Constricting Effect of Leukotrienes on Cerebral Arterioles of Mice

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SUMMARY Three leukotrienes (LTB₄, C₄, D₄) were locally applied to cerebral arterioles in doses varying from 3 × 10⁻⁸ to 4 × 10⁻⁷ M and each produced a dose related constriction. LTC₄ was tested in the presence and absence of FPL-55712, a relatively specific inhibitor of leukotriene receptors. A low dose (10⁻⁸M) of FPL-55712 did markedly reduce the response to the LTC₄ and did not significantly alter an equally pronounced constriction produced by norepinephrine. The constricting properties of the leukotrienes shown by our data support the suggestion that these products of lipoxygenase's action on arachidonic acid have the potential to modify cerebrovascular tone. This potential may be important in view of recent evidence showing that the brain can make significant amounts of leukotrienes from arachidonic acid.

LEUKOTRIENES are a group of polyunsaturated fatty acids synthesized from arachidonic acid by lipoxygenase. They represent, therefore, the product of enzymatic activity in competition with cyclooxygenase and other enzymes which produce prostaglandins (PG) and thromboxane (TX) from arachidonate. While the action of PG and TX on cerebral vessels has been studied (e.g.,) the action of leukotrienes has been given scant attention even though lipoxygenase products including leukotrienes are produced in abundance by brain. The action of leukotrienes on cerebral vessels are of potential importance for several reasons: They are known to be vasoactive in other vascular beds; they may be produced by inflammatory cells and consequently could mediate vascular response in inflammation, their synthesis may be enhanced if the competitive pathway for arachidonate utilization is blocked, as by nonsteroidal antiinflammatory agents used in the treatment of transient ischemic attacks. The following report describes the contractile action of 3 different leukotrienes.

Methods

Male mice, ICR strain were anesthetized with urethane, and their pial arterioles were exposed and observed with a Leitz Ultropak microscope as previously described. In each mouse a single arteriole between 30 and 45 μm in diameter (M ± SD = 36 ± 3 μm) was arbitrarily chosen for monitoring, and its diameter was continuously measured with an image splitter and TV microscope also as previously described. With this technique repeated measurements of the same vessel give identical results (i.e. the mean of 10 consecutive measurements is not significantly different from the mean of the next 10 measurements), and differences as small as 0.5 microns can be detected. Thus for vessels of the size used in this study a random error of approximately ± 1.5% of resting diameter would be expected. This contrasts with the consistent constriction produced by leukotrienes as shown in our results. The preparation was continuously irrigated with artificial cerebrospinal fluid (CSF) at pH 7.35 and 37°C. Drugs such as leukotrienes were topically applied for 30 seconds in a ml bolus of artificial CSF at the same pH and temperature. Different doses, when applied sequentially to a single animal, were applied in random order. This minimizes the chance of a systematic effect of time on the dose-response relationship as the preparation ages. However this precaution was really unnecessary since preliminary studies showed no effect of time on the response to a dose applied 3 times at 15 minute intervals. Thus there was no fatigue of the preparation. Also, the response to a given dose was independent of its place in the sequence. Vessels fully recovered during the 15 minute wash out period. At the end of each experiment the arterial O₂, CO₂ and pH of each mouse was determined with a Radiometer blood gas analyzer. The mean (± SD) values for these parameters were 109 ± 8 mm Hg, 29 ± 3 mm Hg and pH = 7.34 ± 0.03.

The leukotrienes used here were donated by Dr. P. Sheard, of Fisons Pharmaceuticals (Leicestershire, England). LTB₄, LTC₄ and LTD₄ were used in separate studies. In some studies the leukotriene receptor blocker FPL 55712 was also used. FPL 55712 was donated by Dr. A. Taub, Fisons Corp, Bedford, MA. Finally, in order to test the specificity of the blocker, studies of its action on norepinephrine were performed.

Results

A total of 15 mice were used to test the effect of the leukotrienes. The leukotrienes we tested always elicited a constriction even at the lowest doses cited below.

