Leukotrienes C₄ and D₄ Are Potent Endothelium-Dependent Relaxing Agents in Canine Splanchnic Venous Capacitance Vessels

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In the present study, the vasomotor effects of the peptide leukotrienes (LTs) LTC₄ and LTD₄ on isolated canine venous capacitance vessels were evaluated. Both LTs evoked marked concentration-dependent relaxation of norepinephrine-contracted rings of mesenteric and splenic veins and inferior vena cava but had minimal activity in the femoral vein. Relaxation induced by either LT was abolished after physical removal of the vascular endothelium, whereas marked relaxation responses were evoked by glyceryl trinitrate in the same endothelium-denuded rings. The nitric oxide synthase antagonist N⁶-nitro-l-arginine methyl ester (L-NAME) completely abolished LT-induced mesenteric vein relaxation and unmasked a contractile effect of LTC₄. Only partial attenuation of LT-induced relaxation of the inferior vena cava in the presence of L-NAME was observed. In the splenic vein, responses solely to LTC₄ were very slightly reduced in the presence of L-NAME. Reduced hemoglobin (10⁻⁶ and 10⁻⁵ M) inhibited LTC₄-evoked splenic vein relaxation and, in a concentration of 10⁻⁷ M, inhibited LTD₄-evoked relaxation of the splenic vein. On the other hand, methylene blue (10⁻⁶ and 10⁻⁷ M) attenuated splenic vein relaxation produced by both LTs but solely reduced LTD₄-evoked inferior vena cava relaxation. Thus, the peptide LTs, the major components of the slow-reacting substance of anaphylaxis, exert a profound endothelium-dependent relaxant effect on venous capacitance vessels, which is only partially dependent on L-arginine and nitric oxide. A role for LT-evoked capacitance venodilation as a mechanism contributing to the reduced venous return and cardiac output associated with systemic anaphylaxis is postulated. (Circulation Research 1993;73:395-404)

KEY WORDS • anaphylaxis • vasodilation • hypotension • hypersensitivity • veins

It has been recognized for many years that the sulfidopeptide leukotrienes (LTs) are the principal components of what is classically referred to as the slow-reacting substance of anaphylaxis.¹² Systemic anaphylaxis (immediate hypersensitivity) is a pathophysiological response occurring subsequent to exposure to a variety of antigenic stimuli and is characterized by bronchoconstriction and a number of profound cardiovascular changes, the most serious of which is severe hypotension. Studies evaluating human clinical cases and animal models of anaphylaxis indicate that the dramatic fall in cardiac output associated with anaphylactic shock involves a reduction in venous return to the heart secondary to pronounced vasodilation of the splanchic venous capacitance vasculature.³⁴

The peptide LTs are potent bronchial spasmogens³ as well as vigorous vasoconstrictor agents in certain peripheral regional vascular beds.⁶⁷ Paradoxically, the peptide LTs have the capacity to evoke in vitro endothelium-dependent relaxation of precontracted rings of guinea pig pulmonary artery and thoracic aorta³⁸ and canine superior mesenteric and renal arteries.⁹¹⁰ In addition, we have found that the peptide leukotriene LTD₄ can elicit the release of an endothelium-derived relaxing factor (EDRF) from the canine renal artery that is pharmacologically indistinguishable from that released by acetylcholine, ie, endothelium-derived nitric oxide (EDNO).¹¹ However, characterization of the splanchic venomotor effects of the peptide LTs is curiously lacking, especially in view of the potential for their participation in venous vascular dysfunction associated with anaphylaxis.

Recently, we¹² demonstrated that LTD₄ can elicit potent endothelium-dependent vasomotor relaxation of the canine renal vein that appears to be due to a mechanism or mediator dissimilar from the classic EDRF (ie, EDNO). Although the renal vein is not considered a venous capacitance vessel, these results suggest that the LTs can strongly influence venomotor tone. Therefore, the overall goal of the present investigation was to determine whether the peptide LTs, LTC₄ and LTD₄, have the capacity to modulate venomotor tone in three splanchic venous capacitance vessels: the mesenteric vein (MV), splenic vein (SV), and the inferior vena cava (IVC). Because EDNO has been demonstrated to serve as a homeostatic regulator of arterial blood pressure¹³-¹⁵ and is believed to be the terminal mediator of the vascular response to endotoxin- and cytokine-induced hypotension,¹⁶-¹⁸ specific objectives of these studies were to assess whether venomotor responses to these eicosanoids are dependent on a functionally intact vascular endothelium and to begin to characterize this relation. The venomotor...

