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Mesenteric vascular responses to i.v. administration of lipoxin A_4 and lipoxin B_4 in the conscious rat

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Lipoxin A_4 and lipoxin B_4 are newly discovered lipoxygenase-interacting products of leukocytes which might have a role in cardiovascular events associated with anaphylaxis. We have tested this possibility by systemic administration of both LXA_4 and LXB_4 to the conscious rat while monitoring systemic and regional hemodynamic changes. LXA_4 and LXB_4 (1–100 μg/kg) produced dose-dependent constriction of the mesenteric vessels, up to $+123 \pm 23\%$ and $+50 \pm 9\%$ for LXA_4/B_4, respectively. Dose-related changes were not observed in arterial blood pressure, heart rate, renal (LXB_4) and hindquarter blood flow. We suggest that LXA_4 and LXB_4 might affect selective vascular beds, such as the mesenteric vessels, and contribute to variations in blood flow in specific pathophysiological states.

Lipoxin; Anaphylaxis; Mesenteric circulation; Renal circulation; Icosanoid

1. INTRODUCTION

Lipoxin A (LXA_4) and lipoxin B_4 (LXB_4) are lipoxygenase-interacting products of the following respective structure: 5*S*,6*R*,15*S*-trihydroxy-7,9,13-*trans*-11-*cis*-eicosatetraenoic acid and 5*S*,14*R*,15*S*-trihydroxy-6,10,12-*trans*-8-*cis*-eicosatetraenoic acid [1–3]. LXA_4 is produced by activated eosinophil leukocytes [4] and LXA_4 and LXB_4 by polymorphonuclear (PMN) leukocytes [5]. LXA_4 was shown to induce neutrophil chemotaxis, superoxide production, contraction of guinea pig lung strips, arteriolar dilation in the hamster cheek pouch vessels and rat glomerular afferent arterioles [5]. Furthermore, LXA_4 and LXB_4 were shown to inhibit NK-cell activity [3]. Of special interest are recent studies showing activation of protein kinase C by LXA_4 [6] suggesting an intracellular role in signal transduction.

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While these data support a biological role for LXA_4 and LXB_4 in multiple organs and systems, little information is available on the discrete actions of lipoxins on blood vessel tone in vivo. The potential modulation by LXA_4 and LXB_4 of organ blood flow might be of special interest, since LXA_4 is generated by activated eosinophils, which are the source of massive production and release of eicosanoids in acute systemic anaphylaxis. In this latter reaction, we have recently shown a marked decrease in splanchnic organ blood flow which persisted beyond the systemic hypotension [7]. A systematic search for the potential arachidonate mediator(s) of blood flow derangements in acute anaphylaxis yielded only partial information indicating that cysteinyl-leukotrienes ($LTC_4/D_4/E_4$) might be involved in the prolonged mesenteric vasoconstriction [8]. Yet other lipoxygenase products of arachidonate might play a role in this shock syndrome since LTs cannot reproduce all the hemodynamic responses of acute anaphylaxis nor can 5-lipoxygenase inhibitors completely reverse these phenomena [9].

The discovery and further availability of pure synthetic LXA_4 and LXB_4 prompted us to examine

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Table 1

Baseline levels of hemodynamic variables before LXA_4 and LXB_4 administration in the conscious rat

Group	MAP (mmHg)	HR (bpm)	Blood flow (kHz)			Resistance (mmHg/kHz)		
			HQ	R	M	HQ	R	M
Vehicle i.v.	124 ± 5	395 ± 14	6.3 ± 0.9	6.2 ± 0.7	6.1 ± 0.8	25 ± 7	19 ± 2	21 ± 3
LXA_4 i.v.	123 ± 5	390 ± 14	6.4 ± 0.9	6.5 ± 0.8	7.0 ± 0.6	24 ± 6	19 ± 2	18 ± 1
LXB_4 i.v.	119 ± 8	380 ± 15	6.2 ± 1	6.9 ± 0.6	7.1 ± 0.9	25 ± 7	18 ± 1	18 ± 3

MAP, mean arterial pressure; HR, heart rate; bpm, beats per min; HQ, hindquarter; R, renal; M, mesenteric

whether LXA_4 or LXB_4 can produce the type of mesenteric, renal and hindquarter circulatory changes produced by acute systemic anaphylaxis.

2. MATERIALS AND METHODS

Male Sprague-Dawley rats (275–300 g, Taconic Farms, Germantown, NY) were anesthetized with an intramuscular injec-

