Effect of Endogenously Produced Leukotrienes and Thromboxane on Renal Vascular Resistance in Rabbit Hydronephrosis

Christine R. Albrightson, Alex S. Evers, Anthony C. Griffin, and Philip Needleman

Ureteral obstruction in rabbits is characterized by mononuclear cell invasion of the renal cortex and proliferative fibrosis that is associated with exaggerated prostaglandin synthesis in response to vasoactive and inflammatory cell agonists. In this investigation, we studied the effects of the chemotactic peptide N-formylmethionyl-leucyl-phenylalanine (fMLP) and bradykinin (BK) on eicosanoid synthesis and renal vascular resistance in the ex vivo perfused hydronephrotic kidney (HNK). Administration of fMLP resulted in the dose-dependent synthesis of leukotrienes, thromboxane A\(_2\) (TXA\(_2\)), prostaglandin E\(_2\) (PGE\(_2\)), and prostacyclin (PGI\(_1\)). Peptidoleukotriene synthesis was monitored by specific radiolimmunoassay and by guinea pig ileum bioassay and it was then validated by inhibition of the ileal contractile activity with the peptidoleukotriene receptor antagonist FPL-55712. The leukotrienes produced were identified as LTD\(_4\), LTE\(_4\), LTD\(_4\), and LTE\(_4\), by comigration with authentic standards on reverse phase high-performance liquid chromatography (RP-HPLC) and by ultraviolet spectroscopy. BK administration stimulated the synthesis of TXA\(_2\), PGE\(_2\), and PGI\(_1\), but not the synthesis of leukotrienes, in contrast to the results with fMLP, suggesting the involvement of different cell types. Administration of fMLP to the HNK also resulted in a renal vasoconstriction that was partially inhibited by FPL-55712 and that was completely inhibited by the thromboxane synthase inhibitor OKY-1581. Consistent with this result, exogenous administration of LTC\(_4\), resulted in the synthesis of TXA\(_2\), and in a renal vasoconstriction that was inhibited by either FPL-55712 or OKY-1581. These studies provide conclusive evidence that leukotrienes are produced in renal inflammation, that endogenously produced LTC\(_4\), can stimulate the synthesis of TXA\(_2\), and that LTC\(_4\),-induced vasoconstriction is mediated by the production of TXA\(_2\). The vasoconstrictor effects of endogenously produced leukotrienes and thromboxane suggest a pathophysiologic role for these substances in renal inflammation. (Circulation Research 1987;61:514–522)

Previous studies of the ex vivo perfused rabbit hydronephrotic kidney (HNK) have demonstrated that administration of bradykinin (BK) and angiotensin II result in the synthesis of large amounts of prostaglandin E\(_2\) (PGE\(_2\)) and thromboxane A\(_2\) (TXA\(_2\)). The ability of agonists to stimulate prostaglandin production in these preparations increases with the time of ex vivo perfusion. Treatment of hydronephrotic animals with lipopolysaccharide/endotoxin (LPS) 1 hour before removal of the kidney eliminates this time dependence and results in maximal agonist-stimulated synthesis of eicosanoid metabolites at initiation of ex vivo perfusion. The HNK’s exaggerated prostaglandin synthetic response to agonists occurs in temporal association with macrophage infiltration and fibroblast proliferation in the renal cortex. Unobstructed contralateral kidneys (CLKs) taken from rabbits made hydronephrotic are similar both histologically and biochemically to kidneys from rabbits without ureter obstruction. Both the normal kidney and CLK in comparison with the HNK are devoid of inflammatory cells, lack fibroplasia, and exhibit low eicosanoid synthetic capacity in response to agonists. The data from experiments using normal kidneys and CLKs were combined and reported as CLK values.

