Endothelial-Dependent Relaxation Induced by Leukotrienes C₄, D₄, and E₄ in Isolated Canine Arteries

Roberta J. Secrest and Barry M. Chapnick

Leukotriene D₄ has been shown to possess the capacity to relax canine superior mesenteric and renal arterial rings in an endothelial-dependent manner. The present study was designed to determine if the remaining peptidoleukotrienes, leukotrienes C₄ and E₄, share this property. In addition, influences of atripine and of inhibitors of cyclooxygenase and lipoxygenase activities on relaxation produced by leukotriene D₄ and acetylcholine were determined to characterize further leukotriene D₄-induced relaxation and to compare these properties with those of acetylcholine. Vasomotor tone was measured with isometric force transducers. Following induction of tone with norepinephrine, leukotriene C₄ and acetylcholine produced concentration-dependent relaxation of renal and superior mesenteric arterial rings in which the endothelium was intact. Only minimal decreases in tone were produced in response to leukotriene E₄. Neither acetylcholine nor leukotriene C₄ altered tone after the endothelium had been intentionally disrupted. Nitroglycerin relaxed rings both before and after rubbing the endothelium. These results demonstrate that, similar to leukotriene D₄, leukotriene C₄ possesses the capacity to produce endothelial-dependent relaxation in canine renal and superior mesenteric arteries. Relaxation of the superior mesenteric artery produced in response to acetylcholine, but not leukotriene D₄, was inhibited in presence of atropine. Incubation of the rings with meclofenamate had no effect on relaxation induced by either acetylcholine or leukotriene D₄. Thus, it appears that endothelial-dependent relaxation induced by leukotriene D₄ is neither dependent on muscarinic receptor activation nor related to generation of cyclooxygenase metabolites of arachidonic acid. In contrast, 5,8,11,14-eicosatetraynoic acid and nordihydroguaiaretic acid attenuated relaxation in response to leukotriene C₄ and acetylcholine, suggesting that lipoxygenase-derived products may participate in leukotriene D₄-induced as well as acetylcholine-induced relaxation. (Circulation Research 1988; 62:983–991)
precontracted canine renal and superior mesenteric arterial rings in which the endothelial cell layer was intact. No change in vasomotor tone was induced in vascular rings following intentional disruption of the intima. These observations suggested that LTD₄, a lipoxygenase-derived product, probably was not a component of EDRF. Indeed, whether an EDRF was released by LTD₄ remains unknown.

In view of the fact that the other members of the peptide-containing leukotriene family, LTC₄ and LTE₄, are well known to possess vasoactive properties and because LTD₄ is intermediate in the conversion of LTC₄ to LTE₄, the purpose of the present study was to determine whether LTC₄ and LTE₄ produce endothelial-dependent relaxation of canine arteries in vitro. An additional goal was to characterize further the influences of LTD₄ on vasomotor tone of isolated canine arteries in which the endothelial cell layer was intact. Because ACh is the prototypical endothelial-dependent relaxing agent, vasomotor effects of LTD₄ were compared with those of ACh.

Materials and Methods

Adult male mongrel dogs (17–24 kg), fasted overnight but allowed free access to water, were anesthetized with sodium pentobarbital (30 mg/kg i.v.). 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. Four to six ring segments were obtained from each artery. During preparation of the ring segments, special care was taken to avoid touching the luminal surface to ensure integrity of the endothelium. The rings were mounted as previously described in 20-ml jacketed glass organ chambers containing 8 ml of the incubation medium, modified Krebs-Ringer bicarbonate solution. Isometric force was measured with Grass FT.03 force displacement transducers coupled to a Grass polygraph (model 7, Grass Instruments, Quincy, Massachusetts). All equilibrations and experimental procedures were conducted at 37°C. The modified Krebs-Ringer bicarbonate solution was continuously gassed with 95% O₂-5% CO₂ to maintain pH at 7.4 and had the following composition (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. Renal and superior mesenteric arterial rings were suspended under basal tensions of 4 g and 10–12 g, respectively. These loads were near optimum for maximal isometric contraction of the intima. The rings were studied in pairs. Thus, individual rings were exposed to either an inhibitor or its vehicle for 30 minutes. Following the reequilibration period, the rings were studied in pairs. Therefore, in this complete series of experiments, vasomotor responses to ACh, nitroglycerin, and an individual leukotriene were obtained and compared in a single ring segment both before and after rubbing the luminal surface.