Received November 9, 1992; accepted April 22, 1993.

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responses elicited by LTC₄ and LTD₄ in these splanchnic vessels were compared with those produced in the femoral vein (FV).

**Materials and Methods**

**Tissue Preparation**

Adult male mongrel dogs (20 to 30 kg) that had been fasted overnight but allowed free access to water were anesthetized with intravenous sodium pentobarbital (30 mg/kg). The left femoral artery was isolated and cannulated with polyethylene tubing. Heparin sodium (1000 U/kg) was administered intravenously before excision of the animal via the femoral artery. The abdomen was opened by a midline incision, and the MV, SV, and IVC were carefully dissected free of surrounding tissue, excised, and immediately placed in ice-cold modified Krebs-Ringer-bicarbonate buffer (KRB) of the following composition (mM): NaCl, 118.3; KCl, 4.7; CaCl₂, 2.5; MgSO₄, 1.2; KH₂PO₄, 1.2; NaHCO₃, 25.0; Na⁺-EDTA, 0.026; and glucose, 11.1. The same isolation procedure was observed for excision of the FV, which was obtained from the hind limb contralateral to that in which the femoral artery had been cannulated. The MV, SV, FV, and IVC were cleaned of perivascular adipose and connective tissue, exercising care to maintain the integrity of the endothelial cell lining. All blood vessels were used the same way on which they were obtained from the animal.

According to methods previously described in this laboratory,⁹ ¹² ⁵ to 7-mm rings of MV, SV, FV, and IVC were prepared and immediately connected to an isometric force transducer (model FT.03, Grass Instrument Co, Quincy, Mass) coupled to a polygraph (model 7E, Grass) and anchored onto a fixed stainless-steel support. Special care was observed to avoid disruption of the endothelium. The rings were suspended in 25-mL jacketed organ chambers containing 10 mL KRB to which indomethacin (10⁻⁵ M) had been added to inhibit cyclooxygenase activity. The KRB was maintained at 37°C and aerated with 95% O₂-5% CO₂ gas to maintain a pH of 7.4. Tissues were washed every 15 to 30 minutes with 25 mL fresh KRB. Rings were gradually (60 to 90 minutes) stretched to their optimal basal tension (6, 6, 1, and 5 g for the MV, SV, FV, and IVC, respectively) as predetermined by evaluation of tension-force relations. After attaining optimal basal tension, rings were allowed to equilibrate for approximately 15 minutes before proceeding. All rings were initially contracted with 60 mM KCl, which stabilizes subsequent submaximal contractions.¹¹ ¹² Thereafter, submaximal tone (40% to 75%) was induced in the rings with norepinephrine (NE, 10⁻⁴ to 10⁻⁶ M). Submaximal tone in untreated endothelium-intact MV, SV, FV, and IVC rings averaged 5.3±0.3, 4.6±0.4, 3.2±0.2, and 3.1±0.2 g, respectively.

**Effect of Endothelium Removal on LTC₄ and LTD₄-Induced Venomotor Relaxation**

Submaximal (40% to 75%) tone was induced in the MV, SV, FV, and IVC rings with NE (10⁻⁴ to 10⁻⁶ M). When active tension had stabilized, LTC₄ or LTD₄ (10⁻¹₀ to 10⁻⁷ M) was added to the incubation medium in a cumulative manner to determine its effect on venomotor tone. In addition, effects of A23187 (10⁻⁷ M) on venomotor tone in the FV were determined. After each series of agonist additions, the rings were washed with fresh KRB and allowed to reequilibrate. The vascular endothelium was then removed by rubbing the intimal surface of the rings with the roughened shaft of a 23-gauge needle. After rubbing, the rings were allowed to reequilibrate to basal tension for 30 to 60 minutes in KRB, and then venomotor responses to LTC₄, LTD₄, and A23187 were determined. Additionally, these same rings were exposed to the endothelium-independent nitrovasodilator glyceryl trinitrate (GTN, 10⁻⁷ M) after intimal scraping to ensure that any loss of the vasorelaxant response to LTC₄, LTD₄, or A23187 was due to endothelium removal and not to damage of the underlying vascular smooth muscle.

