Leukotriene D₄ Relaxes Canine Renal and Superior Mesenteric Arteries

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SUMMARY. To characterize the influences of leukotriene D₄ on regional vascular smooth muscle, effects of leukotriene D₄ on vasomotor tone of canine renal and superior mesenteric arterial rings were determined. Vascular smooth muscle tone was measured with isometric force transducers. After tone had been induced with norepinephrine, leukotriene D₄, in concentrations of 10⁻⁶ M to 10⁻⁷ M, produced dose-dependent relaxation of renal and superior mesenteric arterial rings. Leukotriene D₄-induced relaxation was observed only in those ring preparations in which care had been taken to avoid damaging the luminal surface. Acetylcholine (10⁻⁷ M) also decreased tone in these same ring segments. Neither acetylcholine nor leukotriene D₄ altered tone of arterial rings after the endothelium had been intentionally disrupted by rubbing with a cotton-tipped applicator. Nitroglycerin (10⁻⁵ M) relaxed rings both before and after rubbing the intimal surface. These results demonstrate that leukotriene D₄ possesses the capacity to relax canine superior mesenteric and renal arterial rings in an endothelial-dependent manner. Because relaxation of renal and superior mesenteric arterial rings in response to leukotriene D₄ was not altered after incubation with indomethacin (10⁻⁵ M), the observed endothelial-dependent relaxation induced by leukotriene D₄ did not appear to be related to release of a cyclooxygenase metabolite(s). In contrast, FPL 55712 (10⁻⁵ M) attenuated the relaxation produced by leukotriene D₄, suggesting that this response was a receptor-linked consequence. (Circ Res 57: 323–329, 1985)

LEUKOTRIENES are a group of arachidonic acid metabolites formed via the lipoxygenase pathway, and the cysteinyl derivatives, leukotriene C₄ (LTC₄), D₄ (LTD₄), and E₄ (LTE₄), have been identified as active components of slow-reacting substances of anaphylaxis (SRS-A) (Lewis et al., 1980; Lewis and Austen, 1981). Vascular tissue was first shown to release SRS-A more than 20 years ago (Brocklehurst, 1960; Chakravarty, 1960), and more recently it has been demonstrated that large peripheral blood vessels obtained from a variety of species, including the dog, possess the capacity to synthesize and release leukotriene-like substances (Piper et al., 1983; Wolbling et al., 1983; Dembinska-Kiec et al., 1984). Although the formation of leukotrienes in vascular smooth muscle has not been unequivocally demonstrated, these derivatives stimulate vascular smooth muscle and may participate in vasomotor control mechanisms.

Although most studies have shown that leukotrienes contract vascular smooth muscle, divergent effects have been observed. Both LTC₄ and LTD₄ decreased coronary blood flow in anesthetized dogs (Panzenbeck and Kaley, 1983; Woodman and Dusting, 1983), but relaxed spiral segments of dog coronary artery in the presence of 27 mM potassium (Burke et al., 1982). In addition, LTC₄, LTD₄, and LTE₄ possess the capacity to produce marked vasoconstriction in the mesenteric vascular bed of both the anesthetized dog and cat, but have little vasoreactivity in the canine kidney (Feigen, 1983; Chapnick, 1984; Lippton et al., 1984). Because in these studies leukotriene-induced mesenteric vasoconstriction was not altered after administration of several inhibitors of cyclooxygenase activity, including indomethacin, ibuprofen, and meclofenamate, it did not appear that cyclooxygenase products of arachidonic acid were participants, either as amplifiers or modulators, in the observed hemodynamic alterations. It was also reported that mesenteric vasoconstriction produced by LTD₄ and LTC₄ was attenuated after administration of the putative leukotriene antagonist, FPL 55712 (Augstein et al., 1973), which suggested a direct effect of the leukotrienes via receptor-mediated processes (Feigen, 1983). However, we have observed that this antagonist produced a marked increase in mesenteric blood flow in the absence of an effect on either mean arterial pressure or renal blood flow (unpublished data). Whether this was or was not a direct effect of FPL 55712 is yet unknown, but it appears that interpretation of results obtained in the presence of this putative antagonist must be viewed with some caution, and further investigation of the mode of action of these newly described products of arachidonic acid metabolism still is required.

To characterize the influences of leukotrienes on regional vascular smooth muscle function more directly, in these studies we attempted to determine the vasomotor effects of LTD₄ on ring preparations of canine superior mesenteric and renal arteries in vitro. Because it has been suggested (Furchgott and...
LTD* is not likely to be a component of the endothelium-derived relaxing factor (EDRF) discovered by Furchgott and Zawadzki (1980). Rather, it is possible that this lipoxygenase-derived product of arachidonic acid may, itself, release an EDRF.