Because LTD₄ had previously been shown to produce endothelial-dependent relaxation of canine superior mesenteric and renal arterial rings, a separate series of studies was conducted to determine effects of atropine, meclofenamate, ETYA, and NDGA on LTD₄-induced vasomotor relaxation. Analogous to the protocol described above, submaximal tone was induced with NE, and control responses to ACh, nitroglycerin, and LTD₄ were obtained prior to addition of an individual antagonist to the incubation medium. After obtaining the control responses, the ring preparations were washed with 60 ml buffer and allowed to reequilibrate for 30 minutes. Following the reequilibration period, the rings were studied in pairs. Thus, individual rings were exposed to either an inhibitor or its vehicle for 30–60 minutes prior to induction of tone with NE. Effects of cumulative additions of ACh, nitroglycerin, and LTD₄ were again determined. If after this procedure, relaxation produced by ACh was decreased by at least 80% of the control response in absence of a decrease in relaxation produced by nitroglycerin, an endothelial-independent relaxing agent, the endothelium was considered to be functionally disrupted. Therefore, the influence of LTD₄ on vasomotor tone of isolated canine arteries in which the endothelial cell layer was intact. Because ACh is the prototypical endothelial-dependent relaxing agent, vasomotor effects of LTD₄ were compared with those of ACh.

Materials and Methods

Adult male mongrel dogs (17–24 kg), fasted overnight but allowed free access to water, were anesthetized with sodium pentobarbital (30 mg/kg i.v.). 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. Four to six ring segments were obtained from each artery. During preparation of the ring segments, special care was taken to avoid touching the luminal surface to ensure integrity of the endothelium. The rings were mounted as previously described in 20-ml jacketed glass organ chambers containing 8 ml of the incubation medium, modified Krebs-Ringer bicarbonate solution. Isometric force was measured with Grass FT.03 force displacement transducers coupled to a Grass polygraph (model 7, Grass Instruments, Quincy, Massachusetts). All equilibrations and experimental procedures were conducted at 37°C. The modified Krebs-Ringer bicarbonate solution was continuously gassed with 95% O₂-5% CO₂ to maintain pH at 7.4 and had the following composition (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. Renal and superior mesenteric arterial rings were suspended under basal tensions of 4 g and 10–12 g, respectively. These loads were near optimum for maximal isometric contraction in response to 20 mM KCl, as assessed from prede-
saline. LTD$_4$, LTC$_4$, and LTE$_4$ (Merck Frosst Canada, Pointe-Claire-Dorval, Quebec, Canada) were obtained as stock solutions in water. The stock solutions were 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.

Meclofenamate (Parke-Davis, Morris Plains, New Jersey) was dissolved in 100 mM sodium carbonate. Nitroglycerin (Parke-Davis) and atropine (atropine sulfate; Sigma; dose in terms of base) were dissolved in saline. ETYA was prepared by dissolving the acid in absolute ethanol and then diluting with 100 mM sodium carbonate to a final concentration of 0.3 mg/ml of the acid in 10% ethanol and sodium carbonate.

NDGA was diluted in 100 mM sodium carbonate to a final concentration of 0.3 mg/ml. All solutions, with the exception of ETYA, which required addition of 240 μl, 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. Values of n indicate the number of rings studied, each obtained from an individual animal. Statistical analyses were performed with Student’s t test for paired or unpaired observations. A p value of 0.05 or less was considered statistically significant.