LTB₄ was tested in 5 mice. Three doses were applied to the pial surface of each mouse, 15 minutes apart, in randomized order. The doses were 3 × 10⁻⁸, 1.5 × 10⁻⁷ and 3 × 10⁻⁷ M. They produced dose dependent constriction equivalent to 6 ± 3, 9 ± 14 and 13 ± 11% (M ± SD) of resting diameter (p < .05 for dose effect on analysis of variance; diameter 38 ± 3 μm).

LTC₄ was tested in 3 mice at a dose of 3 × 10⁻⁷ M and reduced diameter by 20 ± 5%, while a lower dose (3 × 10⁻⁸ M) used in 3 other mice reduced constriction by 7 ± 3%. Diameter in these mice was 33 ± 4 μm.

LTD₄ also constricted pial arterioles. Five mice were tested, each receiving 4 × 10⁻⁸, 2 × 10⁻⁷ and 4
× 10^{-7} M fifteen minutes apart in randomized order. Again a dose-response relationship was found, with diameter reduced by 5 ± 2, 8 ± 1 and 11 ± 6% respectively (p < .01 for dose effect on analysis of variance; diameter 38 ± 2 μm).

Following the experiments above, additional studies were performed using FPL 55712, an antagonist of leukotriene action. In five mice 1.6 × 10^{-7} M LTC_4 constricted diameter (35 ± 5 μm) by 18 ± 6% (M ± SD). This constriction was reduced in the same mice, to only 7 ± 6% when the LTC_4 was applied together with 10^{-8} M FPL 55712. The mean difference between the unblocked and blocked response was 11 ± 7%, and was significant (paired "t" test) at the 0.03 level. The same dose of FPL 55712 (10^{-8} M) failed to alter the response to 5 × 10^{-6} norepinephrine. The latter produced a constriction of 17 ± 3% which was similar to that produced by LTC_4. In the presence of 10^{-8} M FPL 55712, constriction was 14 ± 6%, the mean difference was only 3 ± 4% and was not significant even at the 0.10 level.

Discussion

Leukotrienes are a group of arachidonic acid metabolites which include the powerful bronchoconstrictor once called the "slow reacting substance of anaphylaxis." They have potent vasoactive actions as well, but these may differ in different vascular beds. For example LTC_4, D_4 and E_4 constrict dog mesenteric artery but LTC_4 and D_4 increase renal blood flow in the dog. In the present study dose dependent constriction was observed in the pial arterioles of the mouse. All three leukotrienes used here were at least as potent as either norepinephrine or serotonin. For example diameter was reduced 17% by 5 × 10^{-7} M norepinephrine and (data not presented) 12% by 2.5 × 10^{-6} M serotonin, while lower doses of LTB_4 (3 × 10^{-7} M), C_4 (3 × 10^{-7} M) and D_4 (4 × 10^{-7} M) constricted vessels by 20%, 13% and 11% respectively. The action of LTC_4 was markedly inhibited by FPL-55712 a drug considered to be a specific blocker of leukotriene receptors. A low dose of this drug (10^{-8} M) which did markedly reduce the response to LTC did not significantly reduce the constriction produced by norepinephrine. However much higher doses (10^{-7} M) did inhibit the response to norepinephrine (data not shown), indicating only a relative specificity for the leukotriene receptor. Similar nonspecific inhibitory effects of high doses of FPL-55712 have been reported by others.

We did not test FPL-55712 against LTB_4 or LTD_4 and therefore can make no statements concerning its efficacy as an inhibitor of these leukotrienes in our model.

Since the brain is capable of making large amounts of lipooxygenase products including leukotrienes from arachidonate, others have suggested that leukotrienes might function as mediators of cerebrovascular tone. Our demonstration of vasoconstriction produced by three different leukotrienes provides direct evidence in support of this possibility. The possible action of leukotrienes should be kept in mind whenever cyclooxygenase blockers like aspirin or indomethacin are employed since blockade of cyclooxygenase may shunt any available arachidonic acid to the lipoxygenase pathway. The existence of this shunting effect has been shown in platelets, for example. Whether it also occurs in brain remains a subject for investigation.

References

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