Roles for thromboxane A₂ and leukotrienes in endotoxin–induced acute renal failure

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Roles for thromboxane A₂ and leukotrienes in endotoxin–induced acute renal failure. Bolus i.v. administration of 100 μg/kg of E. coli lipopolysaccharide endotoxin (LPS) to adult male Munich-Wistar rats (N = 18) resulted in a progressive fall in RBF and GFR from 6.9 ± 0.2 se and 1.1 ± 0.05 ml/min to minimal values at 50 minutes of 3.8 ± 0.4 and 0.32 ± 0.08 (P < 0.05, respectively), without a fall in mean arterial pressure. At 50 minutes, renal cortical generation rates of PGE₂ (1075 ± 108 pg/mg tissue), 6-keto PGF₁α (221 ± 41 pg/mg), and TXB₂ (106 ± 12 pg/mg) were significantly higher than those of vehicle-treated control rats (N = 10, PGE₂ = 466 ± 107, 6-keto PGF₁α = 94 ± 3, and TXB₂ = 35 ± 3 pg/mg), and morphologic examination revealed normal histology with notable absence of leukocytes and platelets. Pretreatment of a third group of nine rats with TxA₂ synthetase inhibitor UK-37,248 (dazoxiben, 10 mg/kg) selectively abolished the LPS-induced rise in TXB₂ (29 ± 3 pg/mg), but not PGE₂ (837 ± 62 pg/mg) or 6-keto PGF₁α (179 ± 5 pg/mg), preventing the fall in RBF at 50 minutes (6.3 ± 0.4 ml/min), and allowed for significant preservation of GFR (0.67 ± 0.08 ml/min). In addition, antagonism of endogenously produced LTs with the putative receptor antagonist FPL55712 (N = 10, 500 μg/kg/min × 40 min), while not preventing the LPS-induced rise in TXB₂ generation rate (97 ± 10 pg/mg), significantly ameliorated the fall in RBF at 50 minutes (5.9 ± 0.5 ml/min), whereas GFR was depressed (0.46 ± 0.13 ml/min), but recovered to 0.57 ± 0.08 ml/min at 70 min post-LPS (P < 0.05 vs. LPS alone). These observations point to major roles for TxA₂ and sulfidopeptide LTs in mediating the renal functional impairment of experimental endotoxia.

Acute oliguric renal failure is a major cause of mortality and morbidity during endotoxic shock [1]. The mechanism of endotoxin–induced renal injury is not fully understood although roles for the alpha adrenergic system, the renin angiotensin system, and other humoral factors have been suggested [2–5]. In man, dog, and rat, endotoxin leads to an increase in renal vascular resistance (RVR) and a decrease in glomerular filtration rate (GFR) [1–5].

Endotoxin is a potent stimulus of white blood cells in vitro leading to activation of phospholipase A₂ and release of arachidonic acid [6], resulting in the synthesis of potent vasconstrictor compounds such as thromboxane A₂ (TxA₂) and sulfidopeptide leukotrienes (LTC₄ and LTD₄) [6, 7]. Thromboxane A₂ is a known vasconstrictor whose release in response to endotoxin in non-renal models of endotoxic injury has already been demonstrated [8, 9]. The sulfidopeptide LTs have been shown to increase RVR in the intact animal [10] as well as in the isolated perfused kidney [11]. They have also been shown to contract isolated rat glomeruli [12] and, recently, these same glomeruli have been shown to possess LT binding sites having the biologic characteristics of true receptors [13]. Of interest, in numerous models of endotoxic shock, inhibition of arachidonic acid metabolism, either at the level of cyclooxygenase with aspirin or indomethacin, or through dietary modification, has resulted in improved organ function and whole animal survival [14–16].

In view of possible roles for TxA₂ and LTs in endotoxin–induced renal ischemia, we developed a model of E. coli endotoxin–induced acute renal failure in the rat, and measured the renal hemodynamic changes as well as the renal cortical generation rates of TxA₂, prostaglandin E₂ (PGE₂) and prostaglandin I₂ (PGI₂), in response to endotoxin. Furthermore, we selectively antagonized the effects of TxA₂ and LTs in this model of renal injury. Our results suggest an important role for these products of arachidonic acid in sequentially mediating the increase in RVR and the decrease in GFR during the first seventy minutes following endotoxin administration. Results of histologic studies suggest the release of these eicosanoids from resident renal parenchymal cells rather than from infiltrating cellular elements.