Results

Influence of Leukotrienes on Vasomotor Tone

Vasomotor activity of ACh and peptidoleukotrienes was observed solely after induction of active tone. Therefore, in all studies, the agonists were added to the incubation medium after the rings were precontracted to a stable plateau with NE (10$^{-7}$ to 3 × 10$^{-8}$ M) as described above.

In unrubbed ring preparations, ACh (10$^{-7}$ M) produced relaxations of 69.6 ± 4.0% (n = 32) and 67.9 ± 2.2% (n = 33) in renal and superior mesenteric arteries, respectively. Relaxation induced by ACh was immediate in onset and reached a plateau within 2–4 minutes. Relaxation of the rings in response to LTC$_4$ (10$^{-8}$ to 3 × 10$^{-7}$ M) was somewhat slower in onset (15–20 seconds) than that produced by ACh and reached a plateau approximately 3 minutes later.

Concentration-response curves relating relaxation produced by the leukotrienes in superior mesenteric and renal arterial rings as a function of NE-induced tone are shown in Figures 1 and 2, respectively. LTD$_4$, and LTC$_4$, (10$^{-8}$ to 3 × 10$^{-7}$ M) elicited concentration-dependent relaxations in both vessels. Relaxation of the rings in response to LTD$_4$, ranged from 13.2 ± 2.8% to 33.7 ± 6.2% in the renal artery and from 6.6 ± 1.1% to 34.9 ± 3.1% in the superior mesenteric artery. In comparison, LTC$_4$, produced decreases in tone ranging from 5.2 ± 1.7% to 32.9 ± 7.0% in the renal artery and 3.9 ± 0.9% to 31.8 ± 4.2% in the superior mesenteric artery. Although LTD$_4$, appeared to possess a greater capacity to produce relaxation than LTC$_4$, statistically significant differences between responses were observed only at concentrations of 3 × 10$^{-8}$ M and of both 3 × 10$^{-8}$ M and 10$^{-7}$ M in the superior mesenteric and renal arteries, respectively. In contrast to both LTD$_4$, and LTC$_4$, LTE$_4$, had very little, if any, influence on the precontracted rings. The lower concentrations of LTE$_4$ (10$^{-8}$ and 3 × 10$^{-8}$ M) had no effect on either renal or superior mesenteric arterial rings, while concentrations of 10$^{-7}$ and 3 × 10$^{-7}$ M produced minimal decreases in tone.

To determine whether the LTC$_4$, induced relaxation described above was dependent on an intact functional endothelium, vasomotor influences of LTC$_4$, ACh, and nitroglycerin were determined in a separate group of renal and superior mesenteric arterial rings both before and after disruption of the intimal surface. In this group of tissues, tone induced in response to similar concentrations of NE before and after rubbing the intimal surface was not altered (Table 1). Prior to rubbing the intimal surface, addition of ACh (10$^{-7}$ M) produced a fall in NE-induced tone of 68.9 ± 3.8% in the superior mesenteric artery and 72.8 ± 4.6% in the renal artery. LTC$_4$ (10$^{-7}$ M) decreased tone by 28.7 ± 4.9% in the superior mesenteric and 18.7 ± 2.9% in the renal artery. However, following perturbation of the intimal layer, vasomotor activity of both ACh and LTC$_4$, was completely abolished in both vessels. In contrast to these observations, vasomotor relaxation produced in response to nitroglycerin (10$^{-6}$ M) was slightly, but not significantly, enhanced following endothelial disrup-
FIGURE 2. Concentration-response curves comparing vasomotor effects of leukotrienes D₄ (LTD₄), C₄ (LTC₄), and E₄ (LTE₄) on canine renal arteries (endothelium intact) after induction of submaximal tone with norepinephrine (NE). Concentrations of NE used to induce tone were those required to obtain 40–75% of maximum contraction and ranged between 10⁻⁷ and 3×10⁻⁶ M. In five rings, relaxation was not produced in response to the lower concentrations (i.e., 10⁻⁸ and 3×10⁻⁷ M LTE₄). All values expressed as mean ± SEM. Number adjacent to each symbol indicates number of rings studied, each obtained from an individual animal. *Significantly different from LTC₄-induced relaxation (p < 0.05).

