Effects of Leukotrienes C₄, D₄, and E₄ on Cerebral Arteries of Newborn Pigs

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ABSTRACT. We examined effects of topical application of leukotrienes (LT) C₄, D₄, and E₄ on cerebral arteries of newborn pigs *in vivo*. Diameters of pial arteries were measured using a cranial window method during application of artificial cerebrospinal fluid without drug, and cerebrospinal fluid containing LT C₄, D₄, and E₄ (1, 10, 100, 1000, and 5000 ng/ml). Control diameters ranged from 51–345 μ m. All three LT constricted pial arteries in a dose-dependent manner, with a threshold for detectable response at 10 ng/ml ($7 \pm 3\%$ for LTD₄). The magnitude of constrictor response at the highest dose was $23 \pm 3\%$ for LTC₄, $17 \pm 2\%$ for LTD₄, and $17 \pm 3\%$ for LTE₄. The specific receptor antagonist FPL 55712 blocked the constrictor response to LT. We conclude that LT are potent constrictors of cerebral arteries in newborn pigs. (*Pediatr Res* 20: 973–976, 1986)

Abbreviations

CSF, cerebrospinal fluid
LT, leukotrienes

LT are a group of biologically active compounds that are derived from arachidonic acid via the 5-lipoxygenase pathway (1, 2). The peptidoleukotrienes (LTC₄, D₄, E₄, and F₄) are synthesized by various peripheral tissues in response to injury (1, 2) and have potent effects on vascular tone (3–6) and permeability (7–9). The LT also are synthesized by brain under a variety of pathological conditions (10, 11), but knowledge of the effects of these substances on cerebral vessels is limited and the results are contradictory (12–15). Further, effects of LT on the newborn cerebral circulation have not been investigated. The risk of cerebral trauma during the perinatal period is considerable. LT have potent effects on the pulmonary and systemic circulations in neonatal animals (16). The purpose of this study was to determine effects of LTC₄, LTD₄, and LTE₄ on pial arteries of newborn pigs. Pial arteries are important resistance vessels in the cerebral circulation (17).

MATERIALS AND METHODS

Fifteen piglets (four animals were 1 day old, six were 2 days old, three were 3 days old, and two were 5 days old; 1.1–2.3 kg)

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of either sex were used in these experiments. Piglets were anesthetized with ketamine hydrochloride (33 mg/kg, intramuscular) and acepromazine (3.3 mg/kg, intramuscular), and were maintained on α -chloralose (30–50 mg/kg initially, supplemented with 5 mg/kg/h, intravenous). Following a tracheostomy, a tube was placed into the airway and the animals were intubated and ventilated with room air using a Harvard small animal respirator. A catheter was inserted into a femoral artery to record blood pressure and to sample for blood gases and pH. Blood pressure was determined using a Statham pressure transducer, and the output was displayed on a Gould recorder. Another catheter was inserted into a femoral vein for injection of drugs and fluid. Body temperature was maintained at 37–38° C with water circulating heating pad.

For insertion of cranial window, the scalp was removed and an opening, approximately 2 cm in diameter, was made in the skull over the parietal cortex with bone rongeurs. The dura was cut and reflected over the cut bone edge. A cranial window, similar to that described by Levasseur *et al.* (18) was placed into the hole and cemented into place with dental acrylic. After the dental acrylic hardened, the space under the window was filled with artificial CSF. We used artificial CSF of the following composition: 220 mg KCl, 132 mg MgCl₂, 221 mg CaCl₂, 7710 mg NaCl, 402 mg urea, 665 mg dextrose, 2066 mg NaHCO₃ per liter; pH = 7.33; PCO₂ = 46 mm Hg; PO₂ = 43 mm Hg. Previously we have shown that this artificial CSF does not affect resting pial arterial diameter in piglets (19).

Pial arteries were observed with a Wild M7S trinocular stereomicroscope, television camera (model VC-65SL, Dage-MIT, Michigan City, IN) mounted on the microscope, and video monitor (model CT-1930V, Panasonic Corporation Secaucus, NJ). We selected one artery exposed by the craniotomy in the range of 150–345 μ m and, in some cases, a smaller artery (< 150 μ m) in the viewing field. We never studied more than two vessels per animal. Vascular diameter was measured with a video microscaler (model VPA-1000, For-A-Corp, Los Angeles, CA).