**Effect of N⁵-Nitro-L-arginine Methyl Ester on LTC₄- and LTD₄-Induced Venomotor Relaxation**

A separate series of experiments was conducted to determine the effects of a potent inhibitor of the constitutive form of NO synthase, N⁵-nitro-L-arginine methyl ester (L-NAME), on LTC₄- and LTD₄-induced venomotor relaxation in the MV, SV, and IVC. Each agonist was added to the incubation medium in a cumulative fashion after the NE-induced contraction had reached a steady state. After obtaining control responses to the LTs, venous rings were treated with L-NAME (100 µM) for a period of 60 minutes, at which time venomotor responses to the LTs were redetermined. L-NAME was removed by washing with fresh KRB, and the venous tissues were incubated with L-arginine (1 mM) for a period of 60 minutes. Venomotor responses to the LTs were once again determined after induction of submaximal tone with NE.

The influence of only one agonist and antagonist on venomotor responses was evaluated in each ring. After exposure of each ring to an inhibitory concentration of L-NAME, submaximal tone was induced with NE in a concentration that produced contraction of a magnitude equivalent to that obtained during the control period. This was done to ensure that any effects of the inhibitor were not due to different levels of NE-induced tone.

**Effect of Reduced Hemoglobin and Methyline Blue on LTC₄- and LTD₄-Induced Venomotor Relaxation**

Reduced human hemoglobin (Hb) is known to inhibit the endothelium-dependent relaxation and the associated accumulation of cGMP produced in the bovine intrapulmonary vein and artery in response to bradykinin and acetylcholine, respectively.²⁰ In addition, methylene blue (MB), an inhibitor of soluble guanylate cyclase, is well known to attenuate vasomotor relaxation produced by bradykinin and acetylcholine as well as the nitrovasodilators.²¹ ²² Further, endothelium-dependent relaxation of arterial ring preparations produced by LTD₄ has been shown to be inhibited in the presence of Hb and MB.⁸ ¹² ²³ Therefore, to characterize further LTC₄- and LTD₄-evoked endothelium-dependent venomotor relaxation of the canine MV, SV, and IVC, the influences of Hb (10⁻⁶ and 10⁻⁵ M) and MB (10⁻⁶ and 10⁻⁵ M) on this vasomotor activity were evaluated.

Responses to a single concentration (5×10⁻⁹ M) of LTC₄ and LTD₄, known to produce a decrease in tone in the MV, SV, and IVC of 40% to 60% of the maximum relaxation response, were determined in this series of experiments. The LT was added to the incubation medium
after NE-induced contraction had reached a steady state. In each individual experiment, changes in vasomotor tone evoked by the agonists were determined both before and after treatment of the venous tissue with an individual inhibitor. The influence of only one agonist and inhibitor on venomotor responses was evaluated in each ring. After exposure of each venous ring to an inhibitory agent, submaximal tone was induced with NE in a concentration that produced contraction of a magnitude equivalent to that obtained during the control period. This was done to ensure that any effects of the inhibitor were not due to different levels of induced tone.

Analysis of Data

All values are expressed as mean ± SEM unless otherwise indicated, and n indicates the number of dogs from which venous vascular samples were obtained. Statistical analysis of differences between means of control and treated groups was performed using a two-tailed Student’s t test for paired samples. The study of the inhibitory effects of L-NAME and its reversal by L-arginine on LTC₄- and LTD₄-induced venomotor relaxation was evaluated by means of a one-way analysis of variance and a post hoc test using Bonferroni adjustments for multiple comparisons. The null hypothesis was rejected in all cases where P ≤ 0.05.

Drugs and Chemicals

L-Arterenol (l-NE) hydrochloride, A23187, acetylcholine hydrochloride, L-NAME, MB, human Hb, and indomethacin were purchased from Sigma Chemical Co, St Louis, Mo. GTN was obtained from Parke-Davis Division, Warner-Lambert Co, Morris Plains, NJ. LTC₄ and LTD₄ were generous gifts of the Merck-Frosst Co, Pointe-Claire, Dorval, Quebec.

Reduced human Hb was prepared as previously described. Unless otherwise indicated, all other chemicals were dissolved in normal saline.

Results

Effect of Endothelium Removal on LTC₄- and LTD₄-Induced Venomotor Relaxation

When LTC₄ or LTD₄ (10⁻¹⁰ to 10⁻⁷ M) was added to endothelium-intact rings of MV, IVC, or SV submaximally contracted with NE, a concentration-dependent decrease in tone was observed in each tissue. Maximum relaxation responses evoked by LTC₄ and LTD₄ (10⁻⁷ M) were, respectively, 87 ± 3% and 84 ± 2% for the MV, 93 ± 3% and 87 ± 4% for the IVC, and 83 ± 4% and 75 ± 4% for the SV (Figs 1 through 3, E +). Subsequent to physical disruption of the endothelial cell layer and a reequilibration period, venomotor responses to both eicosanoids were virtually abolished in the MV, IVC, and SV (Figs 1 through 3, E −).

