Comparative airway and vascular activities of leukotrienes C-1 and D *in vivo* and *in vitro*

(guinea pig/pulmonary mechanics/parenchymal strips/tracheal spirals/slow reacting substance of anaphylaxis)

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ABSTRACT The pharmacologic activities of leukotrienes C-1 and D (LTC-1 and LTD), constituents of slow reacting substance of anaphylaxis (SRS-A), were evaluated *in vitro* on airway contractile tissues and *in vivo* on pulmonary mechanical function, mean systemic arterial pressure, and cutaneous microcirculation. *In vitro* both LTC-1 and LTD were potent and selective peripheral airway agonists, being more active than histamine; furthermore, LTD was active on peripheral airways at concentrations 1/100th those of LTC-1. The concentration-effect relationship for LTD and the profile of antagonism by FPL 55712 are consistent with the activity of this molecule at two separate peripheral airway receptors. *In vivo*, LTC-1 and LTD were nearly equally active in their effects on pulmonary mechanics, and the pattern of alterations was consistent with the predominant site of action being in the lung periphery. Furthermore, both agents had a direct systemic arterial hypotensive effect and were vasoactive on the cutaneous microcirculation. Thus, these compounds are likely to be major mediators of the pathologic alterations in immediate type hypersensitivity reactions in which peripheral airway constriction and hypotension are prominent features.

Slow reacting substance of anaphylaxis (SRS-A) (1), which is an activity generated during immediate type hypersensitivity reactions (2), has been found to be composed of leukotrienes C-1 (LTC-1) and D (LTD) (3). Native SRS-A has a unique profile of contractile activity *in vitro* in smooth muscle preparations (4). Both partially and highly purified native SRS-A produced by an anaphylactic reaction in the rat peritoneal cavity (SRS-A^{rat}) exhibit a preferential contractile activity for guinea pig pulmonary parenchymal strips compared to musculus trachealis (5), and LTC-1 and LTD exhibit the same differential effect, at concentrations less than 0.1% of those required for histamine to be active (3). Partially purified SRS-A^{rat} augments vascular permeability when injected into guinea pig skin (6). When administered intravenously into the unanesthetized guinea pig, it produces an alteration in pulmonary mechanics consistent with peripheral rather than central airway action (7). We have now demonstrated that intravenous infusion of LTC-1 and LTD alters pulmonary mechanics in unanesthetized and anesthetized guinea pigs in a manner similar to native partially purified SRS-A^{rat} and that, in addition, these newly described products of arachidonate metabolism (8-10) can differ in their actions on the cutaneous microvasculature and on mean systemic arterial pressure.

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MATERIALS AND METHODS

LTC-1 and LTD were prepared as described (3, 10), sealed in ampoules in 10% methanol under argon, and stored frozen until the day of use. Histamine diphosphate was obtained from Sigma. FPL 55712, a specific SRS-A antagonist, was a gift from P. Sheard (Fisons Pharmaceuticals, Ltd., U.K.).

Tracheal spirals and parenchymal strips were prepared for recording isometric contractile activity (5) and allowed to relax to baseline tensions of approximately 250 mg and 4 g, respectively. Concentration-effect relationships for histamine were determined by exposing the tissues to geometrically increasing concentrations of histamine to a maximum of 100 μ M. The dose-response of tissue to LTC-1 and LTD, respectively, was recorded in terms of the maximal active tension achieved after the serial addition of small amounts of concentrated agonist to obtain final bath concentrations between 10^{-14} and 10^{-5} M. The SRS-A antagonist FPL 55712 (11) at final bath concentrations between 0.1 and 10 μ g/ml (30-min incubation) was examined for its inhibition of the cumulative concentration-effect relationships for both LTC-1 and LTD. The ratio of the concentration of LTC-1 or LTD required to achieve a contraction equal to 30% of that obtained with 100 μ M histamine in the presence of FPL 55712 to that in the absence of FPL 55712 was calculated. This quantity is the "dose ratio" (12).

Hartley strain guinea pigs weighing 350 g were given 1-150 ng of LTC-1 and LTD and 10, 100, and 1000 ng of histamine by intradermal injection into the shaved dorsal skin at 8-10 sites. When augmented cutaneous permeability was being assessed, 0.2 ml of Coomassie brilliant blue R-250 dye (Bio-Rad), 75 mg/ml in phosphate-buffered saline, was administered intravenously 15 min after the intracutaneous injections. When vasoconstriction was being evaluated, 1.0 ml of the dye solution was given 5 min after the intradermal injections of the agonists. The animals were killed under anesthesia, the skins were reflected, and the lesion areas were assessed for diameter and intensity of bluing in the permeability-enhancing protocol and for diameter and absence of bluing in the vasoconstriction studies.