Protocol. Pial arterial diameter was determined 2–4 min following infusion under the window of artificial CSF containing no drug and after infusion of CSF containing 1, 10, 100, 1000, and 5000 ng/ml of LTC₄, (eight vessels from five animals), LTD₄ (11 vessels from six animals), and LTE₄ (eight vessels from four animals). We also measured diameter 10–15 min after flushing the leukotrienes out with CSF containing no drug. We typically flushed 1–2 ml of CSF over 30 s through the window each time. Needles incorporated into the sides of the window allowed infusion of CSF under the window and run-off of excess CSF. One dose response curve to a single LT was performed per animal. We measured the peak response, which was constant over 2–4 min after administration of CSF containing leukotrienes. Subsamples of the synthetic leukotrienes were repeatedly subjected to high-pressure liquid chromatography to confirm that insignificant decomposition had occurred during storage (Fig. 1). Infusion of CSF without LT had no effect on diameter.

In order to determine whether constrictor responses to LT were prevented by LT receptor blockade, we determined effects of LTC₄ (six vessels from three animals) and LTD₄ (three vessels from two animals) at a dose of 5000 ng/ml before and after addition of FPL 55712 (1000–5000 ng/ml) to CSF. Following the application of leukotriene to the cortical surface, we reapplied this dose of LT with FPL 55712. No tachyphalaxis occurred over this time period. We also assessed effects of 10⁻⁴ M clonidine

HCl (Sigma, St. Louis, MO) and 10⁻⁴ M clonidine plus FPL 55612 (1000 ng/ml) on pial arteries (three vessels from two pigs).

Pial arterial diameter and arterial pressure in the dose-response experiment were analyzed using repeated measures analysis of variance. If the F value was significant, the Student-Newman-Kuels test was performed. The relationship between initial diameter and response to leukotrienes (all data from LTC₄, LTD₄, and LTE₄ at 5000 ng/ml were pooled) was analyzed using linear regression analysis and the unpaired *t* test. Control diameter was compared with recovery diameter (after leukotrienes) using paired *t* tests. Effectiveness of receptor blockade was analyzed using a paired *t* test, comparing percent change from control in the absence of FPL 55712 and in the presence of this antagonist. Control diameter was compared to 10⁻⁴ M clonidine with and without FPL 55712 using paired *t* tests. α -Level of *p* < 0.05 was considered significant in all statistical tests. Values are presented as means $\pm$ SEM of raw values or as percent change from control values.

RESULTS

Infusion of LT in CSF under the cranial window did not affect arterial blood pressure (Table 1).

LT C₄, D₄, and E₄ had dose-dependent constrictor effects on pial arteries (Table 1; Fig. 2). At the lowest dose (1 ng/ml), LT did not reduce arterial diameter significantly. At 10 ng/ml, LTD₄ but not LTC₄ and LTE₄, constricted pial arteries. At the higher doses, all three LT constricted pial arteries (LTC₄: 100 ng/ml < 1000 ng/ml = 5000 ng/ml) (LTD₄: 10 ng/ml < 1000 ng/ml = 5000 ng/ml) (LTE₄: 100 ng/ml = 1000 ng/ml < 5000 ng/ml). Magnitude of responses to the three LT was not different statistically except at 1000 ng/ml, where LTC₄ > LTE₄ (Fig. 2). Recovery diameter was 156 $\pm$ 42 μ m for LTC₄ (significantly different from initial control, see Table 1), 155 $\pm$ 24 μ m for LTD₄ (nonsignificant compared to control), and 122 $\pm$ 27 μ m (nonsignificant compared to control).

Initial control diameter of the pial arteries examined had a modest bearing on the magnitude of the constrictor response to LT. Small (< 150 μ m) pial arteries constricted to a slightly greater extent than large arteries (150–345 μ m) (22 $\pm$ 2%; *n* = 14 versus 15 $\pm$ 2%; *n* = 13, for small and large arteries, respectively). For pooled data for all three LT at 5000 ng/ml, the correlation coefficient (initial diameter/percent change in diameter) was 0.46 (*p* < 0.05, compared to zero; *n* = 27) and the equation of the regression line was Y (% change) = 0.04 (initial diameter) – 25.