To ensure that removal of the vascular endothelium did not impair the ability of the vascular smooth muscle to undergo vasomotor relaxation, a single concentration (10⁻⁷ M) of the endothelium-independent nitrovasodilator GTN was administered to the NE-contracted endothelium-denuded MV, IVC, and SV rings. As illustrated by the bars to the right in Figs 1 through 3, GTN produced venomotor relaxation of 87% or greater in each endothelium-denuded venous tissue.

In contrast to these observations, addition of LTC₄ and LTD₄, in the same concentration range, to endothelium-intact rings of FV submaximally contracted

![Graph showing the effect of endothelium removal on leukotriene C₄- and leukotriene D₄-induced relaxation in rings of norepinephrine-contrasted canine mesenteric vein. GTN, glyceryl trinitrate; E+, endothelium intact; E−, endothelium denuded; p[Agonist], negative log molar concentration of leukotrienes C₄ and D₄ and GTN. Removal of endothelium virtually abolished venomotor relaxation evoked by leukotrienes C₄ and D₄. Negative changes in isometric tension reflect relaxation; positive changes in isometric tension indicate contraction. Responses to GTN were obtained after exposure of endothelium-denuded rings to leukotriene C₄ (GTN-C₄) or leukotriene D₄ (GTN-D₄). *Significantly different from corresponding control value (P ≤ 0.05); n = 7 rings, each obtained from an individual dog.](image-url)
with NE was only marginally effective and, at the highest concentration, elicited only slight decreases in tone of 18±2% and 16±5%, respectively (Fig 4). However, these modest relaxation responses were also abolished on physical removal of the endothelium, whereas GTN (10⁻⁷ M) could still evoke marked venomotor relaxation (bars at right, Fig 4). Although not illustrated, the functional integrity of the FV endothelium was confirmed in control studies by exposing the FV rings to the calcium ionophore A23187 (10⁻⁷ M), which elicited a decrease in NE-induced tone of 76±5%. In comparison with these observations, after endothelial disruption venomotor relaxation evoked by A23187 was virtually abolished (7±3%).

Whereas removal of the vascular endothelium did not alter basal tone, it was necessary to slightly (≈10%) reduce the concentration of NE in order to contract the endothelium-denuded venous rings to a level of tone equivalent to that induced during the control period (data not shown).

Effect of L-NAME on LTC₄- and LTD₄-Induced Venomotor Relaxation

To determine the role of EDNO· in LT-induced venous relaxation, the effect of the inhibitor of constitutive NO· synthase, L-NAME, on these responses was determined. After incubation with L-NAME (100 μM) for 60 minutes, LTD₄-induced MV relaxation was markedly attenuated (Fig 5, B), whereas mesenteric venomotor responses to LTC₄ were not only abolished but a contractile effect of the eicosanoid was unmasked (Fig 5, A). After washout of L-NAME and incubation of the tissues with KRB containing l-arginine (the natural substrate for NO· synthase) for 60 minutes, relaxation evoked by the LTs returned to control values (Fig 5, dashed lines). Although not illustrated, in control experiments in which L-NAME was removed from the tissue chamber but no l-arginine was added to the KRB medium (ie, only flushing the L-NAME from the tissue chamber followed by equilibration for 1 hour in l-arginine-free KRB), no reversal of L-NAME-induced inhibition of either LTC₄- or LTD₄-induced MV relaxation was observed. Thus, reversal of L-NAME-induced inhibition of LT-evoked MV relaxation was dependent on provision of excess natural substrate for the NO· synthase enzyme.

Treatment of endothelium-intact IVC rings with the NO· synthase inhibitor also attenuated LTC₄- and LTD₄-induced venomotor relaxation (Fig 6) although to a much lesser extent than in the MV (Fig 5). The inhibitory effects of L-NAME were reversed 60 minutes after addition of excess l-arginine (1 mM) to the incubation medium (Fig 6, dashed lines), and LT-evoked responses were not significantly different from those obtained during the initial control period.

In contrast to the aforementioned studies, L-NAME (100 μM) only slightly attenuated LTC₄-induced venomotor relaxation in endothelium-intact SV rings but did not affect LTD₄-evoked responses (Fig 7). Similar to the MV and IVC, l-arginine (1 mM) completely reversed the L-NAME–induced inhibition of LTC₄-evoked relaxation (Fig 7A, dashed lines). Relaxation of the SV produced by LTD₄ in the presence of l-arginine was not different from the corresponding control value (Fig 7B).

