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Short communication

N-Acetyl-leukotriene E_4 is a potent constrictor of rat mesenteric vessels

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N-Acetyl-leukotriene E_4 administered to conscious freely moving rats produced a dose-dependent vasoconstriction in the mesenteric vessels which led to profound reduction of blood flow to the gut. Renal and hindquarter blood flow and vascular resistance were not affected even by high doses of N-acetyl-leukotriene E_4. N-Acetyl-leukotriene E_4 was 10-fold more potent than the thromboxane analog U-46619 and 1000-fold more potent than prostaglandin $F_{2\alpha}$ but 2-5-fold less potent than leukotriene D_4/E_4 to induce mesenteric vasoconstriction. These data indicate that N-acetyl-leukotriene E_4 is a biologically active metabolite of peptide leukotrienes, and might play a role in cardiovascular derangements mediated by leukotrienes.

Peptide-leukotrienes; N-Acetyl-leukotriene E_4; Prostaglandins; Mesenteric circulation; Anaphylactic shock

1. Introduction

N-Acetyl-leukotriene (N-Ac-leukotriene) E_4 is a recently discovered metabolite of the cysteinyl leukotrienes, leukotriene C_4, D_4 and E_4 (Denzlinger et al., 1985). Although this leukotriene metabolite has been discovered so far only in the bile of some species (e.g. rat), an enterohepatic cycle for leukotriene has already been demonstrated (Denzlinger et al., 1986) which provides a route for leukotriene metabolites such as N-Ac-leukotriene E_4 access to the systemic circulation. The biological significance of such a cycle would however depend on the degree of the biological activities preserved in the leukotriene metabolites.

We have previously reported that leukotrienes have profound vasoconstrictor effect on selected peripheral blood vessels of the rat, especially on the coronary (Zukowska-Grojec et al., 1985) and the mesenteric (Eimerl et al., 1986) circulation. Therefore, we have postulated that if N-Ac-leukotriene E_4 would have any appreciable vascular activity in the rat it would be apparent in the mesenteric circulation.

This hypothesis was tested in the conscious rat in which Doppler flow probes were implanted on the renal and superior mesenteric vessels as well as on the lower abdominal aorta for hindquarter blood flow measurement. Using this technique is the only way to obtain on line, continuous measurements of blood flow changes in the conscious rat for substances which produce instant and short lasting responses.

2. Materials and methods

2.1. Animal preparation

The rats were anesthetized with an intramuscular injection of ketamine (130 mg/kg) and acepromazine (1.3 mg/kg). Miniaturized Doppler flow probes (Valpey-Fisher, MA) were implanted

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to measure hindquarter blood flow, renal blood flow and superior mesenteric blood flow according to the method of Haywood et al. (1981). Twenty-four hours before the experiment, polyethylene catheters (PE50) were inserted into the femoral vessels as described earlier (Eimerl et al., 1986).

Seven days after the flow probe implantation the rat was connected to the flow probe connectors and the arterial line was connected to a pressure transducer (Narco RP1500i). The blood pressure (mean, systolic, diastolic) and heart rate continuously recorded on the computerized Narcotrace 80 physiograph. The regional blood flow was measured with a pulsed Doppler flowmeter (University of Iowa Bioengineering Facility, Model 545c-3) and the blood flow recordings continuously monitored on the Narcotrace 80 physiograph. Vascular resistance was calculated by dividing the mean arterial pressure by blood velocity (Doppler shift in kilohertz). Changes in blood flow and vascular resistance are expressed as a percent of control values.

2.2. *Drugs*

Pure synthetic cysteinyl leukotrienes were provided by Merck-Frosst, Canada. Prostaglandin $F_{2\alpha}$ was purchased from Sigma and the stable throm-

