Systemic and regional hemodynamic effects of leukotrienes D_4 and E_4 in the conscious rat

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EIMERL, J., A.-L. SIRÉN, AND G. FEUERSTEIN. *Systemic and regional hemodynamic effects of leukotrienes D_4 and E_4 in the conscious rat.* Am. J. Physiol. 251 (Heart Circ. Physiol. 20): H700–H709, 1986.—The effect of leukotrienes (LTs) D_4 and E_4 on systemic and regional hemodynamic variables were studied in the conscious rat (n = 5–9). Renal (R), mesenteric (M), and hindquarter (HQ) blood flow (BF) were monitored by directional pulsed Doppler velocimetry, and mean arterial blood pressure (MAP) and heart rate were recorded through a catheter in the femoral artery. In a separate series of experiments, cardiac index (CI) was measured by the thermodilution method. Systemic injection of LTD_4 or LTE_4 (0.1–10 μg/kg) produced dose-dependent pressor responses; BF in the M, HQ, and R vessels declined, due to increased vascular resistance (VR) at the following order: M ≫ HQ > R. Low doses of LTD_4 or LTE_4 produced vasodilation in the HQ area. Infusion of LTD_4 (3 $\mu g \cdot kg^{-1} \cdot min^{-1}$) for 10 min produced progressive and pronounced vascular constriction in the M and HQ regions along with reduction in BF. The LTD_4 infusion also markedly decreased CI with a concomitant rise in total peripheral resistance index (TPRI). Indomethacin (5 mg/kg iv) pretreatment did not modify any of the hemodynamic effects of LTD_4 or LTE_4. FPL 55712 (10 mg/kg iv) and LY 171883 (30 mg/kg iv), two different LT-receptor antagonists, partially blocked the constriction effects of these LTs. LY 171883, but not FPL 55172, blocked the HQ vasodilation produced by LTE_4. LY 171883 alone increased HQ-BF and reduced HQ-VR. These data indicate that LTD_4 and LTE_4 are potent constrictors of the M vascular bed, but at low doses they also produce dilation of the HQ blood vessels. Furthermore, no escape from the effects of prolonged infusion of the LTs was demonstrated in this species. Finally, the hemodynamic responses to LTD_4 and LTE_4 in the conscious rat are independent of cyclooxygenase products of LTs and are only partially blocked by FPL 55712 or LY 171883. These studies taken together suggest a differential distribution of multiple LT receptors in the rat vasculature.

indomethacin; blood pressure; renal blood flow; mesenteric blood flow; leukotriene antagonists; anaphylactic shock

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THE PEPTIDOLEUKOTRIENES (LTs) are 5-lipoxygenase metabolites of arachidonic acid, which have been shown to be the active constituents of the slow reacting substance of anaphylaxis (SRS-A) (27). The potent vasoactive properties of the LTs are well established, but major differences occur between various in vitro vs. in vivo preparations and among diverse species (9). Although most normal vascular preparations constrict in response to in vitro LTs (for review see Ref. 9), some reports show the opposite (14, 18, 19). In most species studied, in vivo LTs produce a pressor response, but hypotension is the dominant outcome in guinea pigs and rabbits (9). Furthermore, LTs were shown to produce renal vascular constriction in the anesthetized pig (24) but to produce dilation in the dog (4, 8). Thus conflicting results on hemodynamic effects of in vivo LTs are also common.

The rat is particularly useful for evaluating hemodynamic effects of LTs, since, in this species, the respiratory system is relatively resistant to the deleterious effects of LTs (10, 30). In the rat, LTs have been shown to increase systemic vascular resistance and vascular permeability and to lower cardiac output (CO; 1, 23, 29). LTs were also shown to differentially affect various vascular beds in the rat (2, 29); for example, the mesenteric (M) artery was found to be more sensitive to LTD_4 administration than was the hindquarter (HQ) or renal (R) vessels (2). However, other studies in anesthetized rats (1) or in the isolated perfused rat kidney (25) demonstrated greater renal constrictive potency for LTs, whereas neither the M nor the R vascular beds were significantly effected in the pithed normotensive rat (29). These conflicting reports might be the result of different techniques of blood flow measurement, rat strain differences, anesthesia, or in vitro vs. in vivo conditions. No study to date has examined blood vessel responses to LTs in the conscious rat.

It is also pertinent to note that most of the LT effects described above were observed after bolus administration. However, continuous infusion of LTs better simulates sustained exposure of vascular tissue under pathophysiological conditions, such as anaphylaxis. Moreover, the coronary arteries of the anesthetized domestic pig (7) show rapid tachyphylaxis to sustained infusion of LTs, presumably due to release of platelet-dependent dilator factor (7, 10). Since rat platelets do not aggregate in response to these LTs (10), we postulated that the hemodynamic responses to sustained infusion of LTs in the rat might be substantially different from the responses seen in the pig. Therefore, we also studied the effect of sustained LT infusion on both and regional hemodynamic responses of the conscious rat.

Finally, the mechanism of the LT-induced vascular responses was studied by using the potent cyclooxygenase inhibitor, indomethacin (Indo), and two LT-receptor antagonists. The SRS-A and LT antagonist, FPL 55712, was shown to block LTs' effects on several vascular and

nonvascular preparations, but several exceptions to this general property of FPL 55712 were reported as well as possible nonspecific effects on some vascular beds (9, 25). A newer agent, LY 171883, is claimed to be an effective, long-acting LTs' antagonist (5, 11), but no data are available on its efficacy on specific blood vessels in vivo.

Our study was designed, therefore, to further investigate the systemic and regional hemodynamic effects of LTD_4 and LTE_4 administered in bolus or infusion to the conscious unrestrained rat. In addition, we evaluated the effects of pretreatment with the cyclooxygenase inhibitor Indo and with the LTs' antagonists, FPL 55712 and LY-171883, on the systemic and local vascular responses induced by LTs. This study also emphasizes the potential role of LTE_4 in hemodynamic modulation, since LTE_4 was poorly studied in the past while new information indicates that LTE_4 might be the primary circulatory LT in shock and trauma (6).

MATERIALS AND METHODS

Male Sprague-Dawley rats (Taconic Farms, Germantown, NY) weighing 250–350 g were used in all studies (n = 26). Surgical procedures were performed under anesthesia with intramuscular solution of ketamine (130 mg/kg) and acepromazine (1.3 mg/kg). Experiments were carried out on the conscious unrestrained rats, 1–3 days after operation. All rats were housed individually in standard plastic cages with food and water ad libitum.