In all rings pretreated with L-NAME (100 μM), a 10-fold lower dose of NE was required to induce a level
Splenic Vein

![Graph showing the effect of endothelium removal on leukotriene C4- and leukotriene D4-induced relaxation in norepinephrine- contracted rings of canine splenic vein. GTN, glyceryl trinitrate; E+, endothelium intact; E-, endothelium denuded; [Agonist], negative log molar concentration of leukotrienes C4 and D4 and GTN. Removal of endothelium virtually abolished venomotor relaxation evoked by leukotrienes C4 and D4. Negative changes in isometric tension indicate relaxation; positive changes in isometric tension indicate contraction. Responses to GTN were obtained after exposure of endothelium-denuded rings to leukotriene C4 (GTN-C4) or leukotriene D4 (GTN-D4). *Significantly different from corresponding control value (P<.05); n=7 rings, each obtained from an individual dog.](http://circres.ahajournals.org/lookup/doi/10.1161/01.RES.83.2.395)

of active tone equivalent to that evoked during the control period (data not shown).

**Effect of Reduced Hb and MB on LTC4- and LTD4-Induced Venomotor Relaxation**

Figs 8 and 9 illustrate the heterogeneous inhibitory effects of reduced Hb (10⁻⁶ and 10⁻⁵ M) and MB (10⁻⁶ or 10⁻⁵ M) on venomotor relaxation produced by 5×10⁻⁹ M (EC₄₀;o₆) LTC₄ and LTD₄.

Results obtained in these studies showed that 10⁻⁶ M Hb attenuated only the relaxation evoked by LTC₄ in the SV (Fig 8A), whereas 10⁻⁵ M Hb markedly attenuated both LTC₄ and LTD₄-evoked SV relaxation (Fig 8). Relaxation produced by LTD₄ in the MV was slightly enhanced in the presence of 10⁻⁶ M Hb (Fig 8B).

In comparison with these observations, pretreatment (15 to 20 minutes) of endothelium-intact rings of MV, SV, and IVC with either 10⁻⁴ or 10⁻³ M MB, an inhibitor of activation of soluble guanylate cyclase, resulted in attenuation of LTC₄- and LTD₄-induced relaxation of the SV and LTD₄-induced relaxation of the IVC (Fig 9).

Control studies (n=4) demonstrated that pretreatment of MV rings with MB (10⁻³ M) significantly attenuated venomotor relaxation evoked by the endothelium-independent nitrovasodilator GTN. In the absence of MB, relaxation evoked by GTN (10⁻⁹ to 10⁻⁷ M) ranged from 36±2% to 97±2%, whereas in the presence of MB, the GTN-evoked responses ranged from 16±3% to 56±3%. Because GTN-evoked relaxation is known to involve activation of soluble guanylate cyclase, these results suggest that MB was efficacious in inhibiting MV soluble guanylate cyclase.

Although incubation with either reduced Hb or MB did not change basal tone in any of the venous rings tested, a reduction (=10% to 30%) in the concentration of NE required to elicit an increase in tone of the identical magnitude to that induced during the control period was necessary (data not shown).

**Discussion**

Results of the present study indicate that the peptide LTs, LTC₄ and LTD₄, have the capacity to evoke marked vasomotor relaxation of submaximally contracted rings of canine MV, SV, and the IVC, ie, the splanchnic venous capacitance vessels. Venomotor relaxation was completely abolished in all vessels after physical elimination of the endothelial cell layer, indicating that a functionally intact vascular endothelium is necessary for these eicosanoids to exert their vasorelaxant influence. In contrast to these observations, both LTC₄ and LTD₄ produced only minimal endothelium-dependent relaxation of the canine FV. Because the cyclooxygenase inhibitor indomethacin was always present in the incubation medium, it is doubtful that LT-induced venomotor relaxation was mediated through the action of an endothelium-derived vasodilator prostanoid(s). The peptide LTs have been identified as the primary constituents of the slow-reacting substance of anaphylaxis. Therefore, these findings, which are compatible with the idea that peptide LTs have the capacity to increase venous capacitance, are suggestive of an additional mechanism of action for these eicosanoids in the production of the hemodynamic
and myocardial consequences associated with anaphylactic shock.

During the anaphylactic response, it has been shown that peripheral vascular resistance is either unchanged or increased in sensitized dogs\(^{24}\) and increased in subhuman primates\(^{25,26}\) and humans.\(^{4}\) These results imply that peripheral arteriolar vasodilation does not play a major role in the pathophysiology of systemic anaphylactic shock.

