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THE ACTION OF LIPOXIN-A ON GLOMERULAR MICROCIRCULATORY DYNAMICS IN THE RAT

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Intrarenal administration of 750 ng/kg/min of LX-A in euvolemic rats resulted in significant increases in single nephron GFR (38.4±1.7 to 45.5±3.0 nl/min) and plasma flow rate (95±6 to 127±9 nl/min). The latter was due to a dramatic fall in afferent arteriolar resistance. Mean transcapillary hydraulic pressure difference increased from 33±1 to 43±3 mmHg ($p<0.05$) and the glomerular capillary ultrafiltration coefficient fell from 0.060±0.013 to 0.033±0.005 nl/(s·mmHg) ($p<0.05$). These responses to LXA in the renal microcirculation are in sharp contrast to those previously observed for the leukotrienes, and thus may represent the first example of counterregulatory (constrictor/dilator) vascular interactions within the lipoxygenase pathways.
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The enzymatic oxygenation of arachidonic acid by a variety of cell types results in the formation of several diverse groups of biologically active compounds (1,2). Lipoxin A (LX-A) (5S,6R,15S-trihydroxy-7,9,13-trans-11-cis-eicosatetraenoic acid) and Lipoxin B (LX-B) (5S,14R,15S-trihydroxy-6,10,12-trans-8-cis-eicosatetraenoic acid) are novel oxygenated derivatives of arachidonic acid originally isolated from human polymorphonuclear leukocytes (3,4). Their complete structure and route of biosynthesis have been characterized recently and several stereoisomers prepared by total organic synthesis (5,6). The lipoxins, formed through the sequential actions of the 5- and 15- lipoxygenase (LO) enzymes (Figure 1), possess a number of biological effects including: activation of protein kinase C (7), inhibition of human natural killer cell cytotoxicity (8), pulmonary smooth muscle contraction (5), and activation of human neutrophils (4). In view of their potential role in

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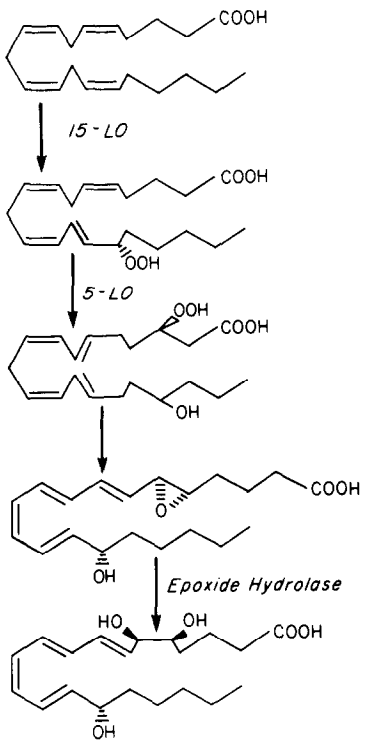

FIGURE 1. Proposed scheme of formation of Lipoxin A by way of a 15(S)-hydroxy-5,6-oxido-7,9,13,-trans-11-cis-eicosatetraenoic acid intermediate. LO: lipoxygenase.

inflammatory reponses, and in light of the described effects of 5-LO pathway products, [leukotrienes (LTs)], on the glomerular microcirculation (9,10) and the recently proposed role of LTs in glomerular inflammatory injury (11), we examined the renal microcirculatory responses to LX-A in the rat.

Methods

Biologically derived LX-A was isolated and purified from activated leukocytes as described (4,5). The Me_3Si derivative of this material eluted as a major component on GC with a C-value of 24.1. The prominent ions were at m/e 203 (base peak), 171, 173, 289, and 379. Ions at lower intensity were at 402, 482, 492, and 582 (M). These ions and C-values are identical to those reported for LX-A (4,5). Synthetic lipoxin A was prepared as in (6). The synthetic and biologically derived materials were matched by published criteria (5). In the present study, the biologically derived and synthetic materials proved to display identical biological actions. LX-A free acid or methyl ester aliquotes were stored in ethanol under argon at -70°C. On the day of the experiment, ethanol was evaporated and the lipoxin resuspended in 0.9% NaCl at the appropriate dilution .