Methods

Physiologic studies

E. Coli endotoxin (LPS) was obtained from List Biological Laboratories (Campbell, California, USA). The preparation used was National Reference Endotoxin (Lot EC-0113) as prepared by Rudbach et al [17]. Detailed description of the chemical and biological properties of this endotoxin has been published [17]. Concentrated solutions of LPS dissolved in distilled water were stored at 4°C and aliquots used as needed. UK-37248-Ol (dazoxiben) was obtained from Pfizer's Pharmaceuticals (Pfizer Laboratories, New York, USA). FPL55712 was obtained from the Fisons Corp (Loughborough, U.K.).

Experiments were performed on adult male Munich-Wistar rats (209 to 305 g) maintained on a standard rat pellet diet and allowed free access to water. Each rat was anesthetized with Inactin (100 mg/kg, i.p.) and placed on a heated surgical table so that body temperature was maintained at a baseline value of 37.0 to 37.5°C. Polyethylene catheters (PE 50) were placed in the left femoral artery and femoral vein. The arterial catheter

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was used for blood sampling and measurement of mean arterial pressure (MAP) with an electronic transducer (Model P23Db, Statham Instruments Division, Gould Inc., Hato Rey, Puerto Rico) connected to a direct writing recorder (Model 7754A, Hewlett Packard Co., Elkhart, Indiana, USA). The venous catheter was used for infusion of homologous rat plasma as required to maintain euvoemia [18]. Following tracheostomy, PE 50 catheters were introduced into both jugular veins for the administration of a 7% inulin solution in 0.9% NaCl at the rate of 1.2 ml/hr, and for the administration of LPS, dazoxiben, FPL-55712, or appropriate vehicles. The left kidney was exposed via a subcostal incision and the left ureter was catheterized with PE 10 tubing. An electromagnetic flow probe (ID = 1.5 min) was placed on the left renal artery and connected to a flow meter (Carolina Medical Electronics, Model 501), the output of which was displayed on a second channel of the Hewlett Packard recorder. To avoid contamination by the laboratory environment, endotoxin was administered in sterilized Hamilton syringes. All other solutions were administered in sterilized disposable plastic syringes.

After a 45 to 60 minute period during which MAP, RBF, and urine flow were constant, two 15 min clearance measurements were performed and control values for MAP and RBF were recorded. Following these measurements, animals received either vehicle injections or LPS, following which RBF was monitored for 70 minutes, and clearance measurements performed between 35 and 50 minutes and, in some animals, between 60 and 70 minutes. The rats were divided into six experimental groups as follows:

**Group I**—vehicle time controls (N = 10). These animals each received an injection of 0.2 ml of 5% dextrose in water as a vehicle control for LPS. In seven animals, the left kidney was perfused with 0.9% NaCl at 50 minutes post-injection and then processed for biochemical assays as described below. Three animals were followed for 70 minutes. This group served as a time control.

**Group II**—LPS alone (N = 18). The rats each received an injection of 100 μg/kg of LPS in 0.2 ml of distilled water over three minutes. Five animals were given 0.25 ml of 0.9% NaCl 10 minutes prior to LPS injection as vehicle controls for dazoxiben–pre-treated rats (see Group IV). Five animals received a continuous infusion of 0.45% NaCl starting 10 minutes before and ending 30 minutes after LPS administration, at the rate of 0.02 ml/min, and served as vehicle controls for FPL-55712–treated rats (see Group V). Since the response to LPS administration was indistinguishable among those animals which received either of these two vehicle protocols and those which received LPS alone, these animals were all considered as a single group and their data pooled. In nine animals in this group, biochemical assays were performed at 50 minutes post-LPS. The remaining nine were followed for 70 minutes.

**Group III**—dazoxiben plus LPS-vehicle (N = 4). These rats each received an injection of 0.2 ml of 5% dextrose in water (LPS-vehicle) 10 minutes following administration of 10 mg/kg of dazoxiben dissolved in 0.25 ml of 0.9% NaCl. In all animals in this group, biochemical assays were performed 50 minutes post-injection of 5% dextrose in water.