Male Hartley strain guinea pigs weighing 300-350 g were anesthetized by the intraperitoneal injection of α -chloralose (50 mg/kg) and urethane (500 mg/kg) and were placed in the

Abbreviations: SRS-A, slow reacting substance of anaphylaxis; LTC-1, leukotriene C-1; LTD, leukotriene D; SRS-A^{rat}, slow reacting substance of anaphylaxis produced by an anaphylactic reaction in the rat peritoneal cavity; G_L , pulmonary conductance; C_{dyn} , dynamic compliance.

FIG. 1. Contractile effects of LTC-1, LTD, and histamine on parenchymal strips (Left) and tracheal spirals (Right) *in vitro*. Results represent the mean $\pm$ SEM of 14 experiments with LTD ($\bullet$ — $\bullet$), 16 experiments with LTC-1 ($\blacksquare$ — $\blacksquare$), and 30 experiments with histamine ($\circ$ — $\circ$) on parenchymal strips, and of 6 experiments with LTD and LTC-1 and 12 experiments with histamine on tracheal spirals.

supine position with cannulas in the trachea, carotid artery, and cephalic vein. Ten percent of the initial anesthetizing dose was given every 2 hr thereafter. The animals were then prepared for recording of pulmonary mechanics as described (13). Pulmonary resistance and dynamic compliance (C_{dyn}) were computed on a breath-by-breath basis by a respiratory analyzer (Hewlett-Packard, model 8816). Pulmonary conductance (G_1) was determined as the inverse of resistance.

Male Hartley strain guinea pigs weighing 280–410 g were lightly anesthetized with ethyl ether, and a small polyvinyl chloride catheter (outside diameter, 0.76 mm; inside diameter, 0.38 mm) was inserted via a skin window into a peripheral vein and threaded centrally into the superior vena cava. The animals were then prepared for recording of pulmonary mechanics according to the technique outlined by Amdur and Mead (14). A polyvinyl chloride catheter (outside diameter, 1.25 mm; inside diameter, 0.76 mm) was placed in a femoral artery and

threaded centrally to allow continuous determination of mean systemic arterial pressure.

A 30-min period was allowed for equilibration after completion of the initial physiologic preparation of anesthetized and unanesthetized animals. Control data were obtained during the next 30 min. Each animal then received a rapid intravenous infusion of a leukotriene in 0.3 ml of saline and then subsequent doses of leukotrienes at 30-min intervals. Pulmonary function and mean systemic arterial pressure were recorded continuously.

RESULTS

LTC-1 and LTD were equally active, on a molar basis, in inducing contraction of tracheal spirals and were approximately 30- to 100-fold more active than histamine (Fig. 1). LTD and LTC-1 were 20,000 times and 200 times, respectively, more potent than histamine in causing contraction of parenchymal

FIG. 2. Competitive antagonism plots for the effects of FPL 55712 on LTC-1 and LTD on parenchymal strips *in vitro*. Dose ratios (DR) were calculated at an active tension equivalent to 30% of the active tension resulting from exposure to 100 μ M histamine.

FIG. 3. Time course of effects of intravenous infusion of LTC-1 (●—●) and LTD (○---○) (0.5 μ g/kg) on mean systemic arterial pressure, C_{dyn} , and G_1 in anesthetized guinea pigs. Results are the mean $\pm$ SEM of four experiments with each compound.

strips, as determined on a molar basis at half-maximal histamine contractions. The concentration-effect relationship for LTD had an initial mean slope of 52 mg of tension per decade of

concentration (10^{-14} M – 10^{-13} M) similar to the mean slope for histamine, 70 mg per decade of concentration. Over the concentration range 10^{-13} – 10^{-10} M, the mean slope was only 17 mg per decade but increased, at higher concentrations, to 54 mg per decade, similar to the mean slope of the LTC-1 concentration-effect relationship of 55 mg/decade.

The effects of FPL 55712 on parenchymal strip constriction induced by LTC-1 and LTD are shown as the logarithm of the concentration of FPL 55712 in the bath plotted against the logarithm of the dose ratio minus 1 resulting from each concentration of FPL 55712 (12) (Fig. 2). Contractions induced by LTC-1 were not antagonized by FPL 55712, as indicated by mean dose ratios near unity over the range of antagonist concentrations 0.1–10 μ g/ml. Contractions less than 35% of maximal histamine response induced by LTD were antagonized by FPL 55712 such that the effective concentration for a 2-fold inhibition of the LTD-induced contraction was 0.057 μ g/ml. Contractions of greater magnitude were not inhibited. The slope of this plot, as determined from least squares linear regression analysis, was 0.723.