Several lines of experimental evidence indicate that the presence of inflammatory cells in the HNK is intimately related to the enhanced prostaglandin synthesis. In HNK models devoid of inflammatory cell infiltrates (i.e., the cat, rabbits made leukopenic with nitrogen mustard treatment before ureter obstruction, and postobstructed HNK rabbits), agonist stimulation does not enhance PGE\(_2\) and TXA\(_2\) production. The activation of macrophages in vitro with LPS and enhanced prostaglandin synthetic capacity of the HNK after LPS treatment suggest that inflammatory cells play a major role in the agonist-stimulated prostaglandin production in the HNK.

Macrophages produce not only prostaglandins but also contain 5-lipoxygenase and have been shown in vitro to synthesize leukotrienes. While it remains unclear which cells are directly responsible for agonist-stimulated eicosanoid synthesis in the rabbit HNK, the presence of inflammatory cells in the HNK led us to hypothesize that leukotrienes would be an important eicosanoid product of the rabbit HNK. The potential pathophysiologic importance of leukotriene synthesis in renal inflammation is suggested by leukotrienes’ ability to cause vascular leakage, contraction of smooth...
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Albrightson et al

Leukotrienes, Thromboxane, and Vasoconstriction in HNK

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Table of Abbreviations

fMLP, N-formyl-methionyl-leucyl-phenylalanine
BK, bradykinin
HNK, hydronephrotic kidney
LT, leukotriene
TXA₂, thromboxane A₂
PG, prostaglandin
PGI₂, prostacyclin
RP-HPLC, reverse phase, high-performance liquid chromatography
UV, ultraviolet
LPS, lipopolysaccharide/endotoxin
HETE, hydroxyeicosatetraenoic acid
CLK, unobstructed contralateral kidney
RIA, radioimmunoassay
ODS, octadecylsilyl
PBS, phosphate-buffered saline solution
EDTA, ethylenediaminetetraacetic acid
PMN, polymorphonuclear leukocytes

muscle, and by their immune cell chemotactic activity. A previous investigation in another model of inflammation, rabbit myocardial infarction, showed that fMLP stimulated the dose-dependent synthesis of peptidoleukotrienes and prostaglandins but that BK stimulated only the synthesis of prostaglandins. Based on this observation, we have tested the hypothesis that the HNK can produce leukotrienes. We now report that the rabbit HNK responds to fMLP with the synthesis of leukotrienes. These endogenously produced leukotrienes stimulate the synthesis of TXA₂ that, in turn, results in increased renal vascular resistance.

Materials and Methods

Chemicals

[³H]-keto-PGF₁α (160 Ci/mmol) and [¹²⁵I]NaI (14 mCi/μg) were purchased from Amersham, Arlington Heights, Ill., and [³H]LTC₄ (40 Ci/mmol) was purchased from New England Nuclear, Boston, Mass. LTC₄ antiserum was kindly supplied by Dr. R. Bell, Riker Laboratories, St. Paul, Minn., and 6-keto-PGF₁α antiserum was provided by Dr. R. Fertel, Ohio State University, Columbus, Ohio. Prostaglandin standards were the gift of Dr. J. Pike of the Upjohn Co., Kalamazoo, Mich.; synthetic LTB₄, LTC₄, and LTD₄ were the gift of Dr. J. Rokach of Merck-Frosst Laboratories, Dorval P.Q., Canada; FPL-55712 was the gift of Fisons Pharmaceuticals, Leicestershire, U.K.; indomethacin was a gift of Merck Sharp & Dohme, Rahway, N.J.; and OKY-1581 was a gift of Ono Pharmaceuticals, Osaka, Japan. LPS (Lipopolysaccharide endotoxin-lyophilized Escherichia coli 055:B5) was purchased from Difco Laboratories, Detroit, Mich.; fMLP was purchased from Sigma Chemical Co., St. Louis, Mo., and BK was purchased from Boehringer-Mannheim, Indianapolis, Ind.