**Group IV**—dazoxiben plus LPS (N = 9). As in Group II, these animals each received an injection of LPS 10 minutes following intravenous administration of 10 mg/kg of dazoxiben dissolved in 0.25 ml of 0.9% NaCl. Five animals underwent biochemical assays at 50 minutes post-injection of the endotoxin, while the remaining 4 animals were followed for 70 minutes post-LPS.

**Group V**—FPL-55712 plus LPS (N = 10). As in Group II, these rats each received an injection of LPS in the presence of a continuous intravenous infusion of FPL-55712 (500 μg/kg/min) dissolved in 0.45% NaCl started 10 min prior to LPS injection and maintained for 30 minutes post-injection. Four animals underwent biochemical assays at 50 minutes post-LPS and the remaining six were followed for 70 minutes.

**Group VI**—morphologic studies (N = 6). As in Group II, these rats each received an injection of LPS (N = 3), or vehicle (N = 3), followed, at 50 minutes post-injection, by perfusion fixation of the kidneys and preparation for morphologic studies as described below.

In Groups I, II, IV, and V, white blood cell counts were obtained, using a Coulter counter, before and 50 minutes after administration of either 5% dextrose in water or LPS.

**Biochemical assays**

In all animals in which biochemical assays were performed, kidneys were perfused with cold saline until the venous effluent was clear, excised, and the capsule gently removed. The kidney was then bisected and the medulla and cortex rapidly separated by fine dissection. Tissues were immediately moistened with Krebs Ringer bicarbonate buffer (KRB) at pH 7.2 and 10 to 30 mg of tissues were finely, uniformly minced with a razor blade and incubated in 2 ml KRB in a 25 ml flask on a shaker platform in a 5% CO2 incubator at 37°C for 30 minutes. All supernatants were immediately stored at −20°C for PGE2, 6 keto PGF1α and/or TXB2 analysis. It was not necessary to extract PGE2, TXB2 or 6 keto PGF1α from tissues, since cyclooxygenase metabolites accumulate in the medium rather than in the tissues [19].

PGE, TXB2, and 6 keto PGF1α, assays. PGE2, TXB2 and 6 keto PGF1α content in supernatant was determined by direct competitive binding radioimmunoassays [20]. Anti-PGE2 serum had a 100% cross reactivity with PGE1 but only 2.7% with PGE2. Therefore, our results can not differentiate PGE1 and PGE2. However, since PGE2 was used as a standard we have expressed our results as PGE2 equivalents. Antiseria to TXB2 did not cross-react (0.04%) with other arachidonic acid metabolites [21]. 6 keto PGF1α, antiseria had a cross reactivity as previously described [22]. 3H-PGE2, 3H-TXB2 and 3H 6 keto PGF1α were purchased from New England Nuclear Corporation (Boston, Massachusetts, USA). Values expressed for PGE2, TXB2 and 6 keto PGF1α represent the mean levels of duplicate determinations. The assay sensitivities for TXB2, PGE2, and 6 keto PGF1α were 26 pg/ml, 30 to 40 pg/ml, and 30 to 40 pg/ml, respectively. Intra-assay coefficients of variations were <2%.

**Morphologic studies**

For light and electron microscopy, kidneys from three vehicle–treated and three endotoxin–treated animals were fixed by perfusion at the measured arterial pressure with 1.25% glutaraldehyde in 0.1 M cacodylate buffer (pH = 7.4) 50 minutes following administration of either vehicle or LPS. The tissue was then rinsed in buffer, postfixed in 1% osmium tetroxide,
were stained with 0.5% toluidine blue in 1% aqueous borax.

Statistical analysis

Since AP, RBF, and GFR were constant over the 45 to 60 minutes prior to administration of vehicle solution or LPS, values for those parameters obtained immediately prior to the administration of either substance were considered as pre-injection controls. These values were compared to those obtained at 50 and 70 minutes post-injection for all groups.

Physiologic parameters as well as biochemical measurements were compared to each other using Wilcoxon's two-sample statistic (Mann-Whitney). This analysis was applied for comparison of experimental to baseline values within individual groups, as well as for inter-group comparisons. A P value of less than 0.05 was considered necessary for statistical significance. All values are reported as mean ± SEM.