FIGURE 3. Effect of atropine on relaxation of canine renal (RA) and superior mesenteric (SMA) arteries produced by acetylcholine (ACh). Following a 30-minute incubation period with either atropine (10⁻⁷ M) or vehicle (0.9% NaCl), submaximal tone was induced with norepinephrine (NE), and responses to ACh were then obtained. Concentrations of NE ranged from 10⁻⁷ to 3×10⁻⁶ M and were those required to obtain 40–75% of maximum contraction. All values expressed as mean ± SEM (n = 7). *Significantly different from vehicle after incubation with atropine (p < 0.05).

TABLE 1. Effect of Intimal Surface Rubbing on Contraction Produced by Norepinephrine

<table>
<thead>
<tr>
<th></th>
<th>Force of norepinephrine-induced contraction (g)</th>
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<tr>
<td></td>
<td>Renal artery</td>
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<tr>
<td></td>
<td>(n = 6)</td>
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<tr>
<td>Renal artery</td>
<td></td>
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<tr>
<td>+ Endo</td>
<td>(2.0 ± 0.7)× 10⁻⁷</td>
</tr>
<tr>
<td>− Endo</td>
<td>(2.0 ± 0.4)× 10⁻⁷</td>
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<tr>
<td>Superior mesenteric artery (n = 8)</td>
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<tr>
<td>+ Endo</td>
<td>(12.6 ± 5.1)× 10⁻⁷</td>
</tr>
<tr>
<td>− Endo</td>
<td>(12.8 ± 4.3)× 10⁻⁷</td>
</tr>
</tbody>
</table>

Values represent mean ± SEM. Concentrations of norepinephrine used ranged between 10⁻⁷ and 3×10⁻⁶ M and were those required to produce 40–75% of maximal contraction. *Significantly different from vehicle (p < 0.05).
FIGURE 4. Effect of atropine on relaxation of canine renal (RA) and superior mesenteric (SMA) arteries produced by leukotriene D₄ (LTD₄) (10⁻⁷ M). Following a 30-minute incubation period with either atropine (10⁻⁷ M) or vehicle (0.9% NaCl), submaximal tone was induced with norepinephrine (NE), and responses to LTD₄ were then obtained. Concentrations of NE ranged from 10⁻⁷ to 3 x 10⁻⁵ M and were those required to obtain 40–75% of maximum contraction. Values expressed as mean ± SEM (n = 7).

Effect of Inhibitors of Cyclooxygenase Activity on LTD₄-Induced Vasomotor Relaxation

Superior mesenteric and renal arterial ring preparations with intact endothelium were incubated with meclofenamate (10⁻⁵ M) or its vehicle for 30 minutes. Submaximal contraction was then induced with NE, and effects of ACh and LTD₄ on vasomotor tone were determined. During incubation with meclofenamate, baseline tone tended to increase slightly, but the change was not significant. However, contractile responses to NE were significantly enhanced in the presence of the inhibitor of cyclooxygenase activity (Table 2). In contrast to its effect on these contractile responses, meclofenamate did not alter relaxation produced by LTD₄ in either renal or superior mesenteric arterial rings (Figure 5). In addition, relaxation induced by either ACh (10⁻⁴ to 10⁻³ M) or nitroglycerin (10⁻⁶ M) was unchanged following pretreatment with meclofenamate (Table 3).