Ureteral Obstruction and Ex Vivo Kidney Perfusion

Unilateral ureteral obstruction was carried out in male New Zealand white rabbits by a previously described procedure. Briefly, animals were anesthetized with sodium pentobarbital (30 mg/kg), the bladder was extruded through a small abdominal incision, and a silk suture was tied around the left ureter. The incision was repaired, and the animals were allowed to recover for 3 days. One hour before sacrifice, some animals received LPS (8 mg/kg i.v.). At the time of sacrifice, the animals were heparinized (500 U/animal), reanesthetized with sodium pentobarbital, and their renal arteries were cannulated with polyethylene tubing. The unobstructed CLK and the HNK or kidneys from rabbits not made hydronephrotic were excised, placed in separate warming jackets, and perfused at a constant flow of 10 ml/min with oxygenated (95% O₂-5% CO₂) Krebs-Henseleit buffer at 37° C. Renal perfusion pressure was monitored with a sidearm in the perfusion apparatus using a P-1000-A pressure transducer (Narco Bio-Systems, Inc., Houston, Tex.) and a Harvard 350 recorder (South Natick, Mass.). Basal perfusion pressure was comparable in both the HNK and CLK, consistent with the relatively low basal eicosanoid metabolite synthesis that occurs in the absence of agonist. The most apparent renal resistance changes between the HNK and CLK are noted during agonist (BK or fMLP) stimulation. The renal venous effluent was allowed to sequentially flow over 2 smooth muscle assay organs: rabbit thoracic aorta strips, to detect TXA₂-like activity, and guinea pig ileum strips, to detect peptidoleukotriene activity. The contraction of the smooth muscle strips was monitored by isotonic myographs and a chart recorder. The assay tissues were also superfused with a mixture of antagonists to render them insensitive to catecholamines, acetylcholine, serotonin, and histamine and superfused with indomethacin to prevent the assay tissue from synthesizing prostaglandins. Before the experimental protocol, the kidneys were perfused ex vivo for 1 hour. At this time, the kidneys were challenged with intra-arterial bolus administration of either BK (30–1,000 ng) or fMLP (30–1,000 ng). Five-minute collections (50 ml) of renal venous effluent were made before and immediately following agonist administration. Aliquots (1 ml) of the effluent were saved (−20° C) for prostaglandin radioimmunoassay (RIA), and the remaining effluent was applied to a C-18 octadecylsilyl (ODS) column (as described below) for subsequent analysis for leukotrienes by RIA and reverse phase high-performance liquid chromatography (RP-HPLC). In inhibitor experiments, OKY-1581 (final concentration, 1 μg/ml) or FPL-55712 (0.4 μg/ml) was administered directly into the renal perfusion fluid.

Radioimmunoassay

Renal venous effluent was assayed for 6-keto-PGF₁α, the stable metabolite of prostacyclin (PGI₂), by a previously described method. PGE₂, and TXB₂, the stable metabolite of TXA₂, levels were measured by RIA using¹²⁵I labelled ligand. Throughout the text and
the discussion of the data, references to detectable PGI₂,
or TXA₂, are based on the 6-keto-PGF₁α and TXB₂ immunoassays, respectively. The [²³¹]PGE₂ and TXB₂ were prepared by the method of Maclouf et al.9 The [¹²⁵]I]PGE₂ assay has previously been described.20 The [¹²⁵]ITXB₂ assay was performed as follows: either standards or samples were added to 50 mM phosphate-buffered saline solution (PBS) containing 0.01 M EDTA, 0.3% bovine γ-globulin, 0.005% Triton X-100, and 0.05% sodium azide (pH 6.8) to a total volume of 100 μl. Fifty microliters of antisera (final dilution of 1:40,000) and 50 μl of [¹²⁵]ITXB₂ containing 10,000 cpm. After overnight incubation at 4°C, 16% polyethylene glycol in 50 mM PBS was added to the tubes. The precipitated bound tracer was separated from the unbound ligand by centrifugation at 3,000 rpm for 30 minutes, and by decanting the supernatant. The radioactivity was counted in a Micro-medic Systems Apex automatic gamma counter. The cross-reactivities of the 6-keto-PGF₁α, PGE₂, and TXB₂ antisera have been previously described.7 Perfusion buffer in volume assayed did not affect the standard curve of these RIAs.