Results

Physiologic studies

Whereas body temperature was stable at a mean value of 37.5 ± 0.08°C in vehicle treated animals of Group I, administration of LPS resulted in a rise in body temperature by an average of 1.1°C from a baseline of 37.5 ± 0.03°C to a mean peak value of 38.6 ± 0.04°C (Group II) at 50 minutes. Neither dazoxiben nor FPL-55712 prevented this hyperthermic response to endotoxin, body temperature averaging 38.7 ± 0.03°C and 38.6 ± 0.05°C in Groups IV and V, respectively, 70 minutes post-LPS. In addition, LPS administration was associated with a significant decrease in white blood cell count. In Group II animals, the baseline value of 15.3 ± 4.0 × 10^3 cells/mm³ fell to 4.2 ± 1.0 × 10^3 cells/mm³ (P < 0.05), a phenomenon not seen in vehicle treated time controls (Group I). A similar fall was observed in rats which received LPS in the presence of either dazoxiben (Group IV) or FPL-55712 (Group V).

The administration of endotoxin resulted in hemococoncentration with a rise in hematocrit (Hct) from 47.2 ± 0.6 to 51.0 ± 0.8 vol% (Group II, P < 0.05). This effect was also seen in the presence of dazoxiben (46.4 ± 0.7 to 49.2 ± 1.1, P < 0.05, Group IV), but not in vehicle-injected time controls (46.2 ± 0.6 to 46.4 ± 0.9 vol%, Group I), animals receiving vehicle injection in the presence of dazoxiben (45.5 ± 0.8 to 46.0 ± 1.0 vol%, Group III), or animals receiving LPS in the presence of the LT antagonist FPL-55712 (46.4 ± 1.1 to 47.7 ± 1.1 vol%, Group V).

Baseline AP was similar in all groups: 112 ± 4, 116 ± 3, 110 ± 5, 115 ± 3, and 108 ± 3 mm Hg in Groups I, II, III, IV, and V, respectively. Neither administration of vehicle solution (Group I), LPS (Group II), dazoxiben plus vehicle (Group III), dazoxiben plus LPS (Group IV), nor FPL-55712 plus LPS (Group V) had any significant effect on AP over the ensuing 50 to 70 minutes of observation.

50 minutes after administration of vehicle, RBF was maintained at 7.1 ± 0.3 ml/min, a value not significantly different from the baseline RBF of 7.0 ± 0.2 ml/min (Group I). By contrast, administration of LPS (Group II) resulted in a progressive fall in RBF, from a baseline of 6.9 ± 0.2 ml/min, reaching a nadir of 3.8 ± 0.4 ml/min at 35 to 50 min (P < 0.05 versus baseline and <0.001 versus Group I). This fall in RBF was maintained 70 minutes post-LPS. The administration of dazoxiben alone followed by vehicle injection (Group III) was without significant effect on RBF, which changed from a control value of 7.0 ± 0.6 ml/min to 6.7 ± 0.7 ml/min at 50 minutes. The fall in RBF associated with endotoxin administration was abrogated when LPS was administered in the presence of dazoxiben (Group IV). In this group of animals, RBF fell from 7.2 ± 0.3 to 6.3 ± 0.4 ml/min at 50 minutes post-LPS, a value not significantly different from baseline (0.1 > P > 0.05), and significantly greater (P < 0.001) than that obtained 50 minutes post-LPS (Group II). RBF was still maintained 70 minutes after endotoxin administration in dazoxiben pre-treated animals. In the presence of FPL-55712 (Group V), RBF fell from a mean control value of 7.1 ± 0.2 ml/min to 4.8 ± 0.8 ml/min at 35 minutes following LPS injection (P < 0.005 versus pre-injection control), but recovered to a value of 5.6 ± 0.5 ml/min at 50 minutes post-LPS. The latter value is still significantly reduced as compared to pre-LPS levels (P < 0.05), but is significantly greater (P < 0.01) than that obtained 50 minutes following injection of LPS alone (Group II). The recovery of RBF in FPL-55712-treated animals continued until 70 minutes post-LPS administration, at which time RBF averaged 5.9 ± 0.5 ml/min, a value not significantly different from pre-LPS control. The time course of changes in RBF for Groups I, II, IV, and V is summarized in Figure 1.

