

## Mesenteric vascular responses to i.v. administration of lipoxin A<sub>4</sub> and lipoxin B<sub>4</sub> in the conscious rat

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Lipoxin A<sub>4</sub> and lipoxin B<sub>4</sub> 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<sub>4</sub> and LXB<sub>4</sub> to the conscious rat while monitoring systemic and regional hemodynamic changes. LXA<sub>4</sub> and LXB<sub>4</sub> (1-100 µg/kg) produced dose-dependent constriction of the mesenteric vessels, up to +123 ± 23% and +50 ± 9% for LXA<sub>4</sub>/B<sub>4</sub>, respectively. Dose-related changes were not observed in arterial blood pressure, heart rate, renal (LXB<sub>4</sub>) and hindquarter blood flow. We suggest that LXA<sub>4</sub> and LXB<sub>4</sub> 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<sub>4</sub>) and lipoxin B<sub>4</sub> (LXB<sub>4</sub>) are lipoxygenase-interacting products of the following respective structure: 5S,6R,15S-trihydroxy-7,9,13-*trans*-11-*cis*-eicosatetraenoic acid and 5S,14R,15S-trihydroxy-6,10,12-*trans*-8-*cis*-eicosatetraenoic acid [1-3]. LXA<sub>4</sub> is produced by activated eosinophil leukocytes [4] and LXA<sub>4</sub> and LXB<sub>4</sub> by polymorphonuclear (PMN) leukocytes [5]. LXA<sub>4</sub> 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<sub>4</sub> and LXB<sub>4</sub> were shown to inhibit NK-cell activity [3]. Of special interest are recent studies showing activation of protein kinase C by LXA<sub>4</sub> [6] suggesting an intracellular role in signal transduction.

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While these data support a biological role for LXA<sub>4</sub> and LXB<sub>4</sub> 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<sub>4</sub> and LXB<sub>4</sub> of organ blood flow might be of special interest, since LXA<sub>4</sub> 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<sub>4</sub>/D<sub>4</sub>/E<sub>4</sub>) 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<sub>4</sub> and LXB<sub>4</sub> prompted us to examine

Table 1

Baseline levels of hemodynamic variables before LXA<sub>4</sub> and LXB<sub>4</sub> 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 <sub>4</sub> 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 <sub>4</sub> 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<sub>4</sub> or LXB<sub>4</sub> 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 injection

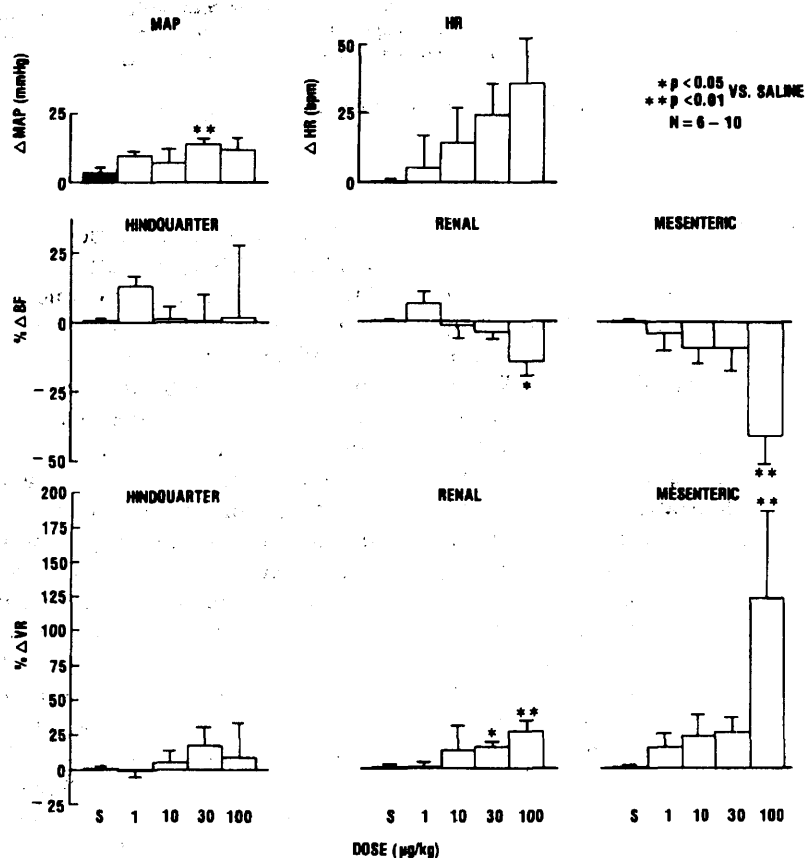


Fig.1. Effect of LXA<sub>4</sub> on systemic and regional hemodynamic variables in the conscious rat. Black columns represent the effect of the vehicle injection while blank columns represent the various doses of LXA<sub>4</sub> from 1 to 100  $\mu$ g/kg (abscissa). % $\Delta$ VR, percent change in vascular resistance; % $\Delta$ BF, percent change in blood flow for the respective organ;  $\Delta$ MAP, change in mean arterial pressure;  $\Delta$ HR, 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 catheters were threaded under the back skin, exited at the nape and further secured by an adhesive collar and spring-wire [11]. The Doppler 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 hind-quarter 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<sub>4</sub> or LXB<sub>4</sub> 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<sub>4</sub> and LXB<sub>4</sub> were purchased from Biomol, Philadelphia, PA. Stock solutions of LXA<sub>4</sub> and LXB<sub>4</sub> 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<sub>4</sub> and LXB<sub>4</sub> 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