Regional blood flow measurements with directional pulsed Doppler technique. Miniaturized Doppler flow probes were implanted around the lower abdominal aorta for HQ blood flow measurement; the superior M and the left R arteries were also implanted with such probes by the method previously described by Haywood et al. (15). Briefly, a midline laparotomy was performed, and the superior M artery, the left R artery, and the lower aorta above its bifurcation were carefully isolated under a dissecting microscope. Miniaturized Doppler flow probes (Valpey-Fisher, Hopkinton, MA) were then loosely sutured (6-0 silk) around each vessel. The insulated wire leads of the probes were tunneled beneath the skin, exteriorized at the nape, and soldered to a receptacle which was then attached to the skull with small screws and dental acrylic. The femoral artery and vein were cannulated with polyethylene catheters (PE-50) which were also tunneled beneath the skin to exit at the nape. Catheters were regularly flushed (0.2 ml) with heparinized saline (100 U/ml) and secured by a soft spring attached to the animal's neck with an adhesive collar.

On the day of the experiment, the arterial line was connected to a pressure transducer (Narco Bio-Systems model RP 1500i) coupled to a strain gauge coupler (Narco Bio-Systems type 7179) and a biotachometer coupler (Narco Bio-Systems type 7032). Mean arterial blood pressure (MAP) and heart rate (HR) were continuously recorded on a Narcotrace 80 computerized dynograph and sampled (automatically or manually) at 15- to 60-s intervals by a Northstar-Hazeltine computer. A connector line was attached between the blood flow receptacle and a directional pulsed Doppler flowmeter (University of Iowa, Bioelectrical Engineering, Iowa). Output signals of Doppler shifts >4 kHz were simultaneously transformed to mean flow velocity (KHz) and recorded continuously on the Narcotrace 80 through universal couplers (type 7178). Local vascular resistance was calculated by dividing the MAP by the Doppler shift in KHz, as previously described (15). It is noteworthy that measurements of MAP from the femoral artery cannula in the rat are virtually the same as the MAP in other arteries (e.g., brachial, mesenteric) in normal, hypotensive, or hypertensive states (22). Changes in regional blood flow and local vascular resistance were further expressed as percent changes from control values. No differences in the basal signal level were found between the various experimental groups, since only signals of >4 kHz for basal levels were considered appropriate.

Experimental protocols. On the day of experiment, each animal was allowed to stabilize for at least 30 min while basal (control) levels of the hemodynamic variables were recorded. Vehicle injections (0.9% NaCl, 300 μl iv) were used as controls for injection effects. To assess the consistency of the various vascular responses, a bolus injection of norepinephrine (3 μg/kg iv) was used; this test dose of norepinephrine caused a prompt increase in MAP, a decrease in HR, and a decreased blood flow in HQ, R, and M. After the stabilization period each animal was scheduled to one of the following protocols: *1*) administration of LTD_4 or LTE_4 (0.1, 1, 3, 10 μg/kg iv) as bolus injections in ascending order; *2*) administration of LTD_4 (10 μg/kg iv) before and 1 min after treatment with the LT antagonist, FPL 55712 (10 mg/kg iv, given over 0.5–1 min); *3*) administration of LTD_4 or LTE_4 (1 and 10 μg/kg iv) before and 15–60 min after treatment with the LT antagonist LY 171883 (30 mg/kg iv). This period (up to 60 min) was necessary to allow stabilization of cardiovascular parameters after LY 171883 administration, since this LT antagonist had definite intrinsic activity; *4*) administration of LTD_4 or LTE_4 (3 μg/kg iv) before and 30–60 min after treatment with Indo (5 mg/kg iv). Each rat received only one LT to avoid any cross tachyphylaxis between the LTs. *5*) Continuous infusion of LTD_4 (3 $\mu g \cdot kg^{-1} \cdot min^{-1}$) over 10 min (27 μl/min), using the Harvard Instruments, MA).

LTD_4 and LTE_4 injections were separated by 15- to 30-min intervals to allow for return to control values. The dose range chosen for administration of LTs D_4 and E_4 and the intervals for complete recovery were based on previous reports from our laboratory (2). Doses of LY 171883 and FPL 55712 were based on previous in vivo studies which showed maximal antagonistic capacity for these agents in several preparations (1, 5, 11).

Measurement of cardiac output. In eight additional rats, the effect of a continuous infusion of LTD_4 on cardiac output and total peripheral resistance (TPR) was investigated. The rats were anesthetized with pentobarbitone (40 mg/kg ip), and PE-50 tubing was inserted into the femoral arteries. These catheters were tunneled beneath the back skin and exited at the back of the neck as described above. Then an incision was made at the midline of the neck from the cricoid to the clavicle, and PE-50 tubing was inserted into the right atrium through

the external jugular vein. Then the left common carotid artery was exposed and ligated, and a thermistor (MX2-780-33 model THMP f 1.5, Teflon reusable, Columbus Instruments, OH) was advanced through the carotid into the ascending aorta (placement above the aortic valve was confirmed in each animal at the end of the experiment and by the shape of the dilution curve before the probe was finally sutured to the neck muscles). The jugular vein catheter and the thermistor were tunneled under the skin to the back of the neck. All lines were secured by a soft spring wire throughout the cage as earlier described. Twenty-four hours after surgery, the arterial line was connected to a blood pressure transducer for hemodynamic recordings. The cardiac output was measured by thermodilution technique as the thermistor was attached to the computerized Cardiomax II (CMX2-780-k with the microprobe option R, Columbus Instruments, OH). The dead space of the venous line was first flushed with 0.05 ml of 0.9% (wt/vol) NaCl (saline) at room temperature (22°C); after a brief stabilization period (10 s to ensure normal core temperature) an additional injection of 0.2 ml normal saline (22°C) was rapidly injected using a 1-ml syringe. Cardiac output was recorded in the following way: a control period of 15 min included two to three cardiac output measurements to test for consistency and placement of the probe, and also to get control values for MAP and HR. Continuous infusion of LTD_4 ($3\ \mu g \cdot kg^{-1} \cdot min^{-1}$) into the jugular vein (27 μl/min) was done by means of the Harvard pump. The timer on the automatic data collection system was started at the beginning of the LTD_4 infusion, and data points were taken at time (t) t_0, t_3, t_5, t_{10}, t_{30} min. TPR was calculated by dividing the MAP by the cardiac output; values of cardiac output and TPR were further indexed per unit of weight (kg).

Drugs. Pure synthetic LTD_4 and LTE_4 (kindly provided by Dr. J. Rokach, Merck Frosst, Dorval, Canada) were aliquoted in distilled water and kept frozen (−70°C) until used. Each aliquot was thawed only once and diluted with 0.9% NaCl to a final volume of 200–300 μl for injection into the animal. FPL 55712 (kindly provided by Fisons Pharmaceuticals, Loughborough, UK) was dissolved in 0.45% saline solution at 40°C and further mixed with normal saline to a final concentration of 20 mg/ml. FPL 55712 was freshly prepared for each experiment. LY 171883 (kindly provided by Dr. J. Fleisch, Eli Lilly, Indianapolis, IN) was dissolved in 0.5 M sodium bicarbonate according to the manufacturer's directions and titrated to pH 8 with 0.1 M HCl. After being aliquoted it was kept frozen until use. Indo (Sigma, St. Louis, MO) was dissolved in 0.1 M sodium bicarbonate and titrated to pH 8.2 with 0.1 M HCl before use. Norepinephrine was also obtained from Sigma.