Methods

Adult mongrel dogs of either sex weighing 17–24 kg were anesthetized with sodium pentobarbital (30 mg/kg, iv). The renal and superior mesenteric arteries were carefully excised, trimmed free of adhering fat and connective tissue, and cut into rings 2–3 mm long. Two ring segments were obtained from each artery. The luminal surface of one set of rings was rubbed gently with a cotton-tipped applicator to remove or disrupt the endothelial cell layer. In the other set of rings, special care was taken to avoid touching the luminal surface to ensure the integrity of the endothelium. Relaxation of vascular smooth muscle in response to acetylcholine (ACh), but not in response to nitroglycerin, has been shown to be dependent on an intact endothelial layer (Furchgott and Zawadzki, 1980). Therefore, disruption of the endothelial cell layer was defined as adequate if, after the luminal surface was rubbed, the relaxation response to ACh, but not to nitroglycerin, was markedly diminished or abolished.

The rings were mounted horizontally between an "L"-shaped fixed stainless steel rod and a freely moving stainless steel triangle in 20-ml jacketed glass organ chambers. The triangle was attached to a Grass FT03 force displacement transducer which was connected to a Grass polygraph (model 7) to record changes in isometric tension. The incubation chambers were filled with 10–20 ml of Krebs-Ringer bicarbonate solution (composition in mm: NaCl 118.3; KCl, 4.7; CaCl2, 2.5; MgSO4, 1.2; KH2PO4, 1.2; NaHCO3, 25.0; Ca-EDTA, 0.026; glucose, 11.1). The incubation fluid was continuously aerated with a gas mixture of 95% O2 plus 5% CO2; pH of this solution was 7.4, and temperature was maintained at 37°C. Before the experiments were begun, the rings were placed at the optimal point of their length-tension relationship, as described by Vanhoutte and co-workers (Vanhoutte and Lesen, 1969; De Mey et al., 1982). Basal tensions of 4–6 g for the renal artery and 8–14 g for the superior mesenteric artery were found to be optimal for inducing maximal isometric contractions to 20 mM KCl. Before the experiment was begun, an equilibration period of 45–60 minutes was allowed, during which time the bath was rinsed every 15 minutes with the control buffer solution, and the tension was adjusted to maintain the appropriate level.

When basal tension was stable, cumulative dose-response curves for norepinephrine (NE) were constructed in all vessels to determine the concentration of the agonist which produced 50–75% of the maximal response. To induce submaximal tone in the renal artery, concentrations of NE ranged from $10^{-8}$ M to $3 \times 10^{-4}$ M and produced a contraction of 7.7 ± 1.0 g. In the superior mesenteric artery, a submaximal contraction of 10.3 ± 2.3 g developed in response to NE, $3 \times 10^{-4}$ M to $10^{-6}$ M. Responses to ACh, LTD4, and nitroglycerin then were obtained under these conditions of active tone. After each series of agonist additions, the ring preparations were washed with 60 ml of buffer and allowed to reequilibrate for 30 minutes.

Norepinephrine (β-norepinephrine hydrochloride, Sigma, dose in terms of base) was dissolved and diluted in saline containing ascorbic acid, 1 mg/ml. Acetylcholine (acetylcholine hydrochloride, Sigma, dose in terms of base) was dissolved and diluted in saline. LTD4 (Merck Frost) was obtained as a stock solution in water. The stock solution was divided into aliquots and stored under an atmosphere of nitrogen in a freezer (So-Low) at ~80°C. For each experiment, an aliquot was thawed, diluted with saline to an appropriate concentration, and kept on ice. Indomethacin (Merck) was dissolved in 100 mM sodium carbonate. FPL 55712 (Fisons) was dissolved in de-ionized distilled water. Nitroglycerin (Parke-Davis) was dissolved in saline. All solutions were prepared in concentrations such that only small volumes, no greater than 100 μl, were added to the incubation chamber.

All values were determined as peak change from control level. Relaxation responses were expressed as percent reduction of NE-induced tone. The results are reported as mean ± SEM. All data were analyzed according to methods for paired comparisons or repeated measures with multiple comparison tests, where appropriate (Daniel, 1978). A P value of 0.05 or less was considered significant.