To assay for leukotrienes by RIA, the renal effluent was processed by a modification of the method of Powell.6 The effluent was acidified to pH 6.2 with HCl and applied to a 1-gram open-bed, preactivated C-18 ODS column (J.T. Baker Chemical Co., Philipsbury, N.J.). The column was washed with water, and the products were eluted with 10 ml of 100% methanol. An aliquot of the methanol was evaporated under nitrogen, and the products were resuspended in the LTC₄-RIA buffer. The LTC₄-antiserum cross reactivities at 50% displacement was 50% for LTD₄ and 8% for LTE₄.

Results

Effect of BK Stimulation on Rabbit Kidney Arachidonic Acid Metabolism

Intra-arterial administration of BK to the ex vivo perfused HNK resulted in the dose-dependent synthesis of PGE₂, PGI₂, and TXA₂. Treatment of the hydronephrotic animal with LPS 1 hour before removal of the ureter-obstructed kidney resulted in enhanced BK-stimulated prostaglandin synthesis during the first several hours of perfusion (Table 1). These differences in peptide-stimulated prostaglandin synthesis were eliminated by prolonged ex vivo perfusion9 (current data not shown). The effects of LPS pretreatment were specific to the HNK because perfused unobstructed control kidneys from rabbits that received LPS pretreatment exhibited a minimal prostaglandin synthetic response to BK (Table 2). Notably, the effluents from BK-stimulated HNK and CLK (±LPS) were devoid of guinea pig ileum contractile activity (bioassay for peptidoleukotrienes) and of detectable LTC₄ immuno-reactivity.

Effect of fMLP Stimulation on Rabbit Kidney Arachidonic Acid Metabolism

Repeated intra-arterial administration of fMLP (100 ng) to the ex vivo perfused HNK stimulated PGE₂, PGI₂, and TXA₂ synthesis; this prostaglandin synthetic response increased in magnitude with time of ex vivo perfusion (Figures 1A and 1B). Treatment of the rabbits with LPS 1 hour before removal and perfusion of the kidney eliminated the time dependent increase in metabolite synthesis and resulted in maximal eicosanoid production with the initial fMLP challenge (Figures 1A and 1B). Tachyphylaxis occurred with frequent (20-minute intervals) fMLP stimulation of the HNK; therefore, fMLP stimulations were performed at hourly intervals. Stimulation of the rabbit HNK with fMLP resulted in the synthesis of peptidoleukotrienes, which were initially observed by bioassay (guinea pig ileum contraction) and subsequently quantitated by RIA for LTC₄. Whereas fMLP-stimulated prostaglandin synthesis was maintained or increased with perfusion time, leukotriene synthesis declined at prolonged perfusion times (Figure 1B). The CLK pretreated with LPS and stimulated with fMLP (1,000 ng) produced far smaller quantities of prostaglandins and leukotrienes than the fMLP-stimulated HNK (Table 2).

Stimulation of the HNK with fMLP (30–1,000 ng) caused a dose-dependent synthesis of PGE₂, PGI₂, (Figures 2A), TXA₂, and LTC₄ (Figure 2B). Treatment

Table 1. The Effect of LPS-Pretreatment on the Maximal Eicosanoids Metabolite Responses of the HNK to Bradykinin and fMLP