Statistics. Data are presented in text and figures as means ± SE for the indicated number of rats. Analysis of variance (ANOVA) with repeated-measures, ANOVA followed by the Student-Newman-Keul test (SNK), or Dunnett's test was used for statistical evaluation of the data. Where appropriate, a two-tailed paired Student's t test was also used. Differences were considered significant when the P value was less than 0.05.

RESULTS

Effect of bolus injection of LTD_4 and LTE_4 on MAP and HR. The MAP and HR of the conscious rats prior to drug administration were 109 ± 2 mmHg and 377 ± 13 beats/min, respectively. Control values did not differ between the various protocols. Systemic bolus administration of increasing doses of LTD_4 and LTE_4 (0.1–10 μg/kg iv) caused a dose-dependent increase in MAP (Fig. 1*A*) that was maximal 30–60 s after injection and gradually returned to control levels (Fig. 1*B*). Lower doses of LTD_4 and LTE_4 (0.1 μg/kg iv) did not affect MAP. Neither LTD_4 nor LTE_4 caused significant changes in HR. LTD_4 was more potent than LTE_4 in its pressor activity (Fig. 1, *A* and *B*).

Effect of bolus injection of LTs D_4 and E_4 on regional blood flow and vascular resistance. Bolus administration of LTs D_4 and E_4 caused a differential response in the various vascular beds (Fig. 2). Both LTs D_4 and E_4 caused a dose-dependent reduction in M blood flow with concomitant increase in vascular resistance (Figs. 3 and 4). At the higher doses (10 μg/kg iv) both LTD_4 and LTE_4 caused a significant increase in R vascular resistance, but LTD_4 showed a greater maximal potency and caused significant reduction of R blood flow at even lesser doses (Fig. 3). The HQ vascular bed showed a more complex response pattern to both LTD_4 and LTE_4. Within the 1st min after injection, both LTs caused an brief initial increase in HQ blood flow (HQ1), due to a decrease in vascular resistance (Figs. 2–5, HQ1 period). Shortly after the first phase of vasodilation, the HQ blood flow and vascular resistance showed an opposite response (reduction in blood flow and an increase in vascular resistance; HQ2). It is important to point out that the secondary constrictor response (HQ2) became obvious only with higher doses (≥3 μg/kg for LTD_4 and 10 μg/kg for LTE_4), whereas lower doses produced only the initial dilator response (Figs. 3 and 4). Also, the HQ1 phase was clearly seen at a dose of LTE_4 (0.1 μg/kg) which had no effect on MAP (Fig. 4). However, the HQ1 phase did not show a dose-response relationship within the range of doses used in this study.

The initial dilator response occurred almost simultaneously with the opposite constrictor responses observed in the R and M vessels and with the developing pressor blood pressure response (Figs. 1, 2, and 5). At doses where MAP showed only minor or no changes (0.1–1 μg/kg) both LTD_4 and LTE_4 caused significant changes in blood flow and vascular resistance, mainly at the M vascular bed. Analysis of peak responses (ANOVA followed by SNK) induced by LTD_4 and LTE_4 (Figs. 3 and 4) showed differential sensitivity of the various vascular beds to their effects as follows: $M \gg HQ \geq R$ ($P < 0.01$; $n = 5$–9).

Effect of continuous infusion of LTD_4 on MAP, regional blood flow, and vascular resistance. Continuous intravenous infusion of LTD_4 ($3\ \mu g \cdot kg^{-1} \cdot min^{-1}$) for 10 min caused gradual increases in MAP, HQ, R, and M vascular resistance. However, only M and HQ blood flow were significantly reduced (Fig. 6). The initial early dilator response (HQ1) was no longer observed in the HQ vessels. Analysis of peak responses revealed the same pat-