Femoral Vein

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FIG 4. Graph showing the effect of endothelium removal on leukotriene C<sub>4</sub>- and leukotriene D<sub>4</sub>-induced relaxation in norepinephrine-contracted rings of canine femoral vein. GTN, glyceryl trinitrate; E+, endothelium intact; E-, endothelium denuded; p[Agonist], negative log molar concentration of leukotrienes C<sub>4</sub> and D<sub>4</sub> and GTN. Removal of endothelium virtually abolished venomotor relaxation evoked by leukotrienes C<sub>4</sub> and D<sub>4</sub> and GTN. Negative changes in isometric tension indicate relaxation; positive changes in isometric tension indicate contraction. Responses to GTN were obtained after exposure of endothelium-denuded rings to leukotriene C<sub>4</sub> (GTN-C<sub>4</sub>) or leukotriene D<sub>4</sub> (GTN-D<sub>4</sub>). *Significantly different from corresponding control value (P≤.05); n=7 rings, each obtained from an individual dog.

Mesenteric Vein

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FIG 5. Graphs showing the effect of pretreatment with N\(^\text{O}\)-nitro-L-arginine methyl ester (L-NAME, 100 \(\mu\)M) for 60 minutes on leukotriene C<sub>4</sub> (LTC<sub>4</sub>)-induced (A) and leukotriene D<sub>4</sub> (LTD<sub>4</sub>)-induced (B) endothelium-dependent relaxation in rings of canine mesenteric vein. p[LTC<sub>4</sub>] and p[LTD<sub>4</sub>], negative log molar concentration of LTC<sub>4</sub> and LTD<sub>4</sub>, respectively. Negative changes in isometric tension indicate relaxation; positive changes in isometric tension indicate contraction. L-NAME virtually abolished venomotor responses to LTD<sub>4</sub> and unmasked a contractile effect of LTC<sub>4</sub> in the presence of the nitric oxide synthase inhibitor. Inhibition was completely reversed 60 minutes after treatment of the mesenteric vein rings with 1 mM L-arginine (L-Arg). *Significantly different from corresponding control value (P≤.05); n=7 rings for each agonist, each obtained from an individual dog.
Inferior Vena Cava

![Graphs showing the effect of pretreatment with N^\text{G}\text{-nitro-L-arginine methyl ester (L-NAME, 100 \mu M) for 60 minutes on leukotriene C_4 (LTC_4) -induced (A) and leukotriene D_4 (LTD_4) -induced (B) endothelium-dependent relaxation in rings of canine inferior vena cava. p[LTC_4] and p[LTD_4], negative log molar concentration of LTC_4 and LTD_4, respectively. Negative changes in isometric tension indicate relaxation; positive changes in isometric tension indicate contraction. L-NAME significantly attenuated venomotor responses to LTC_4 and LTD_4. Inhibition was completely reversed 60 minutes after treatment of the inferior vena cava rings with 1 mM L-arginine (L-Arg). *Significantly different from corresponding control value (P \leq .05); n=7 rings for each agonist, each obtained from an individual dog.](image)

Splenic Vein

![Graphs showing the effect of pretreatment with N^\text{G}\text{-nitro-L-arginine methyl ester (L-NAME, 100 \mu M) for 60 minutes on leukotriene C_4 (LTC_4) -induced (A) and leukotriene D_4 (LTD_4) -induced (B) endothelium-dependent relaxation in rings of canine splenic vein. p[LTC_4] and p[LTD_4], negative log molar concentration of LTC_4 and LTD_4, respectively. Negative changes in isometric tension indicate relaxation; positive changes in isometric tension indicate contraction. L-NAME slightly attenuated venomotor responses to LTC_4. Inhibition was completely reversed 60 minutes after treatment of the splenic vein rings with 1 mM L-arginine (L-Arg). *Significantly different from corresponding control value (P \leq .05); n=7 rings for each agonist, each obtained from an individual dog.](image)