Fig. 1. High-pressure liquid chromatograms of synthetic LT. The purity of each LT [LTC₄ (1 μ g), LTD₄ (2 μ g), and LTE₄ (2 μ g)] was examined using an isocratic high-pressure liquid chromatography system. The system consisted of a u-Bondapak C18 column (3.9 mm $\times$ 30 cm; Waters Assoc., Milford, MA) using a buffer of 45% acetonitrile: 55% water (containing 0.1% acetic acid; buffer pH adjusted to 5.7 with ammonium hydroxide). The solvent was delivered with a Waters liquid chromatography pump (model 6000A; Waters Assoc.). The leukotrienes were detected spectrophotometrically at 280 nm (AUFS = 0.1) using an UV detector (model 450, Waters Assoc.) and recorded on a chart recorder (Series 5000; Fisher Scientific, Lexington, MA) at a constant paper speed of 5 mm/min.

Table 1. Effects of LT on pial arterial diameter in newborn pigs (mean $\pm$ SEM)

	Control	1 ng/ml	10 ng/ml	100 ng/ml	1000 ng/ml	5000 ng/ml
A. LTC₄						
Arterial diameter (μ m)	173 $\pm$ 46	168 $\pm$ 44	164 $\pm$ 44	158 $\pm$ 42*	144 $\pm$ 40*	140 $\pm$ 40*
Arterial blood pressure (mm Hg)	64 $\pm$ 3	61 $\pm$ 3	61 $\pm$ 3	61 $\pm$ 3	60 $\pm$ 3	62 $\pm$ 4
<i>n</i> = 8 vessels						
B. LTD₄						
Arterial diameter (μ m)	159 $\pm$ 24	155 $\pm$ 24	149 $\pm$ 23*	144 $\pm$ 23*	140 $\pm$ 22*	136 $\pm$ 23*
Arterial blood pressure (mm Hg)	59 $\pm$ 5	58 $\pm$ 4	60 $\pm$ 4	61 $\pm$ 3	60 $\pm$ 4	59 $\pm$ 4
<i>n</i> = 11 vessels						
C. LTE₄						
Arterial diameter (μ m)	136 $\pm$ 30	130 $\pm$ 29	128 $\pm$ 28	122 $\pm$ 28*	118 $\pm$ 26*	112 $\pm$ 26*
Arterial blood pressure (mm Hg)	74 $\pm$ 2	72 $\pm$ 3	72 $\pm$ 3	71 $\pm$ 4	71 $\pm$ 4	71 $\pm$ 4
<i>n</i> = 8 vessels						

* *p* < 0.05 compared to control. Means for each drug which were not significantly different from each other are underlined.

For LTC₄, arterial blood pH = 7.31 $\pm$ 0.04, PCO₂ = 34 $\pm$ 2 mm Hg, PO₂ = 88 $\pm$ 3 mm Hg. For LTD₄, arterial blood pH = 7.38 $\pm$ 0.03, PCO₂ = 30 $\pm$ 2 mm Hg, PO₂ = 74 $\pm$ 3 mm Hg. For LTE₄, arterial blood pH = 7.35 $\pm$ 0.04, PCO₂ = 35 $\pm$ 2 mm Hg, PO₂ = 76 $\pm$ 4 mm Hg.

Fig. 2. Effects of LTC₄, D₄, and E₄ on pial arterial diameter. Values are mean $\pm$ SEM. Sample sizes are in parentheses. * $p < 0.05$, compared to control values. † $p < 0.05$, compared to response to LTE₄.

Fig. 3. Effects of leukotrienes (5000 ng/ml) alone or leukotrienes (5000 ng/ml) plus FPL 55712 (1000–5000 ng/ml) on pial arterial response to combined data for LTC₄ ($n = 6$ vessels) and D₄ ($n = 3$ vessels). * $p < 0.05$, compared to control. † $p < 0.05$, compared to response after FPL 55712.