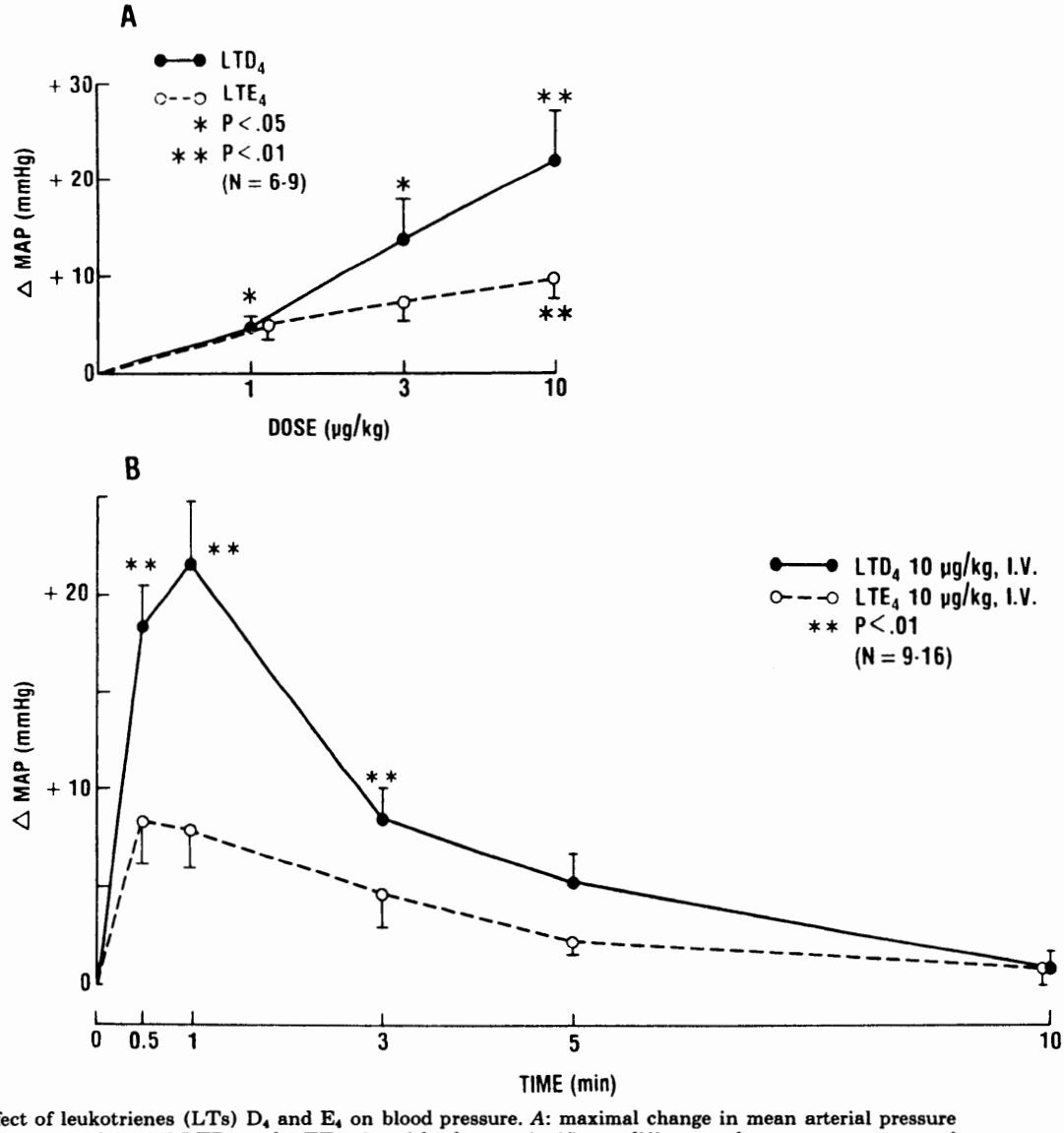

FIG. 1. Effect of leukotrienes (LTs) D_4 and E_4 on blood pressure. *A*: maximal change in mean arterial pressure (ΔMAP) to increasing doses of LTD_4 and LTE_4. *Asterisks* denote significant differences between treatment and control groups (ANOVA followed by SNK). *B*: time course of pressor effect induced by LTD_4 and LTE_4. ΔMAP denotes change in mean arterial pressure from control levels. *Asterisks* denote significant differences between LTD_4 and LTE_4 groups (ANOVA followed by SNK). $F = 10.82$; $P < 0.001$ (ANOVA with repeated measures).

tern of differential sensitivity to LTs for the various vascular beds, as follows: M ≫ HQ > R ($P < 0.01$; $n = 5$). Discontinuation of the infusion was followed by gradual recovery of all the hemodynamic variables toward control levels. However, even 20 min after the cessation of the infusion, M and HQ blood flow, and vascular resistance were still elevated, while MAP, R blood flow, and vascular resistance returned to base line.

Effect of continuous infusion of LTD_4 on cardiac output and TPR (Table 1). The base-line levels of MAP, HR, cardiac index, and TPR before intrajugular infusion of LTD_4 (3 $\mu g \cdot kg^{-1} \cdot min^{-1}$) were 110 ± 4 (SE) mmHg, 422 ± 19 beats/min, 482 ± 21 $ml \cdot min^{-1} \cdot kg^{-1}$, and 0.23 ± 0.01 $mmHg \cdot ml^{-1} \cdot min \cdot kg$, respectively. Infusion of LTD_4 over 10 min induced a sustained fall in cardiac index with a concomitant increase in TPR index. The maximum changes in cardiac and TPR indexes were reached 5 min after the beginning of the infusion. Cardiac index remained low even 20 min after the end of the LTD_4 infusion, whereas TPR index was significantly increased only during the 10 min of infusion. MAP transiently increased with a maximum of +10 ± 4 mmHg 3 min after the beginning of the LTD_4 infusion. Also, LTD_4 infusion induced a sustained tachycardia with a maximum increase in HR of +86 ± 21 beats/min.

Effect of pretreatment with Indo on the vascular response induced by LTD_4 and LTE_4. Intravenous administration of Indo (5 mg/kg iv) 30–60 min before bolus injections of either LTD_4 or LTE_4 (3 μg/kg iv) did not alter the LT-induced pressor or vascular responses (Table 2). Indo did not effect base-line levels of MAP or regional blood flow at any of the monitored vascular beds (ΔMAP: −1 ± 1 mmHg, %Δ blood flow: −2 ± 3, +1 ± 3, +3 ± 3, for HQ, R, and M, respectively; $n = 6$, $P > 0.05$).

Effect of FPL 55712 and LY 171883 on the vascular response induced by LTD_4 and LTE_4. Intravenous admin-