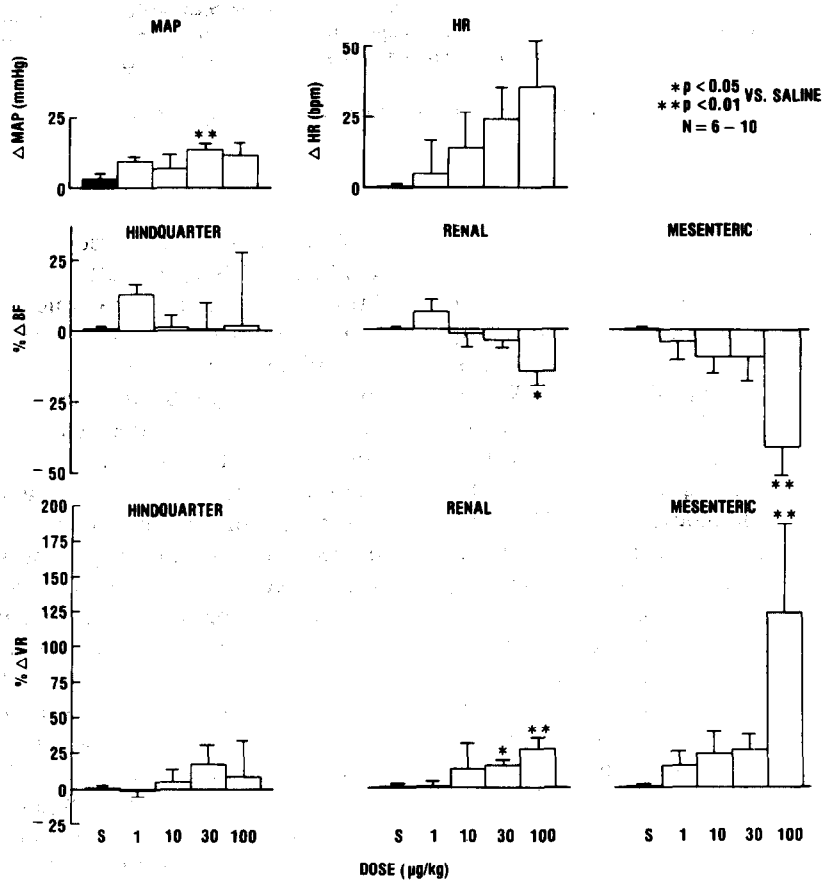

Fig.1. Effect of LXA_4 on systemic and regional hemodynamic variables in the conscious rat. Black columns represent the effect of the vehicle injection while blank columns represent the various doses of LXA_4 from 1 to 100 μg/kg (abscissa). %ΔVR, percent change in vascular resistance; %ΔBF, percent change in blood flow for the respective organ; ΔMAP, change in mean arterial pressure; ΔHR, change in heart rate; *N*, number of rats studied. Asterisks represent statistical significance by ANOVA followed by Student-Newman-Keul test as indicated in the figure.

tion of ketamine (130 mg/kg) and acepromazine (1.3 mg/kg) and Doppler flow probes implanted on the left renal artery, superior mesenteric artery and the lower abdominal aorta, through a mid-laparotomy incision [10]. The rats were allowed to recover for 5–7 days. 24 h prior to the experiment the rats were anesthetized again (2% halothane in oxygen) and PE-50 tubing inserted into the left femoral artery and vein. The cathethers were threaded under the back skin, exited at the nape and further secured by an adhesive collar and spring-wire [11]. The Dopper flow probes were connected to a directional pulsed Doppler flowmeter (model no.545c-3, University of Iowa Bioengineering Facility) and the arterial line to a blood pressure transducer (Narco RP1500i). Mean arterial blood pressure (MAP) and heart rate as well as renal, mesenteric and hindquarter blood flow (RBF, MBF, HQBF, respectively) were continuously monitored by the computerized Narcotrace 80 physiograph. Changes in regional vascular resistance were calculated by dividing the change in MAP by the change in the regional blood flow.

Following 30–60 min of baseline recording for all the above mentioned parameters, LXA_4 or LXB_4 were injected systemically in a bolus of 150 μl volume, in ascending doses of 3, 10, 30 and 100 μg/kg while allowing 30 min for complete recovery between injections.

Pure synthetic LXA_4 and LXB_4 were purchased from Biomol, Philadelphia, PA. Stock solutions of LXA_4 and LXB_4 were kept in ethanol under argon. Samples to be used were evaporated under an argon stream to a volume of 5 μl and further diluted by 145 μl of 0.9% NaCl solution (sterile, pyrogen free) for immediate systemic injections. At no time were diluted samples of LXA_4 and LXB_4 re-frozen nor were they kept at room temperature beyond the few minutes necessary for preparation for injection.

The data in text and figures represent mean ± SE for the indicated number of rats. ANOVA followed by the Student-Newman-Keul test for multiple comparisons was used for statistical analysis of the data and $p < 0.05$ accepted as a significant value.

3. RESULTS

Table 1 shows the basal systemic and regional hemodynamic indices of the rats. The data indicate no significant effect of the vehicle solution (5 μl ethanol and 145 μl of 0.9% NaCl) on any of the hemodynamic variables.