Thus, identification of the site of hemodynamic dysfunction(s) leading to hypotension elicited after exposure to a specific antigen has focused on a fundamental decrease in cardiac contractility. Systemic anaphylaxis is characterized by a decrease in cardiac output secondary to a reduction in stroke volume and a concomitant lack of a positive chronotropic response to the decrease in blood pressure.25,27 These results appear to argue for the existence of a primary dysfunction in cardiac contractility, which may account for the observed hypotension. However, the origin of the hypokinetic state of the anaphylactic myocardium is of considerable debate and may involve myocardial ischemia secondary to intense coronary vasoconstriction induced by various mediators of anaphylaxis, particularly the LTs, a direct effect of these substances on the myocardium itself, or both. Significant variability in the coronary vasomotor properties of the LTs has been reported, ranging from potent vasoconstriction to vasodilation.29 Similarly, evidence for direct myocardial depression produced by the peptide LTs is equivocal, revealing that these eicosanoids can exert both nega-
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dramatic fall in venous return, cardiac output, and right atrial and pulmonary arterial pressures. The reduction in cardiac output was reversed by maintaining constant venous return and left atrial filling pressure by use of an external pump. They also showed that antigen-induced shock could be ameliorated by occluding the descending aorta, implying that the splanchnic vasculature is the locus of blood pooling. Similarly, in the same animal model, Wagner et al. found that anaphylactic shock was accompanied by an increase in the “unstressed volume” (ie, the volume of blood in the visceral capacitance vessels), consistent with dilation of the small veins, in the absence of any acute loss of plasma volume. As suggested above, the present observations may be interpreted to imply that the peptide LTs possess the ability to produce dilatation of venous capacitance vessels and thus provide more specific evidence supporting the concept of splanchnic venous pooling as a mechanism contributing to anaphylactic hypotension.

EDNO has been proposed to be the final common mediator of endotoxin- and cytokine-induced hypotension. Recently, a role for EDNO in anaphylactic shock was postulated by Amir and English, who demonstrated that pretreatment of bovine serum albumin-

**Fig 8.** Bar graphs showing the effect of reduced hemoglobin (Hb) ($10^{-6}$ and $10^{-5}$ M) on endothelium-dependent relaxation in rings of canine mesenteric vein (MV), inferior vena cava (IVC), and splenic vein (SV) evoked by $5 \times 10^{-9}$ M leukotriene C4 (LTC4, A) and leukotriene D4 (LTD4, B). Both concentrations of Hb inhibited LTC4-induced SV relaxation; LTD4-evoked SV relaxation was attenuated solely by $10^{-5}$ M Hb. Numbers in each bar represent the number of rings studied, each obtained from an individual dog. Control (Con) bars represent pooled control values for both concentrations of the inhibitor. For statistical analysis, each ring served as its own paired control. *Significantly different from corresponding control value ($P \leq 0.05$).

**Fig 9.** Bar graphs showing the effect of methylene blue (MB, $10^{-6}$ and $10^{-5}$ M) on endothelium-dependent relaxation in rings of canine mesenteric vein (MV), inferior vena cava (IVC), and splenic vein (SV) evoked by $5 \times 10^{-9}$ M leukotriene C4 (LTC4, A) and leukotriene D4 (LTD4, B). In a concentration-dependent manner the soluble guanylate cyclase inhibitor attenuated LTC4- and LTD4-induced SV relaxation and LTD4-evoked IVC relaxation. Numbers in each bar represent the number of rings studied, each obtained from an individual dog. Control (Con) bars represent pooled control values for both concentrations of the inhibitor. For statistical analysis, each ring served as its own paired control. *Significantly different from corresponding control value ($P \leq 0.05$).
sensitized mice with the NO synthase inhibitor L-NAME substantially reduced murine mortality after antigen exposure. In view of the complete endothelium dependence of LT-induced venomotor relaxation observed in the present study, we investigated the potential role of EDNO as a mediator of relaxation elicited by LTC₄ and LTD₄ in capacitance veins.

Effects of L-NAME on LT-induced relaxation in the MV was heterogeneous inasmuch as venomotor responses evoked by LTD₄ were virtually abolished, whereas a significant contractile response to LTC₄ was unmasked in the presence of the NO synthase inhibitor. Because LTC₄ failed to evoke contraction in endothelium-denuded MV rings, it is postulated that this contractile influence is mediated by an endothelium-derived contractile factor (EDCF), which, in view of the presence of indomethacin in the buffer, is not a product of cyclooxygenase metabolism. Although speculative, potential EDCF candidates could include superoxide anions⁵⁵ and the peptide endothelin.⁶⁶ Peptide LTs have similarly been shown to evoke endothelium-dependent contraction in other vessels,³⁷ although the responsible mediator has not been identified. A possible alternative explanation for LTC₄-evoked MV contraction during endothelial NO synthase inhibition is that it is a consequence of a direct interaction of the eicosanoid with MV smooth muscle. However, a direct effect seems to be unlikely in view of the observed lack of venomotor activity of LTC₄ in the MV subsequent to physical disruption of the endothelium.