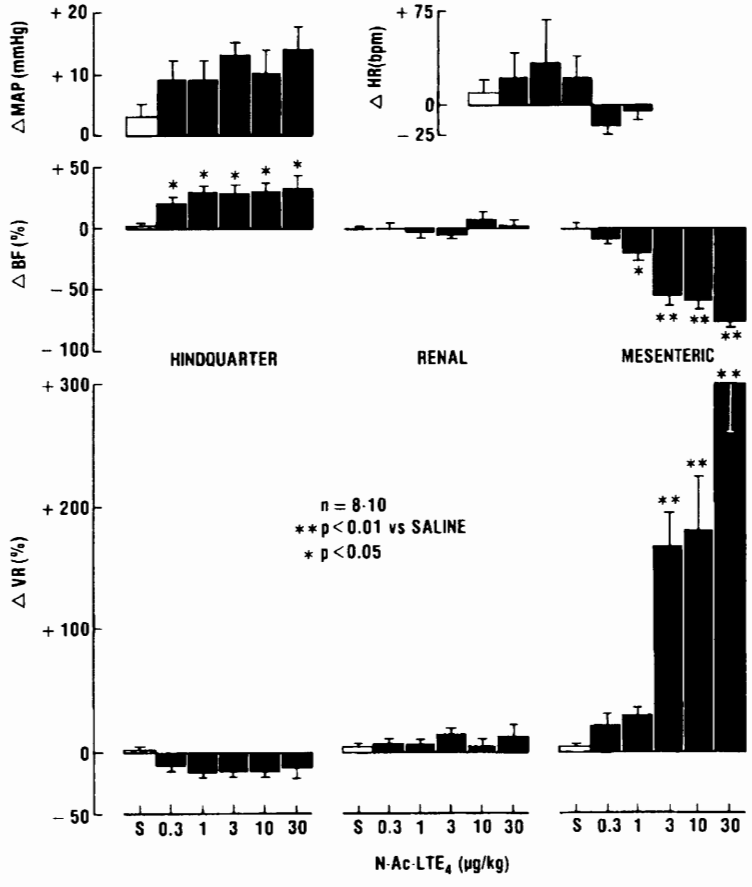

Fig. 1. Effect of N-Ac-leukotriene E_4 on hemodynamic variables in the conscious rat. Saline (25 μl/100 g) or increasing doses of N-Ac-leukotriene E_4 were injected i.v. at 20 min intervals. Values indicate mean ± S.E.M. N = number of animals. MAP = mean arterial pressure, HR = heart rate, bpm = beats per min, BF = blood flow, VR = vascular resistance, S = saline. Asterisks indicate statistical significance from S by Student-Newman-Keuls test.

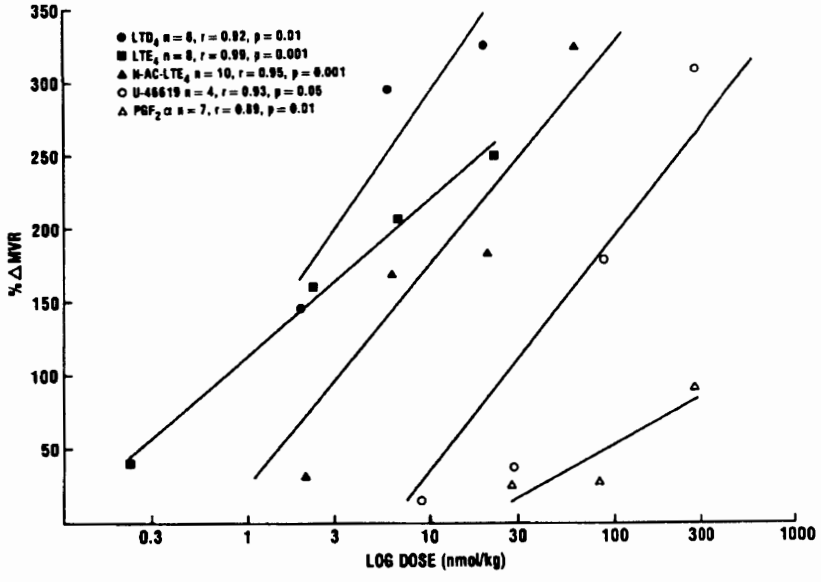

Fig. 2. Log-dose relationship of the mesenteric vasoconstrictor potencies of various leukotrienes, thromboxane analog U-46619 and prostaglandin $F_{2\alpha}$ in the conscious rat. Increasing doses of leukotrienes, U-46619 and prostaglandin $F_{2\alpha}$ were injected i.v. at 20 min intervals. %ΔMVR = percent change in mesenteric vascular resistance. N = number of animals, r = correlation coefficient, p = probability.

boxane analog U-46619 (9,11-dideoxy-9α,11a-methanoepoxy-prostaglandin $F_{2\alpha}$) from Cayman Chemical. The leukotrienes and prostaglandins were stored at −80°C and thawed only once prior to administration in 200 μl of sterile 0.9% NaCl. Saline, leukotrienes and prostaglandins were injected i.v. in a volume of 25 μl/100 g body weight.

2.3. Statistics

All data in text and figures are mean values ± S.E. for the indicated number of rats. ANOVA followed by the Student-Newman-Keuls test for multiple range comparisons was used along with linear regression analysis of the peak responses of the various leukotrienes.