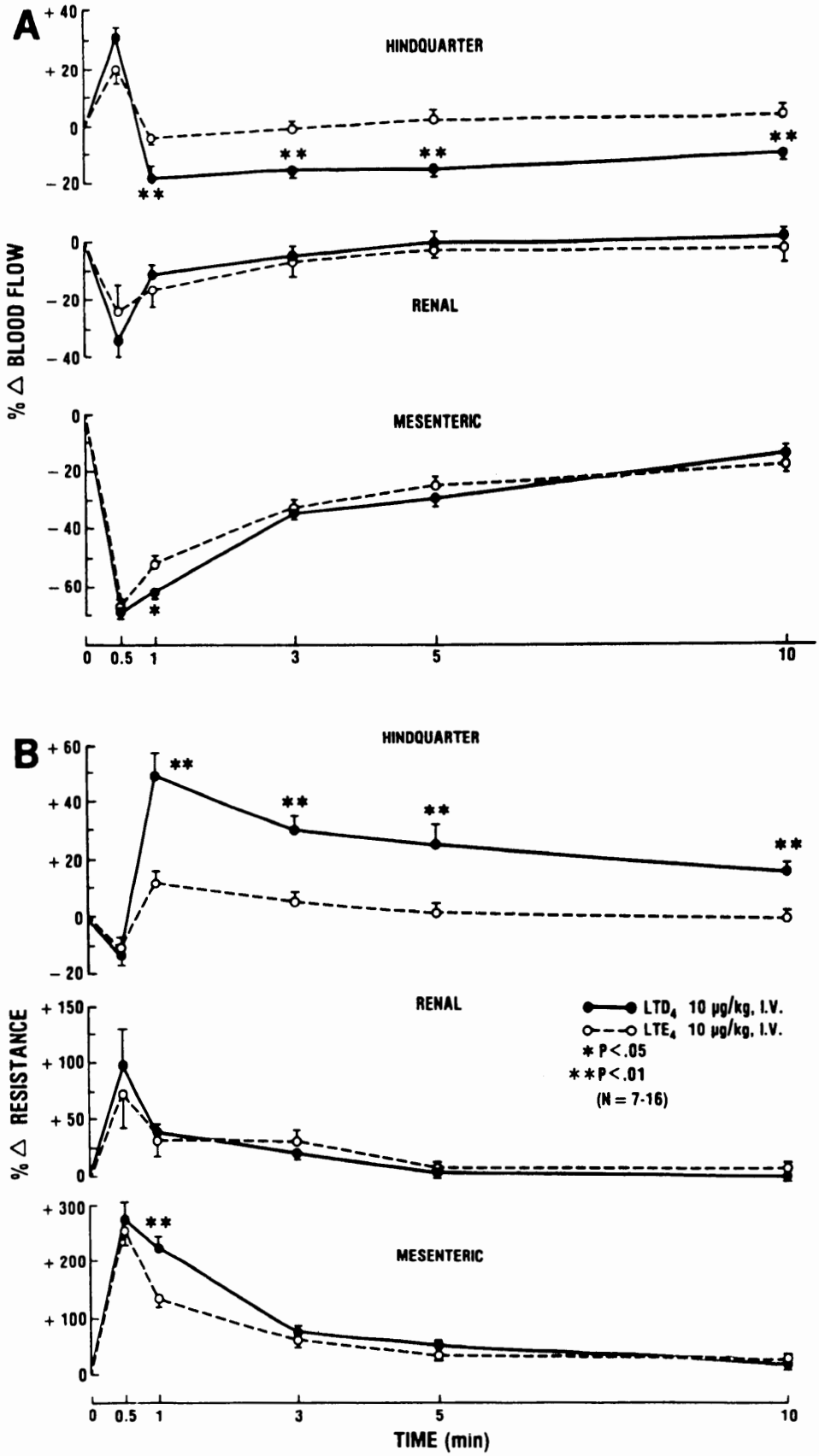

FIG. 2. Time course of the effect of leukotrienes (LTs) D_4 and E_4 on regional blood flow (*A*) and local vascular resistance (*B*) expressed as %changes from control levels (%Δ). *Asterisks* denote significant differences between LTD_4 and LTE_4 groups (ANOVA followed by SNK). ANOVA with repeated measures revealed significant differences for hindquarter blood flow ($F = 7.02$; $P < 0.001$), hindquarter vascular resistance ($F = 5.76$, $P < 0.001$), and mesenteric vascular resistance ($F = 2.60$, $P < 0.05$).

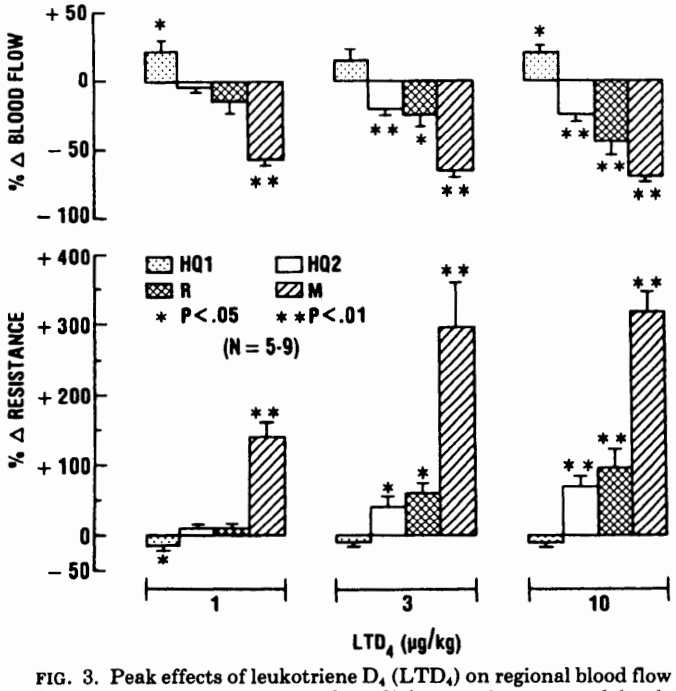

FIG. 3. Peak effects of leukotriene D_4 (LTD_4) on regional blood flow and vascular resistance expressed as %changes from control levels (%Δ). *Asterisks* denote significant differences between treatment and control groups (ANOVA followed by SNK). HQ1; dilatory, or early, phase of hindquarter flow; HQ2, constrictor, or late, phase of hindquarter flow; R and M, renal and mesenteric responses, respectively.

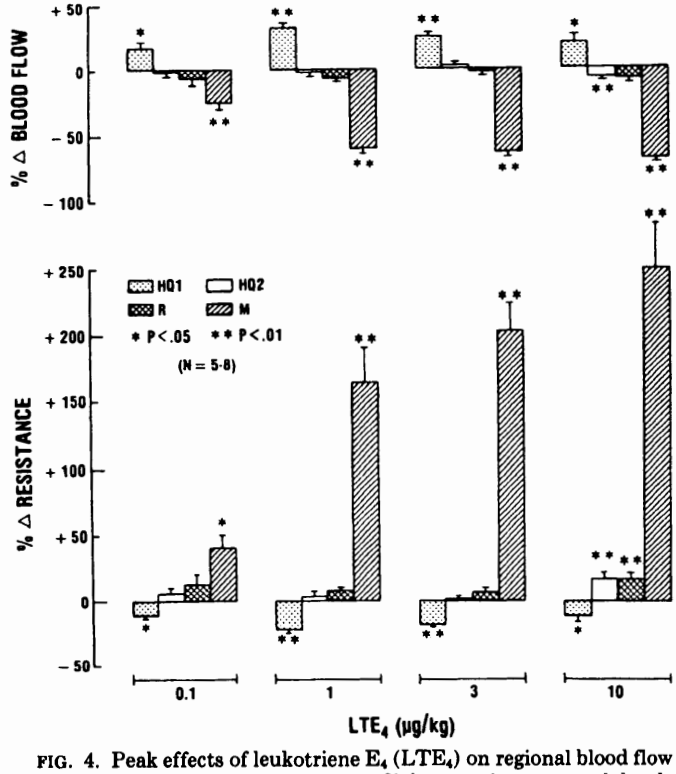

FIG. 4. Peak effects of leukotriene E_4 (LTE_4) on regional blood flow and vascular resistance expressed as %changes from control levels (%Δ). Other symbols are as in Fig. 3.

istration of FPL 55712 (10 mg/kg) produced a small but significant increases in MAP and HQ blood flow, without affecting HQ vascular resistance or R and M circulation (Table 3). FPL 55712 attenuated to the pressor response