<table>
<thead>
<tr>
<th></th>
<th>PGE₂</th>
<th>PGI₂</th>
<th>TXA₂</th>
<th>LTC₄</th>
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<tr>
<td>HNK + LPS</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>fMLP (300 ng) n = 7</td>
<td>545 ± 82*</td>
<td>357 ± 129*</td>
<td>252 ± 48</td>
<td>73 ± 9</td>
</tr>
<tr>
<td>BK (300 ng) n = 7</td>
<td>3,189 ± 788</td>
<td>1,235 ± 212</td>
<td>237 ± 81</td>
<td>...</td>
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<tr>
<td>HNK</td>
<td></td>
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</tr>
<tr>
<td>fMLP (1 μg) n = 3</td>
<td>148 ± 85*</td>
<td>84 ± 13*</td>
<td>25 ± 10</td>
<td>9 ± 2</td>
</tr>
<tr>
<td>BK (1 μg) n = 3</td>
<td>1,343 ± 391</td>
<td>1,207 ± 193</td>
<td>49 ± 18</td>
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Hydronephrotic rabbits were either treated or not treated with LPS 1 hour before sacrifice. The HNKs were then removed and perfused ex vivo for 3–4 hours before stimulation with a maximal dose of either fMLP or BK. Maximal eicosanoid synthetic responses were obtained with 300 ng BK or fMLP in LPS-pretreated animals and with 1 μg BK or fMLP in nonpretreated animals. Values shown were determined by specific RIA and are expressed as nanograms of product measured in a 5-minute (50 ml) collection of effluent ± SEM. Ellipses indicate that the levels were not detectable. The synthesis of each metabolite elicited by BK was compared with the synthesis elicited by fMLP using a Student's t test for unpaired data. The 2 pretreatment groups (±LPS) were analyzed separately, *p < 0.05.
of the hydronephrotic animal with LPS 1 hour before kidney perfusion resulted in a leftward shift of the fMLP dose-response curves for eicosanoid synthesis (Figures 2A and 2B) and in an increase in the maximal response to fMLP (Table 1).

Validation of Leukotriene Production in the fMLP-Stimulated HNK

Since the LTC₄ antisemur cross reacts with LTD₂ and LTE₄, the RIA did not distinguish the species of leukotrienes released from the HNK. RP-HPLC separation of the products released from the fMLP-stimulated HNK revealed the presence of LTB₄, LTC₄, LTD₂, and LTE₄ in the renal venous effluent as detected by comigration with authentic leukotriene standards. RIAs for LTC₄, and LTE₄ contained insufficient material to contract the guinea pig ileum, and LTB₄, did not possess this bioactivity. Effluent subjected to RP-HPLC from unobstructed kidneys and unstimulated or BK-stimulated HNKs served as negative controls. Basal effluent (600 ml) from control kidneys and HNKs (± LPS) processed as described in "Materials and Methods" and subjected to RP-HPLC lacked peak absorbances at 280 nm with retention times similar to leukotriene standards. All of the resulting HPLC fractions lacked leukotrienes as determined by LTC₄ RIA. Similarly, the pooled effluent from BK-stimulated kidneys (600 ml) (CLK or normal and HNK ± LPS) contains neither guinea pig ileum contractile activity nor leukotrienes as detected by HPLC and RIA. The effluent from CLK taken from animals pretreated with LPS showed an attenuated synthesis of LTB₄ and LTC₄ stimulated HNKs served as negative controls. Basal effluent (600 ml) of fMLP (100 ng) from control kidneys and HNKs (± LPS) processed as described in "Materials and Methods" and subjected to RP-HPLC lacked peak absorbances at 280 nm with retention times similar to leukotriene standards. All of the resulting HPLC fractions lacked leukotrienes as determined by LTC₄ RIA. Similarly, the pooled effluent from BK-stimulated kidneys (600 ml) (CLK or normal and HNK ± LPS) contains neither guinea pig ileum contractile activity nor leukotrienes as detected by HPLC and RIA. The effluent from CLK taken from animals pretreated with LPS showed an attenuated synthesis of LTB₄ and LTC₄ after fMLP stimulation. Stimulation of a CLK with 1,000 ng of fMLP produced less than 1/10 of the LTB₄ produced by an HNK stimulated with 100 ng of fMLP.