The time course of effects on pulmonary mechanics resulting from intravenous infusion of LTC-1 and LTD in anesthetized guinea pigs is shown in Fig. 3. After a lag period of 30–45 sec, during which no airway effects could be detected, G_1 and C_{dyn} decreased rapidly and reached their nadir approximately 60–90 sec after completion of infusion. G_1 returned to near pre-infusion levels by 5 min, but the decreased C_{dyn} resulting from LTC-1 or LTD infusion was still significantly below control levels at this time. LTD was more potent than LTC-1 in that the magnitude of the decreases in C_{dyn} was greater throughout the 5-min time course. A similar time course of effects was observed after intravenous infusion of these two agents in unanesthetized animals.

The dose-response relationships for the effects of LTC-1 and

FIG. 4. Dose-response relationships for the effects of LTC-1 (●—●) and LTD (○---○) on mean systemic arterial pressure, C_{dyn} (ml/cm H₂O), and G_1 (ml/sec/cm H₂O) on anesthetized (Left) and unanesthetized (Right) guinea pigs. Results are the mean $\pm$ SEM of four experiments with each compound.

FIG. 5. Time course effects of LTD on mean systemic arterial pressure in anesthetized (O---O) and unanesthetized (●—●) guinea pigs. Results are the mean $\pm$ SEM of four experiments under each condition.

LTD 60 sec after infusion in anesthetized and unanesthetized animals are shown in Fig. 4. In anesthetized animals both LTC-1 and LTD infusions resulted in statistically significant decreases in both G_1 and C_{dyn} . LTD was more potent, on a weight basis, than LTC-1 as indicated by its significant effects on G_1 at 1/10th to 1/3rd the concentrations of LTC-1 required. Whereas a maximal effect on G_1 was achieved with the infusion of LTD in the 500 ng/kg range, C_{dyn} showed a further decrease with increasing doses. The effects of a given dose of LTC-1 and LTD on C_{dyn} and G_1 were less marked in unanesthetized animals than in anesthetized animals. Significant decreases in G_1 were observed only with infusion of either compound at >5000 ng/kg. In both anesthetized and unanesthetized animals the percentage decrease in C_{dyn} was greater than the percentage

decrease in G_1 for a given dose of LTC-1 or LTD. For example, in anesthetized animals LTD at 500 ng/kg resulted in 45.0% decrease in G_1 and a 65% decrease in C_{dyn} ; in unanesthetized animals this dose of LTD resulted in an increase in G_1 and a 38% decrease in C_{dyn} .

Intravenous infusion of either LTC-1 or LTD resulted in a decrease in mean systemic arterial pressure in anesthetized animals. Hypotension in anesthetized animals was most marked 60–120 sec after completion of drug infusion, persisted for at least 5 min (Fig. 3), and was dose-related at 60 sec (Fig. 4). In contrast, in unanesthetized animals LTC-1 or LTD infusion at the higher doses was accompanied by mean systemic arterial hypertension 60 sec after infusion (Fig. 4). After infusion of LTD at 500 ng/kg in unanesthetized animals there was a period of hypertension peaking at 60 sec followed by transient hypotension (Fig. 5). In contrast, in anesthetized animals only hypotension occurred.

The intradermal injection of LTC-1 over the range 5–50 ng elicited vasoconstriction of increasing intensity; in the range 50–150 ng it was associated with complete blanching of the area as assessed in otherwise completely blued guinea pigs (Table 1). The constrictive action of LTC-1 limited detection of this agonist to the periphery of the intradermal injection sites in guinea pigs blued so as to show only areas of increased vascular permeability. In contrast, LTD evoked a dose-related lesional bluing at 5–100 ng in the assay for increased permeability and induced only minimal blanching at 50 and 100 ng in the bioassay for constrictive action.

DISCUSSION

LTC-1 and LTD, major components of SRS-A, are potent pharmacologic agonists that mediate effects on both pulmonary mechanics and systemic arterial pressure of anesthetized and unanesthetized guinea pigs when given intravenously (Figs. 3–5) and on the cutaneous microcirculation when administered intradermally (Table 1). Because protracted peripheral airway involvement is prominent in systemic anaphylaxis (15, 16), leukotrienes may account for the antihistamine-resistant element of such responses.