All experiments were performed on anesthetized adult male Munich-Wistar rats weighing 175-230 gms which were prepared for micropuncture according to protocols described previously (12). In brief, following Inactin anasthesia (100 mg/Kg, i.p), the left femoral artery was catheterized with PE 50 tubing which was used to monitor mean systemic arterial pressure (AP) by means of a

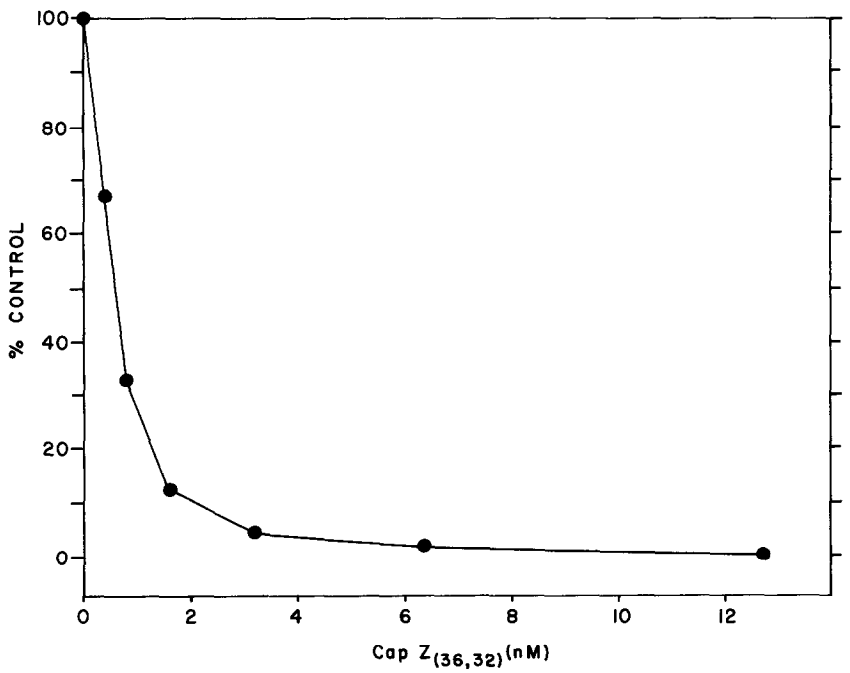

Figure 1. Reduction of the low shear viscosity of actin by Cap $Z_{(36/32)}$. Actin, 11.9 μM, prepared and gel-filtered as described in METHODS, was polymerized in the presence of 116.7 mM KCl, 2 mM $MgCl_2$ and the indicated concentration of Cap $Z_{(36/32)}$ in buffer A plus 3.33% sucrose and 1.67 mM K_2HPO_4. After 2 h and 15 min, the viscosity of the solution was measured in the falling ball viscometer as described in METHODS. Results are expressed as the ratio of experimental to control times.

of actin. A 90% reduction of low shear viscosity was seen at concentrations of approximately 2 nM (0.13 μg/ml) Cap $Z_{(36/32)}$ in 11.9 μM (500 μg/ml) actin. This contrasts with the 7 μg/ml of inhibitor required to see a 90% reduction of low shear viscosity of 500 μg/ml actin in MacLean-Fletcher and Pollard's experiments, suggesting that the specific activity of Cap $Z_{(36/32)}$ is at least 50 times higher than the active material identified in their studies.

To attempt to identify Cap $Z_{(36/32)}$ in actin preparations, pre- and post-column fractions were examined for 1) the ability to reduce the low shear viscosity of actin in the falling ball assay, 2) inhibition of erythrocyte spectrin-band 4.1-actin complex-induced polymerization (the assay originally used to detect Cap $Z_{(36/32)}$ in muscle extracts (2)), and 3) reactivity with the anti-Cap $Z_{(36/32)}$ antibodies on immunoblots. As shown in Fig. 2, the peaks of activity in the falling ball assay and the complex-induced polymerization assays co-localized, and were the same fractions that contained immunoreactive protein bands at the molecular weight of the two subunits of Cap $Z_{(36/32)}$ on immunoblots.

in plasma and urine were determined by the macroanthrone method of Fuhr et al.(15). Protein concentration in arteriolar and femoral arterial plasmas were determined using a fluorometric method developed by Viets et al.(16).

Statistical: Paired t-test was performed to compare, within each group, the changes in various whole kidney and microcirculatory indices which occured from the first to the second study period. Differences were considered significant at a p value ≤ 0.05. All values are reported as mean $\pm$ SEM.

Results

In Group I animals, no significant changes were noted between the first and second period in any of the systemic or renal parameters monitored. In addition, baseline values for these parameters in Group I rats were not statistically different from those of Group II animals described below. Relevant values which remained constant in this Group of animals included: SNGFR (35.9±2.7 to 37.8±1.1 nl/min), Q_A (112±11 to 100±13 nl/min), mean net transcapillary hydraulic pressure difference ($\Delta P = P_{GC} - P_T$) (35±2 to 37±1 mmHg), R_A and R_E (2.34±0.11 to 2.44±0.09 and 1.93±0.12 to 1.88±0.34 10^{10}dyn·s·cm^{-5}, respectively).