Effects of Endogenously Produced Leukotrienes and Thromboxane on Renal Vascular Resistance in the Ex Vivo Perfused HNK

Administration of BK and fMLP to the HNK from animals treated with LPS caused the synthesis of several vasoactive arachidonic acid metabolites. We used the leukotriene receptor antagonist FPL-55712 and the thromboxane synthase inhibitor OKY-1581 to determine the effects of endogenously produced peptidoleukotrienes and thromboxane on renal perfusion pressure. Under constant flow conditions, as performed in these experiments, increased perfusion pressure indicates increased vascular resistance. Administration of fMLP to the LPS-treated HNK resulted in an increase in perfusion pressure, coincident with the
synthesis of thromboxane and peptidoleukotrienes (Figure 4). Intrarenal infusion of the thromboxane synthase inhibitor OKY-1581 inhibited both the fMLP-stimulated TXA₂ synthesis and renal vasoconstriction. Administration of 100 ng of fMLP stimulated TXA₂ production from basal levels of 5.9 ± 1.2 to 121 ± 22 ng (n = 8), which were significantly inhibited by OKY-1581 to levels of 5.8 ± 2.7 ng (n = 5). Perfusion pressure increased with fMLP stimulation by 45 ± 10 mm Hg (n = 12) or by 3 ± 1 mm Hg (n = 5) in the presence of OKY-1581. Interestingly, the LT-receptor antagonist FPL-55712 partially inhibited the fMLP-stimulated TXA₂ synthesis (59 ± 20 ng, n = 7, p<0.05) and the renal vasoconstriction [an increase of perfusion pressure of only 14 ± 4 mm Hg (n = 8)]. Stimulation of the LPS-treated CLK with 1,000 ng fMLP did not cause a change in perfusion pressure. BK administration to the LPS-treated HNK resulted in the production of TXA₂, PGE₂, and PGI₁ in association with a transient renal vasoconstriction. OKY-1581 inhibited BK-stimulated TXA₂ synthesis specifically. Therefore, the renal vasodilation observed after BK administration in the presence of OKY-1581 presumably resulted from the effect of PGE₂ and PGI₁. As would be expected, FPL-55712 had no effect on BK-stimulated vasoconstriction. A representative tracing, along with the corresponding TXA₂ values, from these experiments is shown in Figure 4. In this experiment BK stimulation of the HNK in the absence or presence of inhibitors (i.e., OKY-1581 or FPL-55712) produced 1,683 ± 91 ng PGE₂/5-min collection. Likewise, fMLP-stimulated PGE₂ production was unaffected by the inhibitors and ranged from 68 to 89 ng/5-min collection. BK- and fMLP-stimulated PGI₁ synthesis was also unaltered by OKY-1581 and FPL-55712.

**Effect of Exogenously Administered LTC₄ on Thromboxane Production and Renal Vascular Resistance in the Ex Vivo Perfused Rabbit Kidney**

Intra-arterial administration of LTC₄ (1–10 μg) to the LPS-treated HNK caused a dose-dependent release of TXA₂ (6.8–25.4 ng/50 ml). Only at the highest dose of LTC₄ (10 μg) was the renal arterial perfusion pressure increased above baseline (from 58 ± 9 to 74 ± 10 mm Hg; n = 4, p<0.05). This dose of LTC₄ also stimulated the release of TXA₂ (from 5.1 ± 1.3 to 41.7 ± 11.9 ng/50 ml; n = 4, p<0.05). In the LPS-treated control CLK, LTC₄ did not stimulate the production of TXA₂ or cause a change in perfusion pressure. Administration of LTD₄ (10 μg) to the LPS-treated HNK or CLK did not stimulate TXA₂ production or change renal vascular resistance. Perfusion of the HNK with OKY-1581 or FPL-55712.