Results

The influence of ACh and LTD4 on isolated ring preparations of canine renal and mesenteric arteries was determined by addition of the agonist after the rings were precontracted as described above. Figure 1 shows the effects of ACh and LTD4 on unrubbed ring preparations of renal (panel A) and superior mesenteric (panel B) arteries. Acetylcholine ($10^{-6}$ M) decreased tone by 52 ± 6.9% in the renal artery (n = 15) and 64.1 ± 8.9% in the superior mesenteric artery (n = 7). In both the superior mesenteric artery and renal artery, relaxation induced by ACh was immediate in onset and reached a plateau approximately 4 minutes later. However, the plateau was not well maintained, and, after prolonged exposure to ACh, tone gradually increased. Relaxation of the rings in response to LTD4 ($10^{-6}$ M to $10^{-7}$ M) was somewhat slower in onset (15–20 seconds) than that produced by ACh. The LTD4-induced decrease in tone reached a plateau within 2–3 minutes and, like the response produced by ACh, was not maintained during prolonged exposure to the agonist. Whereas both ACh and LTD4 produced relaxation in NE-precontracted rings, very little, if any, effect of these two substances was observed in the absence of induced baseline tone.

Dose-response curves relating relaxation produced by LTD4 in superior mesenteric and renal arterial rings as a function of NE-induced tone are illustrated in Figure 2. The LTD4-induced relaxation in both the renal and superior mesenteric arteries
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FIGURE 1. Polygraph records illustrating relaxation produced by acetylcholine (ACh) and leukotriene D4 (LTD4) of renal (panel A) and superior mesenteric (panel B) arterial rings. Care was taken during preparation of the rings to prevent damage to the endothelium. After induction of submaximal tone with norepinephrine (NE), ACh or LTD4 was added to the organ chamber. Concentrations of drugs are expressed as logarithm (base 10) of final molar concentration in the organ chamber. After the tracings shown on the lefthand side of the figure had been obtained, the ring preparations were washed and allowed to equilibrate for at least 30 minutes. The tracing on the right hand side of the figure then was obtained.

FIGURE 2. Dose-response curves relating vasomotor effects of leukotriene D4 (LTD4) on unrubbed canine renal and superior mesenteric arteries after induction of submaximal tone with norepinephrine (NE). Doses of NE used to induce tone were those required to obtain 50–75% of maximum contraction, and ranged between 10−8 M and 10−7 M. All values are expressed as mean ± SEM. The number adjacent to each symbol indicates number of individual ring preparations studied.

FIGURE 3. Polygraph records illustrating effect of acetylcholine (ACh), leukotriene D4 (LTD4), and nitroglycerin (GTN) on rings of renal (panel A) and superior mesenteric (panel B) artery after the intimal surface was rubbed. After induction of submaximal tone with norepinephrine (NE), ACh or LTD4 was added to the organ chamber. In the absence of a response to either ACh or LTD4, GTN was then added. Concentrations of drugs are expressed as logarithm (base 10) of final molar concentration in the organ chamber. After the tracing on the lefthand side had been obtained, ring preparations were washed and allowed to equilibrate for at least 30 minutes. The tracing on the righthand side then was obtained.

was dose-dependent. In the renal artery, LTD4 in concentrations of 10−8 M, 3 × 10−8 M, and 10−7 M produced decreases in tone of 13.2 ± 2.8%, 26.5 ± 4.7%, and 37.0 ± 4.2%, respectively. In the superior mesenteric artery, LTD4 (3 × 10−8 M and 10−7 M) produced relaxations of 18.8 ± 7.2% and 37.8 ± 6.6%, respectively.

To determine whether the LTD4-induced relaxation was dependent on an intact endothelium, the intimal surface of one set of ring preparations was rubbed with a cotton-tipped applicator to disrupt the endothelium. Figure 3 illustrates the effect of ACh, LTD4, and nitroglycerin on rings of renal (panel A) and superior mesenteric arteries (panel B) after rubbing. Neither ACh nor LTD4 had any effect on ring preparations in which the endothelium had been disrupted. In contrast, nitroglycerin (10−6 M) produced rapid relaxation both in rings with an undisturbed endothelium (data not shown) and in preparations following rubbing of the intimal surface.

To determine whether a cyclooxygenase derivative of arachidonic acid may be a participant in this apparently endothelial-dependent relaxation, unrubbed ring preparations were incubated with indomethacin (10−5 M), an inhibitor of cyclooxygenase activity, for 30 minutes before induction of tone with NE. During incubation with indomethacin, a slight but not significant increase in baseline tone was observed. In the presence of the cyclooxygenase inhibitor, the influence of NE on renal (Fig. 4, panel A) and superior mesenteric (Fig. 5, panel A) arterial tone was significantly enhanced. In contrast to these contractile responses, indomethacin did not alter relaxation produced by LTD4 of either renal (Fig. 4,
FIGURE 4. Effects of indomethacin on the contractile response to NE (panel A) and the relaxation response to LTD₄ (panel B), in the canine renal artery. Doses of NE ranged from $10^{-7}$ M to $3 \times 10^{-6}$ M and were those required to obtain 50-75% of maximum contraction. All values are expressed as mean ± SEM. * indicates significantly different from control ($P < 0.05$) after incubation with indomethacin.

panel B) or superior mesenteric (Fig. 5, panel B) arterial rings. In addition, relaxation induced by either nitroglycerin ($10^{-6}$ M) or ACh ($10^{-7}$ M) was unchanged following pretreatment with indomethacin (Table 1).