Effect of Inhibitors of Lipoxygenase Activity on LTD₄-Induced Vasomotor Relaxation

Influence of ETYA (3 x 10⁻⁴ M) and NDGA (3 x 10⁻⁵ M), two chemically different lipoxygenase inhibitors, on LTD₄-induced relaxation was evaluated to determine whether vasomotor responses produced by LTD₄ were related to the formation and/or release of lipoxygenase metabolites of arachidonic acid. In this series of experiments, unrubbed ring preparations of superior mesenteric artery were used. After a 60-minute incubation period with ETYA, NDGA, or their respective vehicles, tone was induced with NE as previously described, and vasomotor responses to ACh, LTD₄, or nitroglycerin were obtained. During incubation of the vessel rings in the presence of either inhibitor of lipoxygenase activity, basal tone was unaltered. However, these substances differentially affected the vasomotor response of the rings to NE. In the presence of ETYA, the contractile response produced by NE was slightly enhanced (i.e., approximately 20%), while NDGA had no effect on NE-induced contraction (Table 4). In contrast to these divergent influences on contractile responses, addition of either ETYA or NDGA to the incubation medium resulted in attenuation of relaxation produced by both LTD₄ (Figures 6 and 7) and ACh (Table 5). Relaxation in response to nitroglycerin was enhanced in the presence of ETYA and, although not statistically significant, tended to be larger after addition of NDGA to the incubation fluid (Table 6).

Discussion

In the present study, it was observed that LTC₄ produced relaxation of precontracted renal and superior
TABLE 4. Effect of ETYA and NDGA on Norepinephrine-Induced Contraction in Isolated Canine Superior Mesenteric Arteries

<table>
<thead>
<tr>
<th></th>
<th>Force of contraction (g)</th>
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<tbody>
<tr>
<td>Vehicle (n=16)</td>
<td>17.3 ±1.0</td>
</tr>
<tr>
<td>ETYA (n=17)</td>
<td>20.6 ±0.8*</td>
</tr>
<tr>
<td>Vehicle (n=17)</td>
<td>17.6 ±0.8</td>
</tr>
<tr>
<td>NDGA</td>
<td>15.9±1.0</td>
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ETYA, 3x10^-4 M; NDGA, 3x10^-5 M.

ETYA, 5,8,11,14-eicosatetraynoic acid; NDGA, nordihydroguaiaretic acid. Values represent mean ± SEM. Concentrations of norepinephrine used ranged between 10^-7 and 3x10^-6 M and were those required to produce 40-75% of maximal contraction. n, number of animals.

*Significantly different from vehicle (p<0.05).

Mesenteric arterial rings in which the endothelial layer was intact, while LTE produced little, if any, reduction in vasomotor tone. In contrast, after rubbing the luminal surface of the ring, neither relaxation nor contraction was produced in response to the leukotrienes. Effectiveness of endothelial removal in each ring was assessed pharmacologically. Thus, the influence of ACh and nitroglycerin on vasomotor tone of both rubbed and unrubbed rings was determined. ACh produced relaxation of rings prior to, but not after, rubbing the intimal surface. In contrast, nitroglycerin, a substance known to produce vasomotor relaxation in an endothelial-independent manner, decreased tone in both rubbed and unrubbed ring preparations. These data strongly support the interpretation that rubbing the luminal surface with a cotton swab removed the endothelial cell layer, or at least markedly decreased its capacity to influence vasomotor tone. Results obtained in this study extend our previous observations in that these studies demonstrate LTC also possesses the capacity to produce endothelial-dependent relaxation of canine superior mesenteric and renal arteries, while vasomotor activity of LTE was only marginal.

The endothelial-dependent relaxations produced by the leukotrienes in precontracted canine superior mesenteric and renal arterial rings were dependent on concentration, which ranged from 10^-8 to 3x10^-7 M. In addition, the data obtained in the present study suggest that the vasomotor activity of these products differed. Thus, LTD, appeared to be most active, while addition of LTE to the incubation fluid induced

TABLE 5. Effect of ETYA and NDGA on Relaxation Produced by Acetylcholine in Isolated Canine Superior Mesenteric Arteries

