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

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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 atropine 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)

Although most studies have shown that leukotrienes contract vascular smooth muscle, divergent effects have been observed. Leukotrienes C₄ (LTC₄), D₄ (LTD₄), and E₄ (LTE₄) have been shown to produce marked vasoconstriction in the mesenteric vascular bed of both the anesthetized dog and cat but to have little vasoactivity in the canine kidney. In addition, LTD₄ decreased coronary blood flow in anesthetized dogs, while in anesthetized rabbits were either unresponsive (renal artery, mesenteric artery, thoracic aorta) or only weakly responsive (pulmonary artery). In contrast to the observations of Kito et al., LTD₄ relaxed spiral segments of dog coronary artery precontracted with 27 mM K⁺. Thus, while leukotrienes are generally considered to be vasoconstrictor substances, it appears that these compounds also possess the capacity to produce vasodilation or vasomotor relaxation. Furthermore, LTD₄ has been shown to relax precontracted canine renal and superior mesenteric arterial rings in an endothelial-dependent manner. Arterial relaxation induced by a variety of vasoactive substances has been shown to depend, at least partially, on the presence of a functionally intact endothelium. Although the precise nature of this endothelium-derived relaxing factor (EDRF) remains undefined, cyclooxygenase products of arachidonic acid metabolism do not appear to be responsible for this activity. However, two chemically different lipoxygenase inhibitors, 5,8,11,14-eicosatetraynoic acid (ETYA) and nordihydroguaiaretic acid (NDGA), attenuated or reversed endothelial-dependent relaxation produced by acetylcholine (ACh), arachidonic acid, or bradykinin. Therefore, it was postulated that a product of arachidonic acid metabolism formed via the lipoxygenase pathway subserved endothelial-dependent relaxation. Because peptidoleukotrienes failed to relax strips of rabbit aorta, it was concluded that endothelial-dependent relaxation was not mediated by these lipoxygenase metabolites of arachidonic acid. In addition, we have demonstrated that LTD₄ produced relaxation of...
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. When the NE-induced contraction reached a steady state, ACh, an individual leukotriene, and nitroglycerin were each added to the incubation medium over a wide range of concentrations in a cumulative manner. The tissues were washed with 60 ml buffer and allowed to reequilibrate for 30 minutes after addition of each individual agonist. Effects of only one particular leukotriene were determined in each ring preparation.

Interrelations between endothelial function and vasomotor activity of both LTC₄ and LTE₄ were evaluated in the following manner. In the presence of induced tone, control responses to ACh (10⁻⁷ M), nitroglycerin (10⁻⁴ M), and the particular leukotriene were obtained before disrupting the endothelium. The tissues were then washed with 60 ml buffer and allowed to reequilibrate for 30 minutes. Following equilibration, the luminal surface of the ring was rubbed gently with a cotton-tipped applicator for 45–60 seconds to intentionally remove or disrupt the endothelial cell layer. Thirty minutes later, tone was induced with NE, and influences of ACh, nitroglycerin, and the leukotriene 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, 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 then reetermined in the presence of either the antagonist or its vehicle. Influence of only one blocking agent on vasomotor activity was determined in an individual ring preparation.

NE (L-norepinephrine hydrochloride; Sigma Chemical, St. Louis, Missouri; dose in terms of base) was dissolved and diluted in saline containing ascorbic acid (1 mg/ml). ACh (acetylcholine hydrochloride; Sigma; dose in terms of base) was dissolved and diluted in...
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 $-80\, \text{C}$. 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 dissolving 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 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 $3 \times 10^{-6}$ 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 $3 \times 10^{-7}$ 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 $3 \times 10^{-7}$ 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 $3 \times 10^{-8}$ M and of both $3 \times 10^{-8}$ 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 $3 \times 10^{-8}$ M) had no effect on either renal or superior mesenteric arterial rings, while concentrations of $10^{-7}$ and $3 \times 10^{-7}$ 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 ($10^{-8}$ M) was slightly, but not significantly, enhanced following endothelial disrup-

![Figure 1. Concentration-response curves comparing vasomotor effects of leukotrienes D$_4$ (LTD$_4$), C$_4$ (LTC$_4$), and E$_4$ (LTE$_4$) on canine superior mesenteric 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 $3 \times 10^{-8}$ M. In five rings, relaxation was not produced in response to the lower concentrations (i.e., $10^{-8}$ and $3 \times 10^{-8}$ M LTE$_4$). 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 LTC$_4$-induced relaxation ($p<0.05$).](https://example.com/figure1.png)
Figure 2. Concentration-response curves comparing vaso-motor effects of leukotrienes D4 (LTD4), C4 (LTC4), and E4 (LTE4) on canine renal arteries (endothelium intact) after induction of submaximal tone with noradrenaline (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 \times 10^{-6} M. In five rings, relaxation was not produced in response to the lower concentrations (i.e., 10^{-8} and 3 \times 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).

