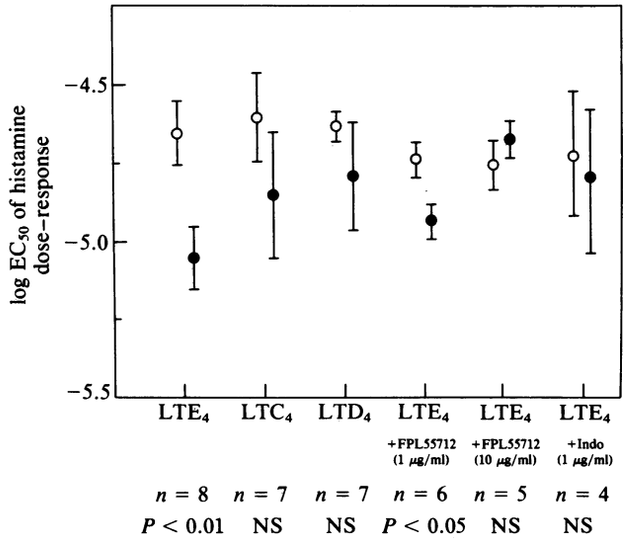

FIG. 4. log EC_{50} of histamine dose–response effect in control tracheas (○) and tracheas (●) pretreated with 23 nM LTE_4, 80 nM LTC_4, 40 nM LTD_4, and 23 nM LTE_4 in the presence of FPL55712 (1 and 10 μg/ml) and of indomethacin (Indo, 1 μg/ml). NS, not significant.

increased responsiveness to histamine despite constricting tracheal tissues by the same degree as LTE_4 (Fig. 4), and indomethacin did not alter the contraction but inhibited the augmentation of reactivity to histamine caused by LTE_4 (Fig. 4). The latter finding suggests that LTE_4 may initiate two postreceptor events: the enhancement of central airway reactivity is indomethacin-inhibitable and, therefore, is probably related to the generation of cyclooxygenase pathway product(s) triggered by LTE_4 exposure; and the LTE_4-elicited contraction is not affected by indomethacin and is mediated by a mechanism independent of prostaglandin synthesis. The LTE_4-induced contraction was markedly inhibited by 1 and 10 μg of FPL55712 per ml (Fig. 1*B*), whereas the LTE_4-induced augmentation of responsiveness to histamine was only inhibited by FPL55712 at 10 μg/ml (Fig. 4). Thus, either the concentration of LTE_4 needed to elicit hyperresponsiveness is less than the dose needed to induce contraction through the same receptor or LTE_4-induced hyperresponsiveness and contraction are mediated by different receptors.

Inhibition of radioligand binding studies have indicated that LTE_4 competes for the LTD_4 receptor but not effectively for the LTC_4 receptor (20, 21, 27), suggesting that the expression of LTE_4 activity may be through the LTD_4 receptor but that there are separate receptors for LTC_4 and LTD_4 (27). The finding that LTE_4, but not LTC_4 or LTD_4, enhances airway responsiveness to histamine suggests that this action of LTE_4 is mediated through a receptor distinct from those for LTC_4 and LTD_4. The capacity of LTD_4 to potentiate histamine responsiveness on tracheal smooth muscle has been observed only in the presence of low extracellular calcium concentrations (0.1 mM) (28), when there may have been an alteration in the properties of the LTD_4 receptor (27). On the basis of our findings and those of other investigators (14, 15, 20–23, 27, 28), we propose that there may be three separate receptors: one for LTC_4, one for LTD_4, and one that is revealed by the preferential contractile action of LTE_4 on tracheal smooth muscle of the guinea pig and the subsequent hyperresponsiveness of that tissue to the spasmogenic action of histamine. The magnitude of the LTE_4-induced enhancement observed in this *in vitro* study was three-fold, whereas asthmatic airways respond to bronchoconstrictor substances at 1/30th the concentration required to elicit a similar physiological response in normal persons (1–3). This difference may be related to the nonlinear relationship of *in vitro* constriction and *in vivo* effects (29), resulting in substantial amplification of the phenomenon *in vivo*, to the participation of inflammatory cells *in vivo*, to

species differences, and to the experimental design, which included extensive washing and resting of normal tissues to remove free agonist and restore normal tone, thereby creating an interval that might lead to partial loss of elicited hyperresponsiveness. If LTE_4 is a major factor in the pathogenesis of asthmatic airway hyperirritability, then inhibition of sulfidopeptide leukotriene production could reduce the airway hyperresponsiveness of asthmatic patients, which subjects them to attacks of wheezing upon exposure to a range of environmental stimuli.

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