<table>
<thead>
<tr>
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<th>Percent decrease in norepinephrine-induced tone</th>
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<tr>
<td></td>
<td>Vehicle ETYA Vehicle NDGA</td>
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<tr>
<td>Acetylcholine (M)</td>
<td>(n=10)</td>
</tr>
<tr>
<td>10^-9</td>
<td>17.1 ±4.4 8.1 ±2.5*</td>
</tr>
<tr>
<td>3x10^-9</td>
<td>33.2 ±6.2 20.8 ±2.6*</td>
</tr>
<tr>
<td>10^-8</td>
<td>42.0 ±6.5 28.8 ±2.8*</td>
</tr>
<tr>
<td>3x10^-8</td>
<td>54.1 ±6.2 37.6 ±3.4*</td>
</tr>
<tr>
<td>10^-7</td>
<td>58.1 ±6.2 40.3 ±3.7*</td>
</tr>
</tbody>
</table>

ETYA, 3x10^-4 M; NDGA, 3x10^-5 M.

ETYA, 5,8,11,14-eicosatetraynoic acid; NDGA, nordihydroguaiaretic acid. Concentrations of norepinephrine ranged from 10^-7 to 3x10^-6 M and were those required to produce 40-75% of maximal contraction. Values represent mean ± SEM. n, number of animals.

*Significantly different from vehicle (p<0.05).
minimal changes in tone that were observed only at the higher concentrations studied (10^-7 and 3 x 10^-6 M). The relaxant capacity of LTC_4 appeared to be intermediate between that of LTD_4 and LTE_4. These differences in activity were most apparent in the renal artery. Although it must be realized that the present study was not designed to reflect differences in potency between the individual leukotrienes, these observations are in agreement with the hypothesis suggested by Bernstrom and Hammarstrom14 that LTC_4 may serve as an active precursor of LTD_4, while conversion of LTD_4 to LTE_4 results in substantial loss of biological activity. Results obtained in other studies under both in vivo and in vitro conditions also are compatible with this hypothesis. For example, in the anesthetized dog and cat, LTD_4 was more active than LTC_4, in producing mesenteric vasodilatation, and both LTD_4 and LTC_4 were considerably more active than LTE_4.9,12 Additionally, both LTD_4 and LTC_4 produced equivalent contractile responses in the distal pulmonary artery of the guinea pig, while LTE_4 was much less potent.12 Furthermore, when the capacities of LTD_4 and LTC_4 to produce contraction of helical strips of rabbit aorta were compared, LTD_4 was more potent than LTC_4.6 Thus, while the present observations were not correlated with these previously reported hemodynamic or vasomotor responses, the rank order of ability to produce relaxation was consistent with that found in earlier studies.

Although most studies have shown that leukotrienes produce contraction of both vascular and nonvascular smooth muscle,15,16 recent observations suggest these substances also have the capacity to relax vascular smooth muscle. Both LTC_4 and LTD_4 have been reported to relax spirally cut segments of dog coronary artery incubated in the presence of 27 mM K.7 Additionally, in the anesthetized pig, an unidentified coronary vasodilator substance was generated during continuous intracoronary infusion of LTD_4.13 Because no attempt was made to determine the presence or functional integrity of the endothelium, it is unknown whether either of these responses was dependent on an intact intimal layer. However, the observations of Burke et al17 and Ezra et al19 are consistent with our findings.

Results of our initial study suggested that indomethacin, an inhibitor of cyclooxygenase activity, had no effect on endothelial-dependent relaxation produced by LTD_4 and ACh.3 To investigate further the possibility that a vasodilator prostaglandin, such as prostacyclin, may subserve LTD_4-induced relaxation, influence of another chemically different inhibitor of cyclooxygenase activity, meclofenamate, on LTD_4-induced decreases in vasomotor tone was determined. In agreement with the previous observation, relaxation of either renal or superior mesenteric arterial rings in response to ACh or LTD_4 was not altered in the presence of meclofenamate. Thus, although LTC_4 and LTD_4 possess the capacity to release cyclooxygenase products, of which prostacyclin is the major component, from human endothelial cells,17 it does not appear that the reduction in tone produced by LTD_4 is dependent on release of a cyclooxygenase-derived product of arachidonic acid metabolism. While relaxation of the rings in response to LTD_4 was not altered in the presence of meclofenamate, NE-induced contraction was enhanced. Similarly, indomethacin also enhanced the contractile response to NE.4 These observations are consistent with the hypothesis that released vasodilator prostaglandins serve as modulators of vasoconstrictor hormones.18