3. Results

N-Ac-leukotriene E_4 produced only a slight increase in the mean arterial pressure (5-10 mm Hg) and no consistant change in heart rate, renal blood flow or renal vascular resistance up to the highest dose used (fig. 1). However, a dose-dependent vasoconstriction was demonstrated in the mesenteric circulation which reached a maximum of +323 ± 55% at the highest dose. When N-Ac-leukotriene E_4 is compared to leukotriene D_4, leukotriene E_4 thromboxane analog U-46619 and prostaglandin $F_{2\alpha}$ (fig. 2) it is 2.1- and 5-fold less potent than leukotriene E_4 and leukotriene D_4 in doubling mesenteric vascular resistance, respectively. N-Ac-leukotriene E_4 is however, approximate 10-fold more potent than U-46619 and 1000-fold more potent then prostaglandin $F_{2\alpha}$ in its capacity to constrict the mesenteric circulation. Also, if the time for blood flow recovery is considered, the area score of N-Ac-leukotriene E_4 effect at 10 μg/kg is 7.6 ± 0.9 Hz · s while that of the same dose of leukotriene D_4 is 4.9 ± 0.8 Hz · s ($P < 0.05$, $n = 6$). All the eicosanoids were, however, far less potent than vasopressin which at doses of 0.03-0.1 μg/kg induced a strong increase in mesenteric vascular resistance with peak responses of +1145 ± 859 and +2177 ± 1164%, respectively.

The hindquarter blood flow showed a slight but significant (up to 30%) increase after N-Ac-leukotriene E_4 administration but the increase in hindquarter blood flow was primarily due to the small increase in mean arterial pressure since hindquarter vascular resistance tended to decrease although not significantly (fig. 1). High doses (30-100 μg/kg) of U-46619 induced pressure and renal vasoconstrictor responses but had no effect on hindquarter blood flow. Prostaglandin $F_{2\alpha}$ at the highest dose (100 μg/kg) increased hindquarter blood flow ($+93 \pm 19\%$) and decreased hindquarter vascular resistance ($-42 \pm 10\%$). This dose of prostaglandin $F_{2\alpha}$ also decreased renal blood flow ($-32 \pm 6\%$) with no significant effect on renal vascular resistance. It also first decreased (-31 ± 3 mm Hg) and then increased ($+26 \pm 3$ mm Hg) mean arterial pressure.

4. Discussion

The data presented in this report clearly demonstrates that N-Ac-leukotriene E_4 is a biologically active metabolite of the peptide-leukotrienes. The potency of N-Ac-leukotriene E_4 (measured as peak response) was 2- and 5-fold less active than leukotriene E_4 and D_4 respectively but due to a somewhat prolonged action the overall activity of N-Ac-leukotriene E_4 in the mesenteric circulation is comparable to that of leukotriene D_4. However, N-Ac-leukotriene E_4 showed no constrictive activity in the hindquarter or renal circulation while leukotriene D_4 is clearly active in constricting these blood vessels at 1/10th of the maximal dose of N-Ac-leukotriene E_4 used in this study (Eimerl et al., 1986). This difference between leukotriene D_4 and N-Ac-leukotriene E_4 might point towards some variance in leukotriene-receptors in different blood vessels even in the same species.

The effects of N-Ac-leukotriene E_4 shown in our study differ from those described in the pig (Foster et al., 1986). In the latter species N-Ac-leukotriene E_4 was found to be virtually inactive in modifying cardiovascular and respiratory parameters. However, N-Ac-leukotriene E_4 is not formed in the pig (Ezra et al., 1987) and therefore the biological actions of N-Ac-leukotriene E_4 in the pig are anyway of little biological significance. In the rat, where N-Ac-leukotriene E_4 is a major leukotriene metabolite (Denzlinger et al., 1985) re-entering to the plasma of this biologically active metabolite could account for the prolonged mesenteric vasoconstriction associated with acute systemic anaphylaxis in this species (Zukowska-Grojec and Feuerstein, 1985). Furthermore, one cannot exclude the possibility of additional formation of N-Ac-leukotriene E_4 in the plasma from leukotriene E_4 absorbed from the bile. This possibility however awaits further clarification.

The data presented in this report also raise the question of which of the leukotriene metabolites is a non active end product. Recent studies point to the formation of several ω-oxidation products of leukotriene E_4 but further studies would be necessary to examine their biological activities.

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