In parallel with its effect on RBF, LPS administration resulted in a progressive fall in GFR in Group II animals, on average from a baseline of 1.10 ± 0.05 to 0.32 ± 0.08 ml/min (P < 0.001) between 35 and 50 minutes after its injection (Table I). This reduction in GFR was still present 70 minutes following LPS (GFR = 0.27 ± 0.07 ml/min). No change in GFR was observed, either at 50 or 70 minutes in vehicle-treated controls (from 1.25 ± 0.05 to 1.25 ± 0.09 ml/min, Group I) or when vehicle injection was preceded by dazoxiben administration (from 1.14 ± 0.02 to 1.11 ± 0.10 ml/min, Group III). Dazoxiben pre-treatment (Group IV) did not prevent the fall in GFR (from
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<table>
<thead>
<tr>
<th>Group</th>
<th>Control GFR</th>
<th>GFR 50 min post-LPS or vehicle</th>
<th>GFR 70 min post-LPS or vehicle</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (Vehicle)</td>
<td>1.25 ± 0.05</td>
<td>1.25 ± 0.09</td>
<td>1.21 ± 0.03</td>
</tr>
<tr>
<td>II (LPS)</td>
<td>1.10 ± 0.05</td>
<td>0.32 ± 0.08*</td>
<td>0.27 ± 0.07*</td>
</tr>
<tr>
<td>IV (LPS + UK-37248)</td>
<td>1.18 ± 0.05</td>
<td>0.67 ± 0.08*</td>
<td>0.65 ± 0.03*</td>
</tr>
<tr>
<td>V (LPS + FPL-55712)</td>
<td>1.05 ± 0.10</td>
<td>0.46 ± 0.15*</td>
<td>0.57 ± 0.08*</td>
</tr>
</tbody>
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Values are mean ± SEM.

* P < 0.05 vs. Control

a P < 0.05 vs. Group II

1.18 ± 0.05 to 0.67 ± 0.08 ml/min, P < 0.001 following LPS, but the GFR value obtained between 35 and 50 minutes post-LPS in dazoxiben pre-treated rats (0.67 ± 0.08 ml/min) was significantly greater (P < 0.005) than that obtained in animals treated with LPS alone (0.32 ± 0.08 ml/min, Group II) and remained higher 70 minutes following endotoxin administration. The administration of FPL-55712 to LPS-treated animals in Group V failed to ameliorate the fall in GFR seen at 50 minutes post-LPS (GFR fell from 1.05 ± 0.10 ml/min to 0.46 ± 0.15 ml/min, P < 0.005). The difference between the values of GFR obtained 50 minutes following LPS alone (0.32 ± 0.08 ml/min, Group II) and that obtained following LPS in the presence of FPL-55712 (0.46 ± 0.15 ml/min, Group V) did not attain statistical significance. At 70 minutes, however, GFR had recovered to 0.57 ± 0.08 ml/min in FPL-55712 treated animals (Group V), a value significantly different from that obtained for rats receiving LPS alone (0.27 ± 0.07 ml/min, P < 0.025). The changes in GFR following LPS administration for the Groups I, II, IV, and V are summarized in Table 1.

Biochemical assays

Baseline renal cortical generation rates of TxB2, PGE2, and 6 keto PGF1α, as measured 50 minutes following administration of vehicle in Group I animals, averaged 35 ± 9, 466 ± 107, and 94 ± 3 pg/mg, respectively. Administration of LPS (Group II) resulted in a significant increase in all three products with generation rates of 106 ± 12 pg/mg for TxB2 (P < 0.005), 1075 ± 108 pg/mg for PGE2 (P < 0.025), and 221 ± 41 pg/mg for 6 keto PGF1α (P < 0.05) (Fig. 2).

Dazoxiben administration alone (Group III) had no significant effect on TxB2 (29 ± 3 pg/mg) or PGE2 (628 ± 51 pg/mg) generation as compared to time controls, but did result in an increase in baseline 6 keto PGF1α levels to 322 ± 166 pg/mg (P < 0.05). When dazoxiben pre-treated animals were given endotoxin (Group IV), TxB2 generation was inhibited to 29 ± 3 pg/mg (not significantly different from Group I, but significantly less than Group II, P < 0.005). LPS-induced increases in PGE2 and 6 keto PGF1α generation, however, were not affected by dazoxiben pre-treatment, values averaging 837 ± 62 pg/mg for PGE2 (P < 0.005 versus Group I and >0.05 versus Group II) and 179 ± 5 pg/mg for 6 keto PGF1α (P < 0.05 versus Group I and >0.05 versus Group II). In rats receiving LPS in the presence of FPL-55712, renal cortical generation rates of TxB2 were not different from those receiving LPS alone (97 ± 17 pg/mg), and were significantly greater than baseline levels observed in Group I (P < 0.005). Figure 2 summarizes the prostanoid generation rates for Groups I, II, and IV.