Thus, in the superior mesenteric artery, nitroglycerin produced a fall in tone of 86.8 ± 4.1% and 90.6 ± 3.6% before and after rubbing, respectively. In the renal artery, nitroglycerin decreased tone 74.3 ± 10.2% before and 80.6 ± 6.0% after endothelial disruption.

Effect of Atropine on LTD4-Induced Vasomotor Relaxation

To determine whether the LTD4-induced relaxation was mediated via interaction with the muscarinic receptor, the influence of atropine, a muscarinic receptor blocking agent, on responses produced by ACh and LTD4 was determined. Prior to incubation of the rings with atropine, ACh (10^{-8} to 10^{-7} M) produced dose-dependent decreases in NE-induced tone (Figure 3). Atropine (10^{-7} M) was added to the bath 30 minutes prior to reinduction of tone with NE. During incubation of the rings solely with atropine, no effect on baseline tone was observed. In addition, the antimuscarinic agent did not alter contractile responses to NE in either the superior mesenteric or renal arterial rings (Table 2). However, in the presence of atropine, ACh-induced relaxation was virtually abolished in both vessels (Figure 3). In contrast, relaxation induced by LTD4 (10^{-7} M) (Figure 4) was unchanged following pretreatment with the muscarinic antagonist.

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

<table>
<thead>
<tr>
<th>Condition</th>
<th>Concentration of norepinephrine (M)</th>
<th>Contraction (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renal artery (n = 6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ Endo</td>
<td>2.0 ± 0.7 × 10^{-7}</td>
<td>12.3 ± 1.0</td>
</tr>
<tr>
<td>- Endo</td>
<td>2.0 ± 0.4 × 10^{-7}</td>
<td>11.5 ± 1.0</td>
</tr>
<tr>
<td>Superior mesenteric artery (n = 8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ Endo</td>
<td>12.6 ± 5.1 × 10^{-7}</td>
<td>13.9 ± 0.6</td>
</tr>
<tr>
<td>- Endo</td>
<td>12.8 ± 4.3 × 10^{-7}</td>
<td>13.8 ± 0.6</td>
</tr>
</tbody>
</table>

+ Endo, endothelium not rubbed; - Endo, endothelium rubbed. Values represent mean ± SEM. n, number of animals.

Table 2. Effect of Atropine and Meclofenamate on Contraction Produced by Norepinephrine

<table>
<thead>
<tr>
<th>Condition</th>
<th>Force of norepinephrine-induced contraction (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Renal artery (n = 7)</td>
</tr>
<tr>
<td>Vehicle</td>
<td>15.8 ± 0.7</td>
</tr>
<tr>
<td>Atropine</td>
<td>16.3 ± 1.5</td>
</tr>
<tr>
<td>Vehicle + Atropine</td>
<td>14.3 ± 1.1</td>
</tr>
<tr>
<td>Meclofenamate</td>
<td>16.9 ± 1.0 *</td>
</tr>
</tbody>
</table>

Atropine, 10^{-7} M; meclofenamate, 10^{-5} M. Values represent mean ± SEM. Concentrations of norepinephrine used ranged between 10^{-7} and 3 \times 10^{-6} M and were those required to produce 40–75% of maximal contraction. n, number of animals. *Significantly different from vehicle (p < 0.05).
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 (10⁻⁸ to 10⁻⁷ M) or nitroglycerin (10⁻⁶ M) was unchanged following pretreatment with meclofenamate (Table 3).

Effect of Inhibitors of Lipooxygenase 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 arteries. This effect was independent of the action of prostaglandins but was sensitive to inhibitors of cyclooxygenase activity. The vasodilator response produced by LTD₄ was also sensitive to inhibitors of lipoxygenase activity, suggesting that both cyclooxygenase and lipoxygenase pathways are involved in the generation of vasomotor responses produced by LTD₄.

Table 3. Effect of Meclofenamate on Relaxation Produced by Acetylcholine and Nitroglycerin

<table>
<thead>
<tr>
<th>Substance</th>
<th>Renal artery (n = 8)</th>
<th>Superior mesenteric artery (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vehicle</td>
<td>Meclofenamate</td>
</tr>
<tr>
<td>Acetylcholine</td>
<td>10⁻⁸</td>
<td>40.2 ± 7.1</td>
</tr>
<tr>
<td></td>
<td>3 x 10⁻⁸</td>
<td>56.1 ± 7.8</td>
</tr>
<tr>
<td></td>
<td>10⁻⁷</td>
<td>62.7 ± 7.7</td>
</tr>
<tr>
<td>Nitroglycerin</td>
<td>10⁻⁶</td>
<td>69.4 ± 5.4</td>
</tr>
<tr>
<td></td>
<td>Meclofenamate, 10⁻³ M</td>
<td></td>
</tr>
</tbody>
</table>