Peripheral lung tissues in the form of parenchymal strips are 200- to 20,000-fold more sensitive to LTC-1 and LTD than to histamine, and central airway tissues (tracheal spirals) are 30- to 100-fold more responsive than to histamine (Fig. 1). Thus, both LTC-1 and LTD have the properties of highly potent selective peripheral airway agonists *in vitro* when compared to histamine. Furthermore, LTD is a more selective peripheral airway agonist than LTC-1, as demonstrated by equal activity of the two leukotrienes on tracheal spirals and the 100-fold greater activity of LTD relative to LTC-1 on parenchymal strips (Fig. 1). The concentration-effect relationships of LTD suggest mediation of its contractile activity through interaction with distinct high- and low-affinity smooth muscle parenchymal receptors. Contractions initiated at low concentrations of LTD via high-affinity receptors plateau at only 30–40% of the maximal contractile activity, and a 1000-fold higher concentration of LTD is needed to recruit a second low-affinity receptor which affords a full contractile response. Further evidence for a dual receptor is the fact that FPL 55712 inhibits LTD in a dose-related manner when dose ratios are calculated for parenchymal strip contractions equal in magnitude to 30% of the maximal histamine response but not when contractions are equal to or greater than 50% of the histamine response or when LTC-1 is the agonist. This finding suggests that the inhibition by FPL 55712 is directed to the high-affinity receptor for LTD in parenchyma and not to the low-affinity receptor for LTD which may be shared with LTC-1. The postulated

Table 1. Cutaneous responses to various doses of LTC-1 and LTD in guinea pigs

Material injected, ng	n*	Major response	Response strength	Response diameter, mm
Saline	29	—	—	—
LTC-1:				
1	4	—	—	—
5	8	C	+	6
10	3	C	+	7
25	12	C	++-+++	10
50	6	C	++++	12
100	4	C	++++	13
150	10	C	++++	14
LTD:				
1	4	—	—	—
5	4	P	+	8
10	4	P	++-+++	10
50	6	P	+++	13
100	4	P	+++	13
Histamine:				
10	4	—	—	—
100	8	P	+	8
1000	15	P	++++	15

Enhanced permeability (P) was assessed from the diameters of the blued areas and the intensities of coloration qualitatively assessed from + (minimal effect clearly distinguishable from uncolored skin) to ++++ (dye intensity completely masking normal skin color at the test site). Animals to be assessed for vasoconstriction at the test sites were prepared so as to produce a generalized bluing of the skin. Vasoconstriction (C) was assessed by the absence of bluing which varied from + (minimal decrease in bluing clearly distinguishable from surrounding skin) to ++++ (injected site completely white) and by the diameter of each positive site.

* n, Number of animals injected with test substance at each dose.

inhibition of a high-affinity receptor of LTD by FPL 55712 is not a simple competitive one as evidenced by a slope of less than unity on the Arunlakshana-Schild plot (12) (Fig. 2).

In both anesthetized and unanesthetized guinea pigs, intravenous infusion of either leukotriene resulted initially in marked decrements in both C_{dyn} and G_1 (Figs. 3 and 4). Two minutes after infusion the increased resistance (inverse of G_1) resolved, whereas the decrease in C_{dyn} persisted through 5 min at the 500 ng/kg dose. In dose-response studies, the decrease in C_{dyn} was consistently greater than the decrement in conductance at the same dose in both anesthetized and unanesthetized animals (Fig. 4); thus, the apparent selective peripheral airway activity of the leukotrienes cannot be attributed to the effects of anesthesia or the breathing pattern. It probably represents a direct effect on airway and airspace contractile elements. Other mechanisms such as peribronchial or alveolar edema could also contribute to this pattern of pulmonary mechanical response.

Although LTD was more active than LTC-1 on pulmonary mechanics on a weight basis, the differences were small, with the largest difference being on G_1 in anesthetized animals. Considered in respect to the greater *in vitro* activity of LTD on parenchymal strips, the *in vivo* observations suggest that LTD may be structurally modified in the circulation between the sites of its introduction and its action in the lung periphery. There may also be differences in terms of uptake, distribution, and metabolism between the two leukotrienes.

In anesthetized animals both leukotrienes were hypotensive agents, and LTD was slightly more potent than LTC-1 (Figs. 3 and 4). In contrast, in unanesthetized animals, LTC-1 and LTD both elicited a transient hypertensive response followed by a return to baseline with LTC-1 and a decrease to a hypotensive arterial pressure with LTD. It seems likely that the hypertensive response observed in the unanesthetized animals is a reflex response and that this response is prevented by general anesthesia. This interpretation implies that, when present, the hypotensive response represents a direct cardiovascular action of the leukotrienes.

In concentrations as low as 5 ng/0.1 ml, or approximately 100 nM, LTC-1 and LTD produced vasoconstriction and increased vasopermeability, respectively, when injected into guinea pig skin (Table 1). Because LTD has recently been shown to be the major component of SRS-A (3), it is not surprising that a previous assay of partially purified SRS-A also showed increased

vascular permeability in guinea pig skin (6). In that study, 5–125 biological units, a range corresponding to 0.4–10 ng of LTD, produced local bluing. It is apparent from the present study that LTD can act to increase vascular permeability at molar concentrations less than 1% that of histamine (Table 1).

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