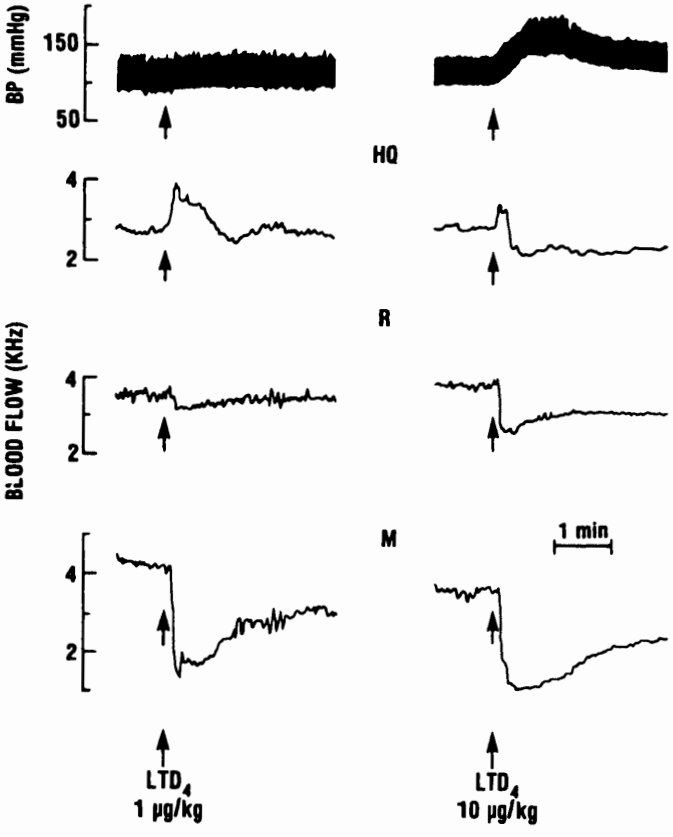

FIG. 5. A typical simultaneous recording of arterial blood pressure (BP) and regional blood flow changes induced by leukotriene D_4 (LTD_4; 1, 10 μg/kg iv). *Arrows* represent injection time.

and the increased vascular resistance induced by LTD_4 but did not effect the dilator HQ response (HQ1) and had only a modest effect on the profound reduction in M blood flow (Fig. 7).

LY 171883 (30 mg/kg iv) produced a significant increase in blood flow with a concomitant decrease in HQ vascular resistance. LY 171883 did not affect R or M circulation and did not change MAP (Table 3). LY 171883 effects on the vascular responses induced by LTD_4 and LTE_4 (1 or 10 μg/kg iv) are summarized in Figs. 8 and 9, respectively. LY 171883 attenuated the pressor response induced by LTD_4 but not by LTE_4. The HQ dilator response (HQ1) induced by the larger dose of LTD_4 (10 μg/kg) and the low dose of LTE_4 (1 μg/kg) and the HQ constrictor response (HQ2) induced by the high dose of LTD_4 (10 μg/kg) were significantly attenuated by LY 171883. Although LY 171883 effectively blocked the M vascular response to LTD_4 and LTE_4, its effects on the profound drop in M blood flow were only minor, especially against the high doses of the LTs. The changes in R blood flow and vascular resistance induced by LTD_4 (10 μg/kg) were significantly blocked by LY 171883. The effect of LY 171883 on the renal effects of LTE_4 (1 and 10 μg/kg) and LTD_4 (1 μg/kg) could not be evaluated, since no significant responses of the R vessels to these LTs were obtained with these doses.

DISCUSSION

This study shows that LTs D_4 and E_4 induce differ-

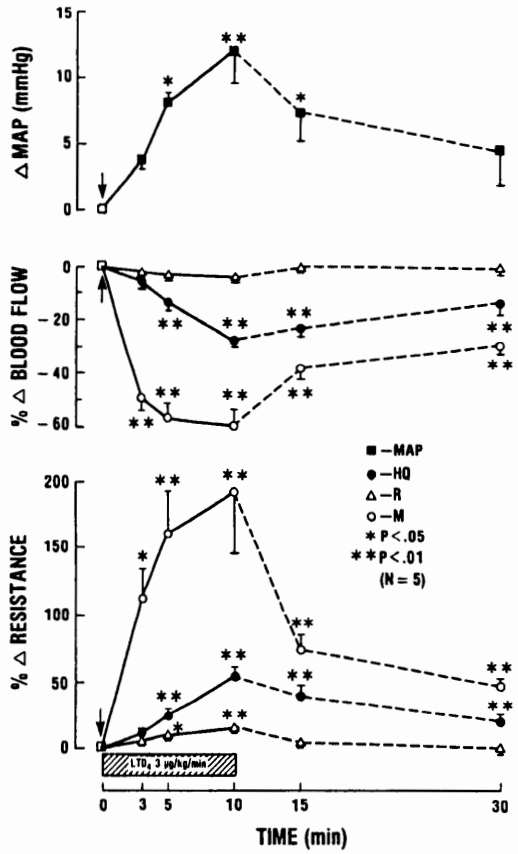

FIG. 6. Effect of continuous intravenous infusion of leukotriene D_4 (LTD_4; 3 $\mu g \cdot kg^{-1} \cdot min^{-1}$) for 10 min on mean arterial pressure (ΔMAP), regional blood flow, and vascular resistance changes expressed as %changes from control levels (%Δ). *Asterisks* denote significant differences between treatment and control groups (ANOVA followed by SNK). *Arrows* with *open squares* represent onset of infusion. *Solid lines* indicate infusion period, and *dashed lines* indicate postinfusion period. HQ, hindquarters; R, renal; M, mesenteric.

ential vascular effects in various blood vessels of the rat. The effects of LTD_4 and LTE_4 were qualitatively similar, but LTD_4 was generally more potent than LTE_4 in all the vascular beds studied. The M artery was found to be most sensitive to the constrictor effect of the LTs, whereas the R artery was generally the least responsive vessel. Unlike previous studies, the present report showed that low doses of LTD_4 and LTE_4, which had no effect on MAP, caused significant increases in M vascular resistance with concomitant decreases in M blood flow. Therefore, these changes can be considered direct ones and not a reflex response to systemic hemodynamic changes. However, larger doses of LTs invariably produced vascular constriction in all the areas studied and LTD_4 was more potent then LTE_4.

These results generally confirm previous reports describing the constrictive vascular effects of LTs in vivo (9). However, in the conscious state, all the vascular beds seem to be more sensitive to the constrictor effects of LTs, especially the R artery, which showed minimal or no response to LTD_4 in the anesthetized or the pithed rat (2, 29). These differences might be related to the use of anesthetics, which were shown to effect LT responses (16) and the better physiological integrity of the conscious rat model.

The results obtained by bolus injection of LTs are further substantiated by data obtained through continuous intravenous infusion of LTD_4, a condition that might better reflect sustained release of LTs during anaphylaxis or trauma situations. Infusion of LTD_4 induced a sustained pressor and differential vasoconstrictor response with the same potency order shown by bolus injections: M ≫ HQ > R. The TPR also markedly increased during LTD_4 infusion, further indicating a potent systemic vasoconstrictor by sustained release of LTD_4. It has recently been shown that antigen-induced anaphylaxis in conscious, sensitized rats can produce a similar pattern of circulatory changes. In the latter study, the M artery was the primary effected vessel, whereas R and HQ blood flow dropped progressively only at later phases of the shock (31).