Both ACh, the prototypical endothelial-dependent relaxing agent, and the peptidoleukotrienes produced relaxation solely in vessels in which the intima was unperturbed; however, significant differences between these responses were observed. Relaxation produced by LTD_4 was not altered in the presence of atropine, a muscarinic receptor antagonist. In contrast, ACh-induced relaxation was markedly inhibited following incubation of the rings with atropine. This observation is in agreement with results of other studies in which endothelial-dependent relaxation produced by ACh was blocked in the presence of atropine. The lack of effect of atropine on LTD_4-induced relaxation suggests that LTD_4 does not interact with the muscarinic receptor to produce vasomotor relaxation. In our previous study, it was found that FPL55712, a putative leukotriene antagonist,19 attenuated LTD_4-induced relaxation but had no effect on that produced by ACh.5 When taken
together, these results suggest that the two endothelial-dependent relaxing substances act through different receptors to initiate vasomotor smooth muscle relaxation. Whether the secondary mechanism(s) and/or mediator(s) involved in the relaxation process are similar for the two agents cannot be determined from these studies.

Relaxation produced by LTD4, and ACh, but not nitroglycerin, was attenuated after incubation with either ETYA, an inhibitor of both cyclooxygenase and lipoxygenase activities, or NDGA, a lipoxygenase inhibitor. These observations are consistent with previous studies demonstrating that both ETYA and NDGA inhibit endothelial-dependent relaxation.8,10,20 When considered together, these results and the lack of effect of blockers of cyclooxygenase activity on either leukotriene- or ACh-induced changes in vasomotor tone support the hypothesis that lipoxygenase metabolites participate in the process of endothelial-dependent relaxation. While the identity of such a metabolite remains neither known nor isolated, such a substance should possess the capacity to produce vasomotor relaxation after removal of the endothelial layer. Although the present observations indicate that endothelial-dependent relaxation is not mediated by these peptidoleukotrienes, they do not preclude a role for some other lipoxygenase-derived product(s) in mediation of endothelial-dependent relaxation. Indeed, Forstermann and Neufang11 and Berkowitz et al12 arrived at the same conclusion when they found that peptidoleukotrienes failed to relax precontracted rabbit aorta.

In contrast to the effects of ETYA and NDGA on decreases in vasomotor tone elicited by LTD4 and ACh, relaxation produced in response to nitroglycerin, an endothelial-independent relaxing agent, was enhanced slightly in the presence of ETYA and, although not statistically significant, tended to be larger during incubation of the arterial rings with NDGA. Consistent with these observations, it has recently been found that either removal of the endothelium21 or inhibition of lipoxygenase activity22 augments vasomotor relaxation produced by nitrovasodilators. Although the effect of NDGA on nitroglycerin-induced responses was not significant, these data appear to be compatible with the hypothesis proposed by Pohl and Busse22 that EDRF possessing the capacity to inhibit cyclooxygenase activity, acts as an inhibitor of relaxation produced in response to nitrovasodilators. Alternatively, because ETYA also enhances the capacity to inhibit cyclooxygenase activity, these data may be interpreted to suggest that a cyclooxygenase product(s) and not EDRF may be responsible for inhibition or attenuation of nitroglycerin-induced relaxation. If this is the case, inhibitors of cyclooxygenase activity, such as indomethacin or meclofenamate, should possess the capacity to enhance relaxation produced in response to nitroglycerin. Results obtained in both this and our previous8 investigations suggested that neither indomethacin nor meclofenamate significantly enhanced relaxation of superior mesenteric or renal arterial rings produced in response to a single concentration (10−6 M) of nitroglycerin. It, therefore, did not appear that a cyclooxygenase product of arachidonic acid was a participant in the relaxation. However, these studies were not specifically designed to answer this question, and, clearly, further studies to evaluate more completely the hypothesis of Pohl and Busse22 are required.