Morphologic studies

Light microscopic examination of kidneys from vehicle-treated and endotoxin-treated animals failed to reveal any differences between the two groups and was consistent with the appearance of normal rat kidney. In particular, there was no increase in glomerular cellularity and platelet adhesion to vascular endothelium was not noted.

Discussion

Administration of LPS was associated with its well known capacity to increase body temperature and decrease circulating white blood cell count [23]. Neither of these effects were abolished by dazoxiben or FPL-55712. The hemoconcentration which followed LPS administration, while being unaffected by TXA2 synthetase inhibition, was abolished by FPL-55712. In previous studies, this same compound was capable of abolishing the rise in hematocrit seen following exogenous administration of leukotriene C4 [10], an effect shown to be secondary to LT-induced plasma volume loss, likely resulting from its vasopermeability—enhancing effect [24]. Since the rise in hematocrit following LPS administration is probably secondary to
plasma volume loss [25], the capacity of FPL-55712 to prevent such losses suggests a role for endogenously produced LTs in mediating this effect. Despite prevention of hemocoagulation, FPL-55712 failed to abrogate the early fall in RBF and GFR seen at 50 minutes following LPS administration (Figure 1 and Table 1). Therefore, in this model of renal injury, factors other than those consequent to a loss of plasma volume play a role in the endotoxin-induced changes in renal hemodynamics.

Despite constancy of AP, RBF fell progressively starting 15 to 20 min after LPS administration reaching a nadir at 45 to 50 min (Fig. 1), thereby implicating an increase in renal vascular resistance as an underlying mechanism. (RVR increased from 17 ± 2 to 31 ± 3 mm Hg/ml-min, P < 0.01). This finding in the rat is in contrast to most studies of renal function during endotoxemia performed in the dog [3, 25] in which a fall in AP was almost always observed whether LPS was administered as a single bolus or by continuous infusion [26]. It is, however, consistent with the findings of Keeler [2] in the rat in which continuous infusion of E. Coli endotoxin resulted in an increase in RVR despite maintenance of AP. The rat has often been regarded as relatively insensitive to the systemic cardiovascular effects of endotoxin, thereby allowing for more rigorous evaluation of its renal actions. In the present study, the increase in RVR was accompanied by an increase in renal cortical generation rates of TXB2, PGE2, and 6 keto PGF1 (Fig. 2). These findings in kidney tissue are consistent with numerous observations in endotoxin-treated animals reported by others in which increases in both vasoconstrictor (TXA2) and vasodilator (PGE2 and PGJ2) products of arachidonic acid have been demonstrated in a variety of tissues [8, 14, 27]. Endotoxin is a potent stimulus of white blood cells in vitro leading to the release of arachidonic acid and its cyclooxygenase and lipooxygenase metabolites [6, 7]. Since TXA2, a potent vasoconstrictor, has been implicated in the pulmonary vasoconstriction following endotoxin in goats [9] and its inhibition shown to result in beneficial effects in various models of endotoxic injury [28–30], we attempted to selectively prevent its synthesis in this model of endotoxin-induced renal ischemia. Dazoxiben was shown to selectively inhibit the synthesis of TXA2 without apparent shunting of arachidonate into increased PGE2 or PGJ2 synthesis (Fig. 2). This inhibition of TXA2 synthesis was associated with prevention of the endotoxin-associated fall in RBF (Fig. 1), thereby assigning a major role for this eicosanoid in the endotoxin-induced increase in RVR. (RVR was unchanged: 16 ± 3 to 18 ± 4 mm Hg/ml-min, P > 0.05). In contrast to dazoxiben pre-treatment, administration of the LT antagonist FPL-55712 in a dose previously shown to totally block the effects of exogenously administered LTC4 in the same strain of rats [10], partially prevented the fall in RBF at 35 to 40 min following endotoxin (Fig. 1). (RVR increased from 15 ± 2 to 23 ± 3 mm Hg/ml-min, P < 0.05). However, antagonism of endogenous LTs, in the presence of FPL-55712, resulted in total recovery of RBF 70 minutes post-endotoxin, an effect not seen in its absence (Fig. 1). That the salutary effect of FPL-55712 was not due to inhibition of TXA2 is shown by the lack of inhibition of the endotoxin-induced increase in TXA2 generation in Group V animals.