In addition to the potent vasoconstriction, continuous infusion of LTD_4 produced a marked long-lasting fall in cardiac index. Our results thus confirm earlier studies that LTD_4 decreases cardiac output in anesthetized rats (1, 26, 29). However, the maximal fall in cardiac index was 29%, whereas TPR index increased almost 70%. Thus the changes in peripheral vascular resistance were much more pronounced than the fall in cardiac index. Interestingly, a profound tachycardic effect accompanied the LTD_4-induced fall in cardiac index. Thus a decrease in cardiac contractility by LTD_4, rather than a negative chronotropic effect on the heart, seems to account for the decreased cardiac index. Myocardial ischemia due to the well known coronary vasoconstrictor action by LTD_4 could explain the decreased contractility, but a direct myocardial depressor effect might also contribute (for review see Ref. 9).

The special value of the conscious animal model is further demonstrated by the original finding of a dilator capacity for both LTD_4 and LTE_4 in the HQ vascular bed. The transient dilator phase in the HQ vascular bed

TABLE 1. *Effect of continuous intravenous infusion of LTD_4 on cardiac output of the conscious rat*

	Minutes after Start of LTD_4 Infusion				
	3	5	10	15	30
ΔMAP, mmHg	+10±4*	+9±4	+5±3	−1±2	−2±2
ΔHR, beats/min	+65±19*	+86±21†	+61±13*	±41±19	+24±20
ΔCI, $ml \cdot kg^{-1} \cdot min^{-1}$	−123±21†	−139±28†	−135±16†	−108±4†	−64±12*
ΔTPRI, $mmHg \cdot ml^{-1} \cdot kg \cdot min$	+0.11±0.02*	+0.16±0.07†	+0.12±0.02*	+0.07±0.01	+0.04±0.02

Values are means ± SE for 8 rats. Leukotriene D_4 (LTD_4; 3 $\mu g \cdot kg^{-1} \cdot min^{-1}$) was infused over a 10-min period only. HR, heart rate; MAP, mean arterial pressure; CI, cardiac index; TPRI, total peripheral resistance index. Statistical significance from basal levels (Dunnett's test) were determined as follows: * $P < 0.05$; † $P < 0.01$.

TABLE 2. *Effect of pretreatment with Indo on changes in vascular resistance induced by LTD_4 and LTE_4 in conscious rat*

	n	ΔMAP, mmHg	%Δ Resistance			
			HQ1	HQ2	R	M
LTD_4						
Before Indo	4	+7±2	−15±3	+16±8	+5±5	+338±170
After Indo	4	+10±3	−16±2	+12±5	+15±6	+293±82
LTE_4						
Before Indo	6	+7±2	−6±3	+15±4	+7±4	+144±25
After Indo	6	+8±3	−10±4	+27±12	+15±5	+106±15

Values are means ± SE measured before and 30–60 min after administration of indomethacin (Indo; 5 mg/kg iv). Changes in vascular resistance are expressed as %changes (%Δ) from base-line levels for each group. ΔMAP presents change in mean arterial pressure. Paired Student's t test for groups before and after Indo did not reveal significant differences. n = no. of rats in each group. HQ1 and HQ2, hindquarter dilator and constrictor responses, respectively; R and M, renal and mesenteric arterial flow, respectively. Leukotrienes (LTs) D_4 and E_4 were administered at 3 μg/kg iv.

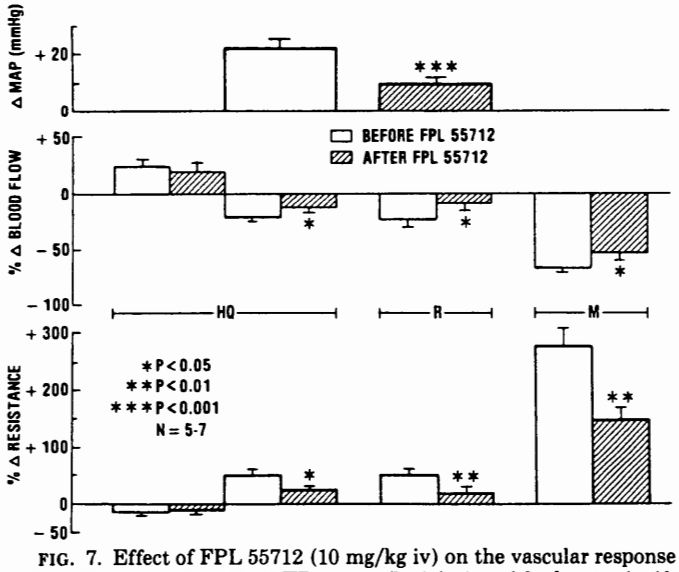

FIG. 7. Effect of FPL 55712 (10 mg/kg iv) on the vascular response induced by leukotriene D_4 (LTD_4; 10 μg/kg iv). *Asterisks* denote significant differences between groups before and after pretreatment calculated for %changes from control levels (two-tailed paired Student's t test). Blood flow and vascular resistance are expressed as %changes from control levels (%Δ).

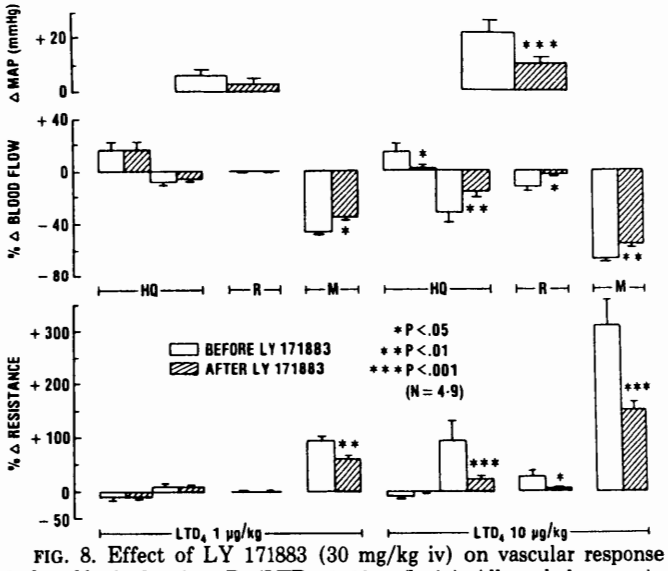

FIG. 8. Effect of LY 171883 (30 mg/kg iv) on vascular response induced by leukotriene D_4 (LTD_4; 1, 10 μg/kg iv). All symbols are as in Fig. 7.