In Group II rats, administration of LX-A was not associated with significant changes in AP (103±3 to 102±3 mmHg) or Hct (46.7±0.6 to 46.1±0.7 vol%). Despite constancy of these systemic parameters, GFR increased from 1.00±0.7 to 1.33±0.12 ml/min ($p<0.05$) as did renal plasma flow (RPF) which increased from 3.09±0.21 to 3.91±0.22 ml/min ($p<0.05$). Micropuncture measurements revealed similarly significant increases in SNGFR and Q_A from 38.4±1.7 to 45.5±3.0 and from 95±6 to 127±9 nl/min, respectively. The increase in Q_A was due to a selective fall in pre-glomerular (afferent) arteriolar resistance (R_A) which fell from 2.55±0.16 to 1.75±0.20 10^{10}dyn·s·cm^{-5} ($p<0.05$), while post-glomerular (efferent) arteriolar resistance (R_E) was unchanged from 1.70±0.22 t0 1.50±0.08 10^{10}dyn·s·cm^{-5}. The constancy of R_E, coupled with the increased Q_A, resulted in a significant rise in intraglomerular capillary hydraulic pressure (P_{GC}) from 46±2 to 53±3 mmHg ($p<0.05$) which, in conjunction with a small, but significant fall in P_T (13±1 to 10±1 mmHg, $p<0.05$) led to an

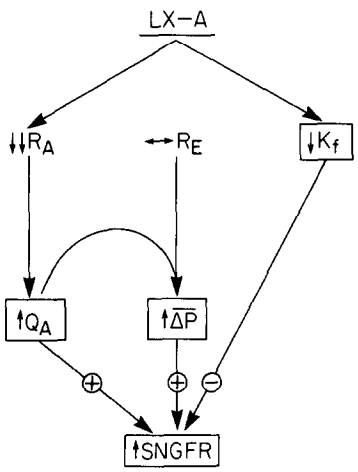

FIGURE 2. The actions of LX-A on the glomerular microcirculation in the rat. LX-A selectively reduces afferent (R_A), but not efferent (R_E) arteriolar resistance thereby leading to increases in glomerular plasma flow rate (Q_A) and net transcapillary hydraulic pressure differnce (ΔP). This results in an increase in single nephron (SN) GFR, an effect partially offset by a simultaneous fall in the glomerular capillary ultrafiltration coefficient (K_f).

increase in ΔP from 33±1 to 43±3 mmHg ($p<0.05$). The increases in glomerular perfusion (Q_A) and the net ultrafiltration pressure (ΔP) were jointly responsible for the observed LX-A-induced increase in single nephron and whole kidney GFR. The latter effect, however, was partially offset by a concomitant significant reduction in the glomerular capillary ultrafiltration coefficient (K_f) which fell from 0.060±0.013 to 0.033±0.005 nl/(s·mmHg) ($p<0.05$). Figure 2 summarizes the effects of LX-A on the glomerular microcirculation.

Discussion

In these experiments, LX-A induced dramatic and selective relaxation of pre-glomerular resistance vessels in the rat with resultant augmentation of the GFR. These vasorelaxant effects of LX-A are in sharp contrast to those reported previously for the sulfidopeptide LTs. The latter potently constrict arteriolar and mesangial smooth muscle, depress the GFR, and may play a role in mediating the reductions in glomerular perfusion and filtration during experimental glomerulonephritis (1, 9-11, 17,19). The present experiments therefore raise the interesting possibility that the net functional response to the activation of the LO pathways during inflammatory injury may depend, in

part, on the relative predominance of the biological activities of their principal vasoactive end-products: LTs and LXs. Although a receptor-mediated activation of the 15-LO is at present unknown, it is clear that the 15-LO is a major pathway for the oxygenation of arachidonic acid in a number of tissues and cell types (20,21). Recent studies indicate that 15-hydroxyeicosa-tetraenoic acid (15-HETE) can be transformed to lipoxins by activated human leukocytes (5) suggesting that cell-cell interactions may play a role in the generation of LXs and related compounds. This is further highlighted by the observations of Soberman et al (22) that the leukocyte 15-LO is preferentially activated under specific conditions, and by the recent demonstration (23) that endogenously produced mono-HETEs are potent stimulants of the 15-LO pathway in the human leukocyte. The response to LX-A of the glomerular microcirculation may involve the release of other mediators. This issue is highlighted by the effect of LX-A on K_f. The fall in K_f, usually interpreted as representing contraction of mesangial cells, contrasts with the relaxant effect of LX-A on the afferent arteriole, and raises the possibility that LX-A may stimulate the release of a K_f-lowering mediator. In summary, these experiments demonstrate that LX-A, in sharp contrast to LTs, induces glomerular hyperperfusion and hyperfiltration. They thus may represent the first demonstration of counterregulatory (costrictor/dilator) vascular interactions within the two major LO pathways (5 and 15-LO).

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