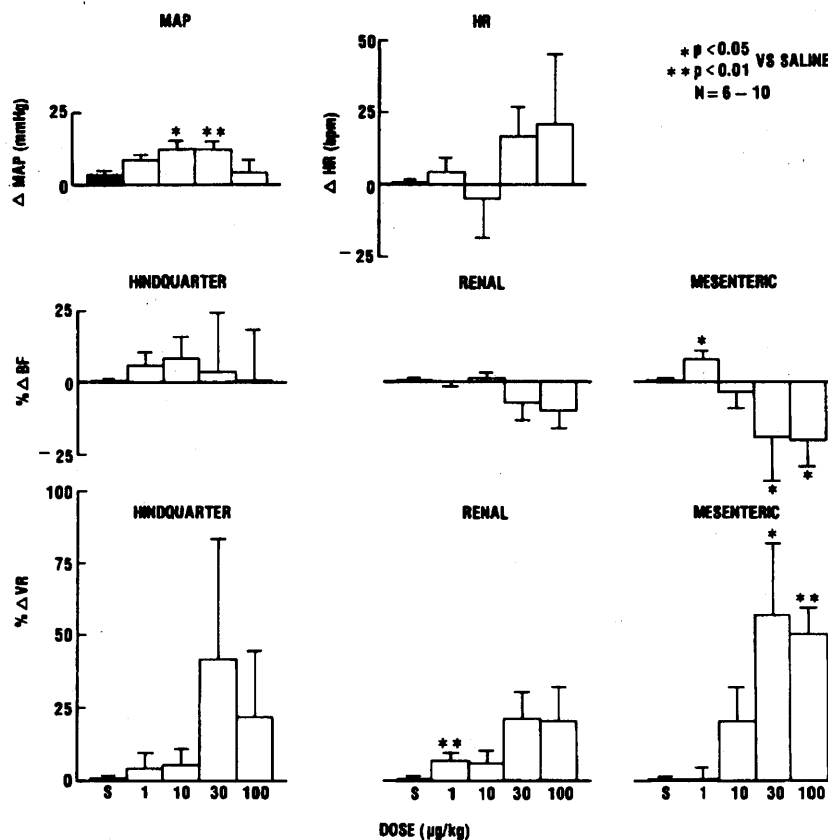


Fig.2. Effect of LXB<sub>4</sub> on systemic and regional hemodynamic variables in the conscious rat. All symbols are explained in the legend to fig.1.

various doses of LXA<sub>4</sub> and LXB<sub>4</sub>. LXA<sub>4</sub> and LXB<sub>4</sub> 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<sub>4</sub> or LXB<sub>4</sub> produced mesenteric vasoconstriction which resulted in reduction of MBF. LXA<sub>4</sub> at the highest doses also slightly increased RVR, but LXA<sub>4</sub> and LXB<sub>4</sub> 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<sub>4</sub> and lipoxin B<sub>4</sub> 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<sub>4</sub> and LXB<sub>4</sub> 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<sub>4</sub> had no significant effects on the renal or hindquarter vascular tone while LXA<sub>4</sub> also produced a significant increase in renal vascular resistance. The contractile nature of LXA<sub>4</sub> and LXB<sub>4</sub> on the mesenteric vessels is similar in potency to the spasmogenic effect of LXA<sub>4</sub> in the guinea pig lung strips [12]. In the only two studies available so far in which vascular smooth muscle responses were examined, LXA<sub>4</sub> produced vasodilation. In one case, LXA<sub>4</sub> 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<sub>4</sub> 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<sub>4</sub> and LXB<sub>4</sub> demonstrated in this model are also substantially different from those of the cysteinyl-leukotrienes. Low doses (0.1–1 µg/kg) of leukotrienes D<sub>4</sub>/E<sub>4</sub> produced short lasting vasodilation of hindquarter blood vessels, mesenteric constriction and change in renal vascular tone; higher doses (10–100 µg/kg) pro-

duced a significant vasoconstriction of all vascular beds along with elevated blood pressure [8].

Since LXA<sub>4</sub> and LXB<sub>4</sub> are products of activated leukocytes and LXA<sub>4</sub> 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<sub>4</sub> and LXB<sub>4</sub> 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<sub>4</sub> and LXB<sub>4</sub> blockers would be added. Further studies are necessary to confirm in vivo production of LXA<sub>4</sub> and LXB<sub>4</sub> in such situations by measurement of circulating levels of these lipoxins. Nevertheless, it is clear from the results of the present study that LXA<sub>4</sub> and LXB<sub>4</sub> are bioactive compounds which produce responses of interest in vascular physiology.

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