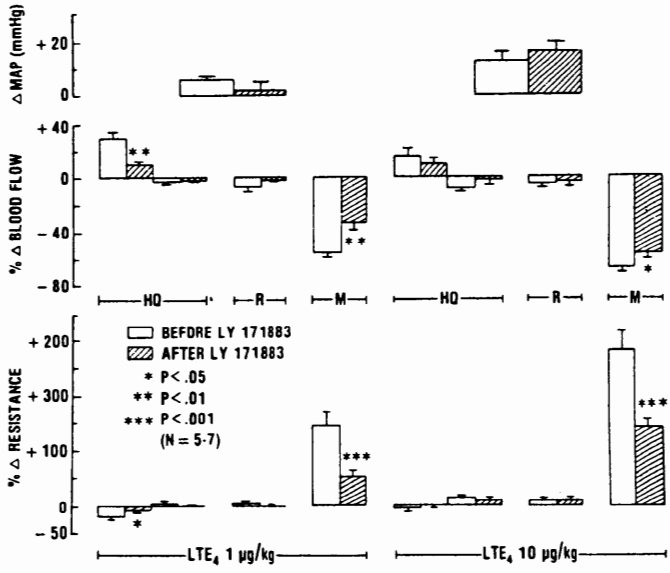

FIG. 9. Effect of LY 171883 (30 mg/kg iv) on vascular response induced by leukotriene E_4 (LTE_4; 1, 10 μg/kg iv). All symbols are as in Fig. 7.

preceded the more sustained constrictor phase and was more apparent at low doses of LTs, which had only minor constrictor activity. This dilator phase was rapidly overridden by the potent constrictor effect of higher doses of LTs. Since the HQ vascular bed consists primarily of skeletal muscles it is conceivable that this dilator phase originates in the skeletal muscle vessels. Previous studies demonstrated the dilator capacity of LTD_4 in the canine renal artery, the hypoxic ductus arteriosus of the fetal lamb, and the human skin (9). These studies, however, did not demonstrate the dual capacity for LTs to induce opposite responses in the same vascular bed. A recent study showed a very similar pattern of hindquarter and carotid blood flow changes in the anesthetized pig, but only for LTC_4 and not for LTD_4 (13). In the latter study, vascular resistance was not evaluated, and increased blood flow could have been merely the result of increased MAP. Species differences may also account for the observed differences (9). The dilator response presented in this study may best be correlated with the single report of the effect of LTC_4 on human vasculature in vivo (17). In humans, LTC_4 dilates the pulmonary and brachial arteries and increases blood flow; no vasoconstriction was observed.

LTs were previously shown to release cyclooxygenase products from various tissues which in turn mediate part of their action in some vascular preparations (9). In the present study, Indo did not effect the dilator or the constrictor properties of LTD_4 or LTE_4 in the conscious rat. This finding generally confirms previous studies in the rat species (1, 25, 30), but furthermore, it suggests that not only systemic but regional hemodynamic responses to LTs as well are probably not mediated by cyclooxygenase metabolites of arachidonic acid. In other

TABLE 3. *Effect of FPL 55712 and LY 171883 on MAP, regional blood flow, and vascular resistance in conscious rat*

	ΔMAP, mmHg	%Δ Blood Flow			%Δ Resistance		
		HQ	R	M	HQ	R	M
FPL 55712 (n = 5–7)	+8±3*	+17±6*	+6±3	+4±6	−6±6	+4±5	+10±8
LY 171883 (n = 6–7)	+2±2	+24±2†	−6±6	+7±4	−19±2*	+9±8	−4±4

Values are means ± SE measured 1 and 15 min after intravenous administration of FPL 55712 (10 mg/kg) and LY 171883 (30 mg/kg), respectively. ΔMAP represents change in mean arterial pressure. Changes in blood flow vascular resistance are expressed as %changes from control levels (%Δ). n, no. of rats in each group. HQ, hindquarter; R, renal artery; M, mesenteric artery. * and † denote $P < 0.05$ and $P < 0.01$, respectively for differences from control levels (ANOVA followed by SNK).

species (e.g., the domestic pig), LTD_4 was shown to produce a vasodilator response in vivo that was mediated by a platelet-dependent factor which was not an arachidonate metabolite (7, 20). Moreover, recent studies showed endothelium-dependent relaxing capacity for LTD_4 on precontracted blood vessels (28). Again, the relaxing activity of LTs in such preparations were independent of arachidonate metabolites. Thus it is possible that the dilator effect of LTs D_4 and E_4 on HQ vessels in our study might be mediated by a still unknown dilator factor derived from blood-borne or endothelial cells.

It was previously suggested that several LT receptors mediate the various effects of LTs on various cells and organs (12). It was also suggested that vascular receptors to LTs are differentially distributed in various vascular beds (2, 4, 8, 25, 29). The similar pattern of the vasoconstrictor response induced by LTD_4 and LTE_4 in our study may suggest activation of one type of vascular receptor to LTD_4. The somewhat greater potency of LTD_4 vs. LTE_4 suggests that this receptor is primarily a LTD_4 receptor. This is in accord with most of the previous studies that showed greater potency for LTD_4 vs. LTE_4 (3, 4, 8, 25). However, the initial dilator response at the HQ vascular bed of the conscious rat was the same for both LTD_4 and LTE_4 and suggests the existence of yet another type of a vascular receptor for these LTs. However, it should be pointed out that the dilator response to LTs in the HQ was not dose dependent, probably owing to the rapid onset of the constrictor phase (HQ2) at somewhat higher doses. This suggestion is supported by the demonstration that FPL 55712 did not effect this dilator response, though it significantly attenuated the constrictor responses.

The recently developed LT antagonist LY 171883 produced vasodilation in the HQ vascular bed. Therefore, its antagonistic action on both the dilator and constrictor responses induced by LTD_4 and LTE_4 in the HQ may be not entirely specific. Nevertheless, without affecting other vascular beds or MAP, LY 171883, in a relatively high dose, attenuated the pressor response induced by LTD_4 but not by LTE_4; the constrictor effects of both LTs on the M artery was also significantly suppressed. This may further support the suggestion that one type of LT receptor mediates their constrictor response and is in accord with previous studies that suggest activation of one receptor site for LTD_4 and LTE_4 (21) and showed the capacity of LY 171883 to antagonize both (11). However, it may also suggest that the pressor response induced by LTE_4 in the conscious rat is mediated by a different receptor from LTD_4. Moreover, if the attenuation of the HQ dilator response by LY 171883 for LTE_4 is rather specific, one could argue that the pressor, dilator, and constrictor effects of LTE_4 are all mediated by different receptors. Clarification of these possibilities awaits for more selective LT antagonists.

N-acetyl-LTE_4 has recently been found to be the predominant endogenous plasma metabolite of LTs after various trauma in the anesthetized rat (6). In the conscious rat, LTE_4 was equipotent to LTD_4 to cause a profound drop in M blood flow. Thus the metabolic process that converts LTC_4 to LTD_4 to LTE_4 in vivo (27) does not result in effective reduction of their overall deleterious hemodynamic potency. Therefore, LTE_4 might also have a role in the pathophysiological situations where LTs are released. The limited capacity of both FPL 55172 and LY 171883 to protect the mesenterium from the sustained ischemia by LTs in the conscious rat should encourage an active search for better LT antagonists.

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