**Figure 1. Time-dependent effect of fMLP on eicosanoid synthesis in the isolated perfused HNK.** The 3-day ureter-obstructed kidney (HNK) with (closed symbols; n = 7) or without (open symbols; n= 5) in vivo LPS treatment were perfused ex vivo (constant flow of 10 ml/min) for 1 hour before agonist stimulation. Samples of the renal effluent were collected for 5 minutes (50 ml) before agonist stimulation (basal) or immediately following intra-arterial bolus administration of fMLP (100 ng). Aliquots of effluent were analyzed by radioimmunoassay for TXB₂ (•, ○) and 6-keto-PGF₁α (◆, ◆), the stable metabolites of TXA₂ and PGI₁, respectively, and for PGE₂ (□, ◻) and LTC₄ (▲, △). Basal release of eicosanoids increased with time of perfusion; therefore, basal values (<10% of stimulated values) were subtracted from the fMLP-stimulated eicosanoid levels. LTC₄ antiserum cross reacted 50% with LTD₄ and 8% with LTE₄, and graphed values thus represent LTC₄-like immunoreactive material. All agonist-stimulated values were significantly increased over basal values and are expressed as nanograms of product in a 5-minute collection of effluent (minus basal values) ± SEM. Statistical comparison of eicosanoid levels between HNK and HNK pretreated with LPS were performed at each time point using Student’s t test for unpaired data: *p<0.05.
inhibited the LTC₄-mediated TXA₂ production and vasoconstriction (Figure 5). Full recovery of the TXA₂ synthetic capacity and vasomotor activity of the HNK after administration of each inhibitor was monitored by the ability of fMLP to stimulate TXA₂ synthesis and increase perfusion pressure.

Discussion

This study demonstrates several novel aspects of arachidonate metabolism in renal inflammation using the rabbit hydronephrotic kidney model. Leukotrienes were produced by the ex vivo perfused HNK in response to fMLP stimulation. This effect was greatly exaggerated by treating the hydronephrotic animal with LPS 1 hour before kidney perfusion. Peptidoleukotriene production in the HNK was initially detected in the renal effluent by bioassay. The presence of LTC₄, LTD₄, and LTE₄ in the renal effluent was further confirmed by RP-HPLC and RIA. Both endogenous and exogenous LTC₄ were shown to stimulate TXA₂ synthesis in the HNK but not in the CLK. This LTC₄-stimulated TXA₂ synthesis resulted in renal vasoconstriction.

Although the cellular source of agonist-stimulated leukotriene synthesis in the HNK is unknown, there are several compelling lines of evidence suggesting an inflammatory cell origin. The fMLP stimulation of leukotriene and prostaglandin production in the HNK is most likely due to the infiltration of inflammatory cells since fMLP receptors have been demonstrated only on inflammatory cells such as polymorphonuclear...
leukocytes and macrophages. Inflammatory cells in vitro stimulated with fMLP also produce leukotrienes. The unresponsiveness of the CLK to fMLP stimulation (little or no leukotriene production) is consistent with the low numbers of resident tissue macrophages. The hypothesis that the HNK-produced leukotrienes originate from inflammatory cells is further supported by the unmasked leukotriene production in the fMLP-stimulated HNK from rabbits treated with LPS, an inflammatory cell agonist. This novel production of leukotrienes is likely to result from LPS activation of the inflammatory cells recruited in vivo by the primary stimulus of tissue damage (in this case caused by ureter obstruction). Alternatively, LPS might stimulate inflammatory cell adherence to endothelium and migration into damaged organs. This seems less likely since fMLP stimulation of the CLK from rabbits treated with LPS causes minimal leukotriene production. The data obtained in this study suggest that fMLP and BK stimulate different cell types in the HNK and that the involved cells are present in the injured (HNK) but not the control (CLK) kidney. Three observations support this hypothesis. First, we are unaware of any evidence that demonstrates that BK directly stimulates macrophages or monocytes; we have treated cultured resident peritoneal macrophages with up to 1 μM BK and not observed the release of eicosanoids (data not shown). Others have reported similar results. Secondly, BK (up to a 3,000-ng bolus) did not release detectable levels of leukotrienes from the HNK (± endotoxin in vivo pretreatment) even when assayed with a very sensitive immunoassay (10−30 pg LTC/D readily analyzed) following extraction of large volumes of renal effluent. Finally, the dose-response comparison of the agonist effect on the HNK demonstrates differential quantitative metabolite production, such that BK stimulation induces the release of 5−10 times more PGE and 6-keto-PGF than fMLP. Furthermore, BK also stimulates much less leukotriene production (BK-undetectable versus fMLP approximately 50 ng/50 ml) than fMLP.