To characterize further the possible role of EDNO in LT-evoked venomotor relaxation, the effects of two well-known inhibitors of endothelium-dependent relaxation on these evoked responses were determined. Reduced Hb has been shown to interact with EDNO, resulting in attenuation of both endothelium-dependent relaxation responses and accumulation of the second messenger cGMP.⁶⁰,⁶¹ MB, which inhibits activation of the soluble form of guanylate cyclase, is known to attenuate vasomotor relaxation produced in response to nitrovasodilators and those endothelium-dependent relaxing agents that release EDNO.⁴⁰ In addition, MB has also been demonstrated to spontaneously generate O₂⁻ radicals,⁶² which have the property of binding to and inactivating EDNO.⁴⁰

In contrast to the aforementioned observations regarding the effects of L-NAME on LT-evoked venomotor relaxation, after treatment of MV rings with reduced Hb or MB, venomotor relaxation evoked by both LTC₄ and LTD₄ was unaltered. The enhancement of LTD₄-induced MV relaxation in the presence of Hb is presently inexplicable but represents an increase in responsiveness of the MV to LTD₄ of only 13%. Thus, when taken together, these results indicate that LTD₄-evoked endothelium-dependent relaxation in the MV is l-arginine dependent but that the putative mediator is chemically dissimilar from EDNO and is not dependent on activation of soluble guanylate cyclase. This is not unlike previously reported results obtained in this laboratory demonstrating that LTD₄-evoked endothelium-dependent relaxation of the canine renal vein is refractory to inhibition by Hb, MB, and N⁶-acetyl-l-arginine, another argininoxylic compound.¹²

In the SV, L-NAME had little effect on LTC₄-evoked and no effect on LTD₄-evoked venomotor relaxation. The reason for the minimal effectiveness of L-NAME on SV relaxation elicited by these eicosanoids is unclear but may involve restricted access of the NO synthase inhibitor to the interior of SV endothelial cells when compared with other tissues. However, consistent with the observation that L-NAME did not affect LTD₄-evoked and only partially inhibited LTC₄-evoked relaxation of the SV, it was also found that a significant component of LT-induced SV relaxation also was produced in the presence of concentrations of Hb and MB that nearly abolish endothelium-dependent relaxation in arterial tissue.¹²,²³ This incomplete inhibition of LT-evoked SV relaxation could be interpreted to be consistent with the postulated⁴¹ existence of multiple and distinct isozymic forms of constitutive NO synthase. Alternatively, it is possible that the relaxation evoked by LTC₄ and LTD₄ is also dependent on other mechanisms or mediators in addition to EDNO.

Heterogeneous inhibition of venomotor relaxation evoked by LTC₄ and LTD₄ in the IVC was also observed. When the effects of L-NAME, Hb, and MB on these responses were compared, it was found that L-NAME inhibited both LTC₄- and LTD₄-induced IVC relaxation by approximately 50%. The inhibitor of guanylate cyclase activation, MB (10⁻⁶ and 10⁻⁵ M), slightly attenuated relaxation elicited by LTC₄, whereas reduced Hb (10⁻⁶ M) altered neither LTC₄ nor LTD₄-induced IVC relaxation. These observations, like those described above, appear to be consistent with the postulate of multiple forms of constitutive NO synthase. Indeed, these data also may indicate that the observed venomotor relaxation of the IVC evoked by both LTC₄ and LTD₄ may also be dependent on a mechanism or an EDRF distinct from EDNO.

In conclusion, results of the present study demonstrate that the peptide LTs, LTC₄, and LTD₄, the principal components of the slow-reacting substance of anaphylaxis, have the capability to evoke marked endothelium-dependent venomotor relaxation of canine splanchic venous capacitance blood vessels. Depending on the vessel, the venomotor effects of these eicosanoids appeared to be partially dependent on EDNO generation and to involve an additional l-arginine-dependent mechanism (possibly consequent to the activity of multiple forms of constitutive NO synthase) and/or non-EDNO mediator(s). These results are compatible with the postulate that peptide LT-induced capacitance venorelaxation may contribute to the reduced venous return and resultant hypotension associated with systemic anaphylaxis.

Acknowledgments

This study was supported by National Institutes of Health Grant HL-34036 (B.M.C.) and a Grant-in-Aid of Research from Sigma Xi, The Scientific Research Society (J.R.P.).

References


Leukotrienes C4 and D4 are potent endothelium-dependent relaxing agents in canine splanchnic venous capacitance vessels.

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Circ Res. 1993;73:395-404
doi: 10.1161/01.RES.73.2.395

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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