Influence of the SRS-A antagonist, FPL 55712, on LTD₄-induced relaxation was evaluated to determine whether vasomotor responses produced by LTD₄ were related to a receptor-mediated process. Responses to LTD₄ were obtained both 3 and 30 minutes after incubation with FPL 55712 ($10^{-5}$ M). At this concentration, the antagonist neither altered the contractile response of ring segments to NE, nor produced relaxation after induction of tone. When obtained in the presence of the blocker, vasomotor responses to LTD₄ were significantly reduced in both the renal and superior mesenteric arteries (Fig. 6). Relaxation produced by LTD₄ following the 3-minute incubation period with FPL 55712 was not significantly different from that obtained after the 30-minute exposure. In contrast, the SRS-A antagonist had no effect on relaxation produced by ACh ($10^{-8}$ M) in either arterial preparation (Table 2).

Discussion

Vasomotor relaxation produced by ACh and a variety of other agents, including bradykinin, histamine, ATP, and ADP, has been associated with the release of an unknown mediator postulated to be formed in the endothelium (Furchgott and Zawadzki, 1980; Furchgott, 1983). Whereas the precise nature of this factor remains undefined, cyclooxygenase products of arachidonic acid metabolism do not appear to be responsible for this activity (Furchgott and Zawadzki, 1980). However, it has been reported that either 5,8,11,14-eicosatetraynoic acid (ETYA) or nordihydroguaiaretic acid (NDGA), two chemically different lipxygenase inhibitors, attenuated or reversed relaxation produced by acetylcholine, arachidonic acid, or bradykinin in NE-precontracted arterial rings (Furchgott and Zawadzki, 1980;
Chand and Altura, 1981; Cherry et al., 1982; DeMey et al., 1982). In view of these observations, it was postulated that a product of arachidonic acid metabolism formed via the lipoxygenase pathway subserved endothelial-dependent relaxation. Because peptidoleukotrienes failed to relax rabbit aorta (Berkowitz et al., 1984; Forstermann and Neufang, 1984), it was concluded that endothelial-dependent relaxation was not mediated by these arachidonic acid metabolites.

The present investigation not only provides further evidence that LTD₄ is not a component of EDRF, but, in addition, suggests that LTD₄ may, itself, produce relaxation of vascular smooth muscle via release of an endothelial-derived factor. Thus, LTD₄ only produced relaxation of norepinephrine-precontracted renal and superior mesenteric arterial rings with an intact intima. In contrast, after rubbing the luminal surface of the ring, neither relaxation nor contraction was observed in response to the leukotriene. Although no attempt was made to assess endothelial removal histologically, pharmacological criteria were applied. Therefore, the influence of ACh and nitroglycerin on vasomotor tone in both rubbed and unrubbed rings was determined. Acetylcholine produced relaxation of vessels prior to, but not after, the intimal surface was rubbed. In contrast, nitroglycerin, a substance known to produce vasomotor relaxation in a nonendothelial-dependent manner (Furchgott and Zawadzki, 1980), decreased tone in both unrubbed and rubbed preparations. These data strongly support the interpretation that rubbing the luminal surface with a cotton swab removed the endothelial layer, or at least markedly decreased its capacity to release an EDRF. Therefore, results of the present investigation indicate that LTD₄ possesses the capacity to produce relaxation of canine superior mesenteric and renal arterial rings in an endothelial-dependent manner. Whether or not the mechanism or mediator is identical to that discovered by Furchgott and Zawadzki (1980) is unknown.

Relaxation produced by LTD₄ was attenuated after incubation with FPL 55712 in the absence of a direct effect of the antagonist on vasomotor tone. This observation suggests that the relaxation produced by LTD₄ in isolated canine renal and superior mesenteric arteries was the result of a receptor-mediated process. Furthermore, the lack of effect of FPL 55712 on ACh-induced relaxation suggests that the two relaxing substances may be acting through different mechanisms and/or mediators. In addition, the observation that relaxation produced by ACh was not affected by FPL 55712 provides further evidence that the EDRF described by Furchgott and Zawadzki (1980) is not a leukotriene, and is consistent with results obtained in the rabbit aorta (Berkowitz et al., 1984; Forstermann and Neufang, 1984).