Figs 1 and 2 show the hemodynamic effects of

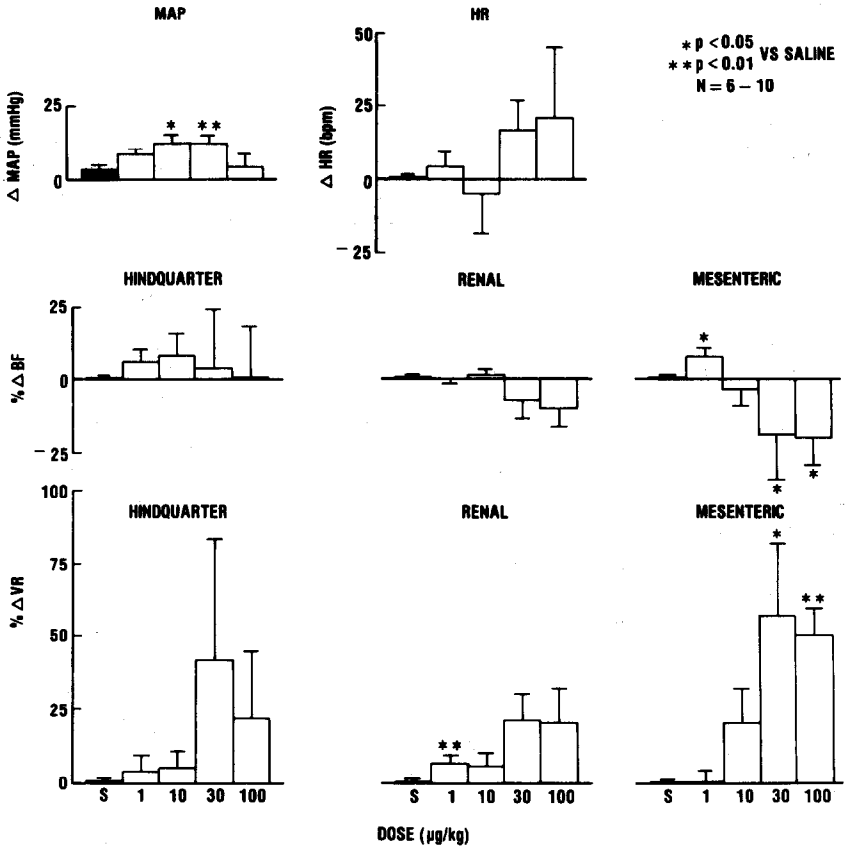

Fig.2. Effect of LXB_4 on systemic and regional hemodynamic variables in the conscious rat. All symbols are explained in the legend to fig.1.

various doses of LXA_4 and LXB_4. LXA_4 and LXB_4 at the doses of 10 and 30 μg/kg induced a slight increase in MAP with no significant changes in heart rate. The higher doses of either LXA_4 or LXB_4 produced mesenteric vasoconstriction which resulted in reduction of MBF. LXA_4 at the highest doses also slightly increased RVR, but LXA_4 and LXB_4 had no effect on HQBF. All the lipoxin-induced cardiovascular changes reached their maximum 30 s after the injection and subsided in 2–3 min.

4. DISCUSSION

The data reported here clearly show that both lipoxin A_4 and lipoxin B_4 possess significant vasoconstrictor activity on the mesenteric vessels of the rat when administered in i.v. bolus injections. The constriction of the mesenteric vessels by LXA_4 and LXB_4 resulted in decreased blood flow which was not accompanied by any significant changes of blood pressure or heart rate. Therefore, the vascular changes most probably reflect a direct action on the given blood vessels. LXB_4 had no significant effects on the renal or hindquarter vascular tone while LXA_4 also produced a significant increase in renal vascular resistance. The contractile nature of LXA_4 and LXB_4 on the mesenteric vessels is similar in potency to the spasmogenic effect of LXA_4 in the guinea pig lung strips [12]. In the only two studies available so far in which vascular smooth muscle responses were examined, LXA_4 produced vasodilation. In one case, LXA_4 produced arteriolar dilation of the hamster cheek pouch vessels when added topically to the cheek pouch [12]. In the second case, selective reduction in the afferent renal arteriolar resistance to LXA_4 was seen during intra-renal arterial administration at a dose of 750 ng/kg per min at a rate of 25 μl/min for approx. 25 min [13]. The studies reported here are therefore in contrast to previously reported vascular effects of lipoxins.

The effects of LXA_4 and LXB_4 demonstrated in this model are also substantially different from those of the cysteinyl-leukotrienes. Low doses (0.1–1 μg/kg) of leukotrienes D_4/E_4 produced short lasting vasodilation of hindquarter blood vessels, mesenteric constriction and change in renal vascular tone; higher doses (10–100 μg/kg) produced a significant vasoconstriction of all vascular beds along with elevated blood pressure [8].

Since LXA_4 and LXB_4 are products of activated leukocytes and LXA_4 primarily of eosinophils [4] it is conceivable that they might contribute to cardiovascular derangements which are mediated by activated leukocytes. Of special importance are vascular responses to acute anaphylaxis which are characterized by severe reduction of splanchnic organ blood flow due to blood vessel constriction [7]. In this respect, LXA_4 and LXB_4 could act in concert with other mediators such as the leukotrienes. Therefore, blockade of the leukotriene action per se may not be sufficient to protect certain blood vessels unless LXA_4 and LXB_4 blockers would be added. Further studies are necessary to confirm in vivo production of LXA_4 and LXB_4 in such situations by measurement of circulating levels of these lipoxins. Nevertheless, it is clear from the results of the present study that LXA_4 and LXB_4 are bioactive compounds which produce responses of interest in vascular physiology.

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