Leukotriene-stimulated TXA2 production has previously been demonstrated in guinea pig lungs and cultured bovine aortic endothelial cells. This leukotriene-stimulated TXA2 production is abolished by pretreatment with the leukotriene receptor antagonist FPL-55712. We demonstrated that the ex vivo perfused HNK (but not the CLK) from rabbits treated with LPS responds to fMLP stimulation with the synthesis of leukotrienes that, in turn, caused production of thromboxane. These experiments represent a unique example of an inflammatory state in which leukotrienes acquire the ability to stimulate TXA2 synthesis. Likewise, this is a novel example of endogenously produced leukotrienes stimulating the production of TXA2. These results indicate that inflammation confers on a previously unresponsive tissue the capacity for leukotriene synthesis and that endogenous leukotrienes in the HNK cause a response not detected in normal tissue.

**Figure 4.** Effect of OKY-1581 and FPL-55712 on the BK- and fMLP-stimulated eicosanoid production and their effect on renal vasoconstriction in the isolated perfused HNK. Ureteral-obstructed rabbit kidneys were perfused at a constant flow of 10 ml/min, and renal perfusion pressure was monitored with a side arm in the perfusion apparatus. BK (300 ng) and fMLP (100 ng) were administered by a bolus intrarrenal injection, and OKY-1581 (1 μg/ml) and FPL-55712 (0.4 μg/ml) were perfused at 0.1 ml/min through the kidney. After inhibitor administration, sufficient time was allowed for recovery of the agonist-stimulated vascular response and TXA2 production. This figure presents a representative experiment, parts of which were repeated 4 to 8 times with the same result.

**Figure 5.** Effect of OKY-1581 and FPL-55712 on the LTC4-induced renal vasoconstriction in the HNK. Arterial perfusion pressures of the ex vivo perfused contralateral control and hydronephrotic rabbit kidneys were monitored after the exogenous administration of LTC4 (10 μg). Recovery after administration of inhibitors was monitored by fMLP-stimulated vasoconstriction and thromboxane production. This figure presents a representative experiment that was repeated 4 times with the same results.
The effect of leukotrienes (through TXA₂ synthesis) on renal vascular resistance was also altered by the in vivo inflammatory state. Only in the LPS-treated hydronephrotic animal did the ex vivo perfusion pressure of the HNK increase with administration of fMLP and LTC₄. This newly acquired response to leukotrienes, mediated in part by the level and condition of inflammatory cells, may have an important impact on renal function. The renal vasocstriction could reduce renal blood flow, cause tissue ischemia, and exacerbate tissue damage. The enhanced leukotriene production in the rabbit HNK also has the capacity to alter physiologic effects not examined in these studies. Potentially, altered leukotriene production in the HNK could enhance vascular leakage and stimulate inflammatory cell chemotaxis. These effects could, in turn, cause further inflammatory cell activation and further tissue damage.

The exaggerated PGE₂ and PGI₂ production induced by agonists in the HNK may conceivably provide a negative feedback modulation of the metabolic processes initiated by the invading inflammatory cells. PGE₂ suppresses: 1) the expression (as does PGI₂) of the Class II histocompatibility molecule, Ia, on macrophages; 2) the macrophage production of interleukin-1, a factor that we earlier demonstrated to stimulate eicosanoid metabolism in fibroblasts, endothelial cells, and vascular smooth muscle cells; and 3) macrophage cytotoxicity. Collectively, these observations suggest that regional production of PGE₂ at the site of injury may negatively modulate local macrophage metabolism and function.

In these studies, we demonstrate the production of leukotrienes in a renal model of inflammation, the HNK. The endogenously produced leukotrienes stimulated TXA₂ synthesis, resulting in renal vasocstriction. The synthesis of leukotrienes and the ability of leukotrienes to stimulate thromboxane synthesis and subsequent renal vasocstriction occurs only in the injured kidney, demonstrating both enhanced leukotriene production and enhanced leukotriene-mediated effects in hydropneophrosis. Collectively, these data demonstrate the synthesis of leukotrienes in hydropneophrosis and suggest their pathophysiologic importance in renal inflammation.

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