Although derivatives formed via the cyclooxygenase pathway did not appear to mediate endothelial-dependent relaxation (Furchgott and Zawadzki, 1980), both LTC₄ and LTD₄ possess the capacity to release cyclooxygenase products, of which prostacyclin is the major component, from human endothelial cells (Cramer et al., 1983; Pologe et al., 1984). Therefore, the possibility that a vasodilator prostaglandin, such as prostacyclin, subserves LTD₄-induced relaxation was considered. However, because LTD₄-induced relaxation of both renal and superior mesenteric arterial rings was not altered in the presence of indomethacin, it did not appear that the

### Table 2

Effect of FPL 55712 (10⁻⁶ M) on Relaxation Produced by Acetylcholine in Isolated Canine Renal and Superior Mesenteric Arteries

<table>
<thead>
<tr>
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<th>% Decrease in NE-induced tone</th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Renal artery (n = 5)</td>
<td>42.9 ± 11.9</td>
</tr>
<tr>
<td>Superior mesenteric artery (n = 6)</td>
<td>42.4 ± 10.2</td>
</tr>
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NE, norepinephrine. Values represent the mean ± SEM.
reduction in tone was dependent on release of a cyclooxygenase-derived product of arachidonic acid metabolism. Whereas relaxation of the rings in response to LTD₄ was not altered in the presence of indomethacin, NE-induced contraction was enhanced. This observation is consistent with the hypothesis that released vasodilator prostaglandins serve as modulators of vasoconstrictor hormones (Brody and Kadowitz, 1974; Messina et al., 1976). This finding also supports the conclusion that cyclooxygenase activity was indeed reduced after the addition of indomethacin.

It is generally recognized that leukotrienes produce constriction of both vascular and nonvascular smooth muscle (Piper, 1983; Feuerstein, 1984). In addition, these substances produced marked mesenteric vasoconstriction in both the anesthetized dog and cat, but only slightly increased blood flow in the dog kidney (Feigen, 1983; Chapnick, 1984; Lippton et al., 1984). Although the present observations were completely unexpected, both LTC₄ and LTD₄ have been reported to relax spirally cut segments of dog coronary artery incubated in the presence of 27 mM potassium (Burke et al., 1982). Because this relaxation was not blocked by FPL 55712, it was concluded that the change in tone induced by either LTC₄ or LTD₄ probably was not due to direct action on the muscle. The decrease in vasmotor tone also did not appear to be related to release of a cyclooxygenase product(s), since the addition of indomethacin did not alter LT-induced relaxation. Because no attempt was made to determine responses of the segments to ACh or to assess histologically the condition of the endothelium, it is unknown whether the observed relaxation of the dog coronary artery was dependent on an intact endothelium. However, these observations of Burke et al. (1982) are consistent with our results.

Whereas the present data do not appear to agree with previous observations in intact animals, it must be emphasized that these studies were performed on relatively large arteries. Furthermore, effects of LTD₄ on small arteries and veins, as well as resistance vessels, are as yet undetermined. Although the biological significance of EDRF release in vivo remains essentially unknown, Angus et al. (1983) have found that both ACh and substance P require an intact endothelium to elicit vasodilation in the femoral artery of the anesthetized dog. Their observation suggests that such a factor may not only serve as a mediator of vasodilation, but also may act as a modulator of vasoconstriction. Clearly, considerable investigation will be required to define such interrelationships between LTD₄ and endothelial-dependent relaxation.

The secretarial help of Linda Russell is gratefully acknowledged.

The gifts of leukotriene D₄ (provided by Dr. Joshua Rokach of Merck Frosst) and FPL 55712 (provided by Dr. Phil Sheard of Fisons) are greatly appreciated.

Circulation Research / Vol. 57, No. 2, August 1985

This study was supported by HL 34036 and a grant from the American Heart Association, Missouri Affiliate.

B.M. Chapnick is the recipient of National Heart, Lung and Blood Institute Research Development Award K04-10572.

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Received October 29, 1984; accepted for publication April 29, 1985.

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INDEX TERMS: Endothelial-dependent relaxation • Leukotriene D4 • Vascular smooth muscle • Canine renal artery • Canine mesenteric artery
Leukotriene D4 relaxes canine renal and superior mesenteric arteries.
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Circ Res. 1985;57:323-329
doi: 10.1161/01.RES.57.2.323

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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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