Values represent mean ± SEM. n, number of animals.
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>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle (n=16)</td>
<td>17.3 ± 1.0</td>
</tr>
<tr>
<td>ETYA 3 × 10^-4 M</td>
<td>20.6 ± 0.8*</td>
</tr>
<tr>
<td>Vehicle (n=17)</td>
<td>17.6 ± 0.8</td>
</tr>
<tr>
<td>NDGA 3 × 10^-5 M</td>
<td>15.9 ± 1.0</td>
</tr>
</tbody>
</table>

ETYA, 3 × 10^-4 M; NDGA, 3 × 10^-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 3 × 10^-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 LTC4 also possesses the capacity to produce endothelial-dependent relaxation of canine superior mesenteric and renal arteries, while vasomotor activity of LTE4 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 3 × 10^-7 M. In addition, the data obtained in the present study suggest that the vasomotor activity of these products differed. Thus, LTD4 appeared to be most active, while addition of LTE4 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>
<th></th>
<th>Percent decrease in norepinephrine-induced tone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=10)</td>
</tr>
<tr>
<td>Vehicle</td>
<td>17.1 ± 4.4</td>
</tr>
<tr>
<td>ETYA 3 × 10^-4 M</td>
<td>8.1 ± 2.5*</td>
</tr>
<tr>
<td>Vehicle</td>
<td>33.2 ± 6.2</td>
</tr>
<tr>
<td>NDGA 3 × 10^-4 M</td>
<td>20.8 ± 2.6*</td>
</tr>
<tr>
<td>10^-8</td>
<td>42.0 ± 6.5</td>
</tr>
<tr>
<td>3 × 10^-8</td>
<td>28.8 ± 2.8*</td>
</tr>
<tr>
<td>10^-7</td>
<td>54.1 ± 6.2</td>
</tr>
<tr>
<td>3 × 10^-7</td>
<td>37.6 ± 3.4*</td>
</tr>
<tr>
<td>10^-9</td>
<td>58.1 ± 6.2</td>
</tr>
<tr>
<td>3 × 10^-6 M</td>
<td>40.3 ± 3.4*</td>
</tr>
</tbody>
</table>

ETYA, 3 × 10^-4 M; NDGA, 3 × 10^-5 M.
ETYA, 5,8,11,14-eicosatetraynoic acid; NDGA, nordihydroguaiaretic acid. Concentrations of norepinephrine ranged from 10^-9 to 3 × 10^-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).
either of these responses was dependent on an intact integrity of the endothelium, it is unknown whether was made to determine the presence or functional of which prostacyclin is the major component, from human endothelial cells," it does not appear that the reduction in tone produced by LTD₄ is dependent on release of a cyclooxygenase-derived product of arachidonic acid metabolism. While relaxation of the rings in response to LTD₄ was not altered in the presence of meclofenamate, NE-induced contraction was enhanced. Similarly, indomethacin also enhanced the contractile response to NE. These observations are consistent with the hypothesis that released vasodilator prostaglandins serve as modulators of vasoconstrictor hormones. 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 LTD₄ 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 LTD₄-induced relaxation suggests that LTD₄ does not interact with the muscarinic receptor to produce vasomotor relaxation. In our previous study, it was found that FPL 55712, a putative leukotriene antagonist, attenuated LTD₄-induced relaxation but had no effect on that produced by ACh. When taken
together, these results suggest that the two endothelial-dependent relaxing substances act through different receptors to initiate vascular 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. 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 acts as an inhibitor of relaxation produced in response to nitrovasodilators. Alternatively, because ETYA also possesses 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 previous⁵ 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 monoxygenases.²⁵-²⁷ Indeed, the presence of a cytochrome P-450-dependent mixed-function oxidase has been described in vascular tissue, including the endothelium.²⁸-³⁰ Although the identities and biological activities or roles of arachidonic acid metabolites arising from cytochrome P-450–dependent monoxygenases are virtually unknown, initial studies suggest that these products may have the ability to alter vasomotor tone.²⁹ Evidence supporting the hypothesis that products of the cytochrome P-450 system may participate in endothelial-dependent relaxation has been obtained in rabbit aorta²² 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 depletion of vascular cytochrome P-450–dependent enzymatic activity enhanced or attenuated, respectively, endothelial-dependent vasomotor relaxation in response to arachidonic acid.³⁰ At high concentrations, ETYA has been shown to inhibit cytochrome P-450–mediated arachidonic acid metabolism in the renal cortex.³¹ It has also been suggested that high concentrations of both ETYA and NDGA may inhibit arachidonate metabolism by cytochrome P-450 in anterior pituitary cells.³² 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.

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