The specific receptor antagonist FPL 55712 was effective in blocking pial arterial constriction to leukotrienes (Fig. 3). For these experiments, diameter was $189 \pm 40 \mu\text{m}$ (arterial pressure = $63 \pm 3 \text{ mm Hg}$) during control, $168 \pm 38 \mu\text{m}$ (arterial pressure = $59 \pm 5 \text{ mm Hg}$) in the presence of LTC₄ or D₄, and $186 \pm 39 \mu\text{m}$ (arterial pressure = $58 \pm 5 \text{ mm Hg}$) in the presence of LTC₄ or LTD₄ plus FPL 55712. In three vessels from two other animals, diameter during control was $173 \pm 14 \mu\text{m}$, was significantly reduced to $138 \pm 15 \mu\text{m}$ during 10^{-4} M clonidine, and was still significantly reduced to $150 \pm 16 \mu\text{m}$ during 10^{-4} M clonidine plus FPL 55712.

DISCUSSION

The present study demonstrates that the peptidoleukotrienes C₄, D₄, and E₄ have potent constrictor effects on pial arteries of newborn pigs. Each of these LT lead to a dose-dependent decrease in diameter, and responses were blocked by the specific antagonist FPL 55712. These results, indicating that the responses resulted from activation of LT receptors, provide direct evidence that pial arteries are responsive to products of 5-lipoxygenase in the immediate neonatal period.

LT have been shown to constrict resistance vessels in coronary (3, 5, 6), pulmonary (16), and mesenteric (4) circulations, although contradictory evidence has been reported (4, 20). In addition, LT also have been reported to increase vascular perme-

ability in skin (7–9, 21). The effects of LT on the cerebral circulation have not been studied extensively, and the results are inconclusive. Von Holst *et al.* (15) found that LTC₄ and LTD₄ had no effect on human cerebral arteries *in vitro*. In contrast, Tagari *et al.* (14) reported that human cerebral arteries contracted in response to LTD₄, but this response was not blocked by FPL 55712, implying that the response was not due to activation of the LT receptors. Tagari *et al.* (14) also found that LTD₄ constricted rat intracranial arteries. Rosenblum (13) found that LTB₄, C₄, and D₄ constricted small (30–45 μm) pial arteries by 7–13% in mice. There was no evidence of tachyphylaxis, and constrictor responses to LTC₄ were attenuated by FPL 55712. Recently, Kamitani *et al.* (12) found that LTC₄ and LTD₄ did not constrict small (32–82 μm) pial arteries of rabbits, while LTB₄ constricted these vessels by ~5%. In our experiments, we looked at responses of pial arteries over a large range of sizes (*i.e.* 51–345 μm initial diameter) and found that LTC₄, D₄, and E₄ constricted these vessels from 17–23% at the highest doses. This response is relatively large, as we have found that arterial hypertension and exogenous norepinephrine ($2 \times 10^{-4} \text{ M}$) constrict piglet pial arteries by 10 and 15%, respectively (19). On the basis of Poiseuille's law, constriction of the magnitude induced by LT would have substantial effects on resistance. We also found that the threshold for detectable constriction was 10 ng/ml for LTD₄. The present report is the first one to document that LTE₄ is a constrictor agent in the cerebral circulation. Potency was LTC₄ = LTD₄ and LTC₄ > LTE₄. We also examined the relationship between initial diameter and response to LT. There was a modest tendency for small pial arteries (< 150 μm) to constrict to a greater extent than large arteries (150–345 μm), but this difference was only about 7%. It should be emphasized that pial arteries of the sizes used in the present study are important resistance vessels in the cerebral circulation (17). The reason for differences in findings is unclear, but may be due to different methods of administration of LT (intraarterial *versus* topical application), different doses, and species variation. Our results are consistent with preliminary findings of Piper and Stanton (22), in which they report that LTC₄ and LTD₄ reduced intracranial blood flow in juvenile pigs.

It has been reported that LT can be synthesized in brain during pathological conditions such as cerebral ischemia, subarachnoid hemorrhage, and concussive brain injury, and levels of LT can reach as high as 12 ng/g of brain (10, 11). We have found that LT begin to constrict cerebral arteries at doses as low as 10 ng/ml CSF. The risk of cerebral trauma during the perinatal period is considerable. Infants can become hypoxic-ischemic at birth, and a large percentage of low birth weight ($\leq 1000 \text{ g}$) infants suffer from intraventricular hemorrhages. Under conditions of cerebrovascular stress in neonates, LT may be released, which would have possible detrimental effects on the cerebral circulation.

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