While ETYA and NDGA had similar inhibitory effects on relaxation produced by LTD4 and ACh, their influence on NE-induced contraction differed. ETYA enhanced NE-induced contraction, while NDGA had no effect on the contractile response. These findings were most likely due to a reduction in cyclooxygenase activity produced by ETYA and were consistent with results obtained in the presence of the cyclooxygenase inhibitors, meclofenamate (described above) and indomethacin.8 Indeed, it may be possible that the slightly enhanced superior mesenteric and renal arterial relaxation produced by nitroglycerin in the presence of ETYA was a consequence of enhanced NE-induced tone.

In addition to serving as a substrate for cyclooxygenase and lipoxygenase enzymatic systems, arachidonic acid may be oxidized via cytochrome P-450 monooxygenases.22–25 Indeed, the presence of a cytochrome P-450–dependent mixed-function oxidase has been described in vascular tissue, including the endothelium.26,27 Although the identities and biological activities or roles of arachidonic acid metabolites arising from cytochrome P-450–dependent monooxygenases are virtually unknown, initial studies suggest that these products may have the ability to alter vasomotor tone.28 Evidence supporting the hypothesis that products of the cytochrome P-450 system may participate in endothelial-dependent relaxation has been obtained in rabbit aorta29 and pulmonary artery.30 In these studies, endothelial-dependent relaxation induced by methacholine, A23187, or arachidonic acid was attenuated in the presence of either metyrapone or SKF 525-A, two inhibitors of cytochrome P-450. In addition, in vivo induction or deletion of vascular cytochrome P-450–dependent enzymatic activity enhanced or attenuated, respectively, endothelial-dependent vasomotor relaxation in response to arachidonic acid.31 At high concentrations, ETYA has been shown to inhibit cytochrome P-450–mediated arachidonic acid metabolism in the renal cortex.24 It has also been suggested that high concentrations of both ETYA and NDGA may inhibit arachidonic acid metabolism by cytochrome P-450 in anterior pituitary cells.31 Thus, the inhibitory effects of ETYA and NDGA could conceivably be mediated, at least in part, by actions on cytochrome P-450.

In summary, our observations indicate that relaxation produced by the leukotrienes in isolated canine superior mesenteric and renal arteries is dependent on an intact endothelium. However, it must be realized that actual release of an endogenous relaxant factor from endothelial cells in response to the leukotrienes has not been demonstrated in these studies. Indeed, if the leukotrienes do release a vasodilator from endothelial cells, it is unknown whether this substance and the
mechanism of relaxation are the same as that described for acetylcholine. Although the vasomotor effects of leukotrienes described in the present study do not appear to be consistent with results obtained in intact anesthetized animals, it must be emphasized that the present study was performed on relatively large arteries and may not reflect influences of these substances on small arteries and veins as well as on resistance vessels.

Acknowledgments

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References

11. Forstermann U, Neufang B: C-6-sulfidopeptide leukotrienes are unlikely to be involved in the endotheliun dependent relaxation of rabbit aorta by acetylcholine. Prostaglandins 1984; 27:181–193
26. Juchau MR, Bond JA, Benditt EP: Aryl 4-monooxygenase and endothelial-dependent relaxation of canine mesenteric artery • endothelial-dependent relaxation • canine mesenteric artery • leukotrienes • canine renal artery • endothelial-dependent relaxation
Endothelial-dependent relaxation induced by leukotrienes C4, D4, and E4 in isolated canine arteries.

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