The administration of endotoxin was associated with a dramatic reduction in GFR, measured as inulin clearance, between 35 and 50 minutes as well as between 60 and 70 minutes after its administration. The fall in GFR was not due solely to the fall in renal plasma flow since filtration fraction fell from 0.31 to 0.17 (at 50 minutes) and 0.14 (at 70 minutes). Factors other than renal hypoperfusion were therefore involved in mediating the fall in GFR in this model of acute renal injury. Of interest is the fact that despite prevention of the fall in RBF in dazoxiben treated animals, GFR, though significantly ameliorated, was not totally preserved. Furthermore, the LT antagonist FPL-55712, while resulting in significant recovery of RBF at 70 minutes, was also associated with attenuation of the fall in GFR at this time, but not its abrogation (Table 1). Of note, in the presence of either dazoxiben or FPL-55712, filtration fraction remained depressed at 70 minutes post-endotoxin, averaging 0.21 and 0.18, respectively. Antagonism of TXA2 and vasoconstrictor LTs, therefore, had greater protective effect on RBF than on GFR, though both were significantly improved relative to values in untreated animals. These observations suggest that, in addition to the fall in nephron perfusion caused, at least in part, by the presence of vasoconstrictor eicosanoids, other determinants of GFR must be depressed. Possible mechanisms include a fall in transcapillary hydraulic pressure related to afferent arteriolar constriction as well as a fall in the glomerular ultrafiltration coefficient. The latter may result from the intrarenal release of angiotensin II, a well known response to renal hypoperfusion.

In view of the above findings implicating the vasoconstrictor properties of TXA2 and sulfidopeptide LTs in endotoxin-induced renal ischemia, the issue of the source of these compounds during this model of injury becomes relevant. When kidneys were fixed perfused at 50 minutes post-endotoxin, (the time of maximal fall in RBF) using a method of perfusion fixation previously shown to preserve cellular elements adherent to the endothelial surface [31], a remarkable absence of platelet or other cellular infiltration was noted. Since sections of cortical tissue used for biochemical assays were also free of platelet or other cellular infiltrates, it seems possible that the major source of the increased levels of TXA2, PGE2, and PGJ2 in this form of renal injury is from indigenous renal parenchymal cells. In this regard, mesangial cells of macrophage–monocyte origin seem the most likely candidates to respond to the endotoxic stimulus [32–34]. Isolated glomeruli from kidneys subjected to antiglomerular basement membrane–antibody–induced injury are known to produce increased amounts of TXA2 and other eicosanoids [32]. Furthermore, recent evidence has suggested that the isolated perfused rat kidney is capable of synthesizing sulfidopeptide leukotrienes when stimulated with the calcium ionophore A23187 [35] and the enzymatic pathway leading to the synthesis of leukotrienes has been identified in renal tissue [35–37]. Our studies raise the possibility that these products play a role in mediating renal vasoconstriction in this model of acute renal failure.

In summary, the present experiments in the rat demonstrate the capacity of E. Coli endotoxin to induce an acute increase in RVR in the absence of systemic hypotension, and to decrease RBF, GFR, and filtration fraction. As in other organ beds, endotoxin seems to stimulate the synthesis of TXA2 and other cyclooxygenase products in the renal cortex, and this vasoconstrictor appears to play a major role in the reduced renal
perfusion and filtration rate seen during the early phase (35 to 50 minutes) of endotoxemic injury. In addition, evidence is presented to suggest that endogenously produced sulfidopeptide leukotrienes, previously shown to be potent constrictors of the renal vasculature, also play a role in perpetuating renal vasoconstriction at 60 to 70 minutes following endotoxin administration. While our methodology cannot totally exclude the possibility of extracellular or platelet elements as sources for the increased release of these eicosanoids, histologic examination failed to demonstrate the presence of such elements in the kidney at the height of the renal injury, thereby suggesting an intrinsic renal source.

Acknowledgments

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References

32. Schreiner GF, Kielty JM, Cotran RS, Unanue ER: Characterization of resident glomerular cells expressing la determinant and...

