Renal Vasoconstriction With U-46,619; Role of Arachidonate Metabolites

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ABSTRACT
Thromboxane A2 (TxA2) or its stable mimic U-46,619 can increase the generation of arachidonate metabolites. Therefore, these studies were designed to investigate the role of prostaglandins, TxA2, and leukotrienes in the renal vascular response to U-46,619. Anesthetized rats were studied during a basal period and during an intra-aortic infusion of vehicle or U-46,619 (1 μg/kg per minute). U-46,619 reduced the GFR and the RBF without changing the mean arterial pressure or the femoral vascular resistance. All of the effects of U-46,619 were blocked by pretreatment with the TxA2/prostaglandin H2 receptor antagonist SQ-29,548. Pretreatment with the cyclooxygenase inhibitor indomethacin did not modify the renal vascular response to U-46,619. However, pretreatment with the TxA2 synthesis inhibitor UK-38,485 or with the leukotriene D4/E4 antagonist LY-163,443 markedly blunted the U-46,619-induced increase in renal vascular resistance and the decrease in GFR. These results indicate that the renal vascular response to U-46,619 is receptor mediated and is promoted by TxA2 and leukotriene D4/E4.

Key Words: Thromboxane A2, prostaglandins, RBF, GFR, leukotrienes

Thromboxane A2 (TxA2) or other vasoconstrictor prostaglandins (PG), such as PGH2, are released by the kidney or blood vessels during the infusion of angiotensin II (1,2) and contribute to the increases in systemic and renal vascular resistances (RVR) (1–5). TxA2 and/or PGH2 have also been implicated in renal vasoconstriction or hypertension in Dahl salt-sensitive hypertension (6), renal parenchymal hypertension (7), renovascular hypertension (2,8), autologous immune nephritis (9), and ureteral occlusion (10). In these models, there is evidence of widespread production of TxA2 or PGH2 in the blood vessels or kidneys.

Infusions of TxA2 (11) or its stable analogue U-46,619 (12–15) reduce the RBF in the rat, dog, and pig. These actions may entail binding to the high-affinity receptors identified in rat renal glomeruli that are activated by TxA2 or PG endoperoxides such as PGH2 (16,17). However, there is considerable variability in the vascular response to TxA2 or its mimetics (18), suggesting that the response is heavily modulated. One set of modulators may be arachidonate metabolites because TxA2 mimetics can release PG and TxA2 itself from blood vessels (19,20). Therefore, the TxA2-induced release of arachidonate vasoconstrictor metabolites such as TxA2 and PGH2 or certain leukotrienes (LT) could augment the vasoconstrictor response to PGH2/TxA2 receptor activation. Indeed, inhibition of endogenous TxA2 generation in isolated aortic rings has been found to diminish the contractile response to U-46,619. This suggests that endogenous TxA2 release contributes to the response to U-46,619 (19). The aim of these studies was to investigate the role of PG, TxA2, and LT in modulating the renal and systemic vasoconstrictor actions of the TxA2/PGH2 mimetic U-46,619.

METHODS

Animal Preparation

Studies were performed on male Sprague-Dawley rats weighing 150 to 250 g maintained on a standard rat chow (Rodent Laboratories Chow 5001; Ralston Purina, St. Louis, MO). Anesthesia was induced with ip Inactin (100 mg/kg; BKY Gulden, Konstanz, Germany). Animals were maintained at 37°C on a rodent operating table. After tracheostomy, one external jugular vein was cannulated for iv infusions, and one carotid artery was cannulated retrogradely with the tip of the catheter passed into the root of the aorta for intra-aortic drug infusions. The right femoral artery was cannulated for blood sampling and for the measurement of mean arterial blood pressure (MAP) from the electrically damped output of a pressure transducer (Model P23; Gould, Oxnard, CA). The other femoral artery was cleaned and encircled by an electromagnetic flow probe (Carolina Instruments, Inc., Winston-Salem, NC) to measure femoral blood flow (FBF). The bladder was catheterized for the collection of urine samples.

Inulin (2 g/dL; Taylor Pharmacology, Decatur, IL), para-aminomhippuric acid (0.2 g/dL; Merck, Sharp, and Dohme, West Point, PA), and albumin (3 g/dL; Sigma Chemical Co., St. Louis, MO) in 0.154 M NaCl were given in a priming dose of 0.5 mL and as a maintenance infusion at 0.5 mL/100 g body wt per hour.

Thirty minutes after the completion of surgery, there was a basal period of 30 min while the rat received an intra-aortic infusion of 0.15 M NaCl solution at 1 mL/h. Thereafter, the
intra-aortic infusion was changed to a solution of U-46,619 or its vehicle. After 10 min, a second (experimental) period of 30 min was undertaken. Blood samples (0.6 mL) were obtained at the end of each clearance period and replaced with equal volumes of 3% albumin-in-saline solution. MAP and FBF were measured at the beginning, midpoint, and end of each clearance period, and the data were averaged.

Experimental Protocols

Series 1. Renal and Femoral Responses to U-46,619: Effects of SQ-29,548. The aim of this series was to investigate the effects of the TXA2/PGF2 mimetic U-46,619 on MAP and renal and femoral hemodynamics and to assess whether any actions could be blocked by a specific competitive antagonist of the TXA2/PGF2 receptor SQ-29,548 (21,22). A previous full dose response study in anesthetized rats had shown that the intra-aortic infusion of U-46,619 at 1 µg/kg per minute produced a half-maximal reduction in RBF without consistent effects on blood pressure (3).

In addition to the albumin-saline infusion, all rats received an iv infusion of drugs or vehicle added to a 0.15 M NaCl solution infused at 1 mL/h and an intra-aortic infusion of the TXA2/PGF2 mimetic U-46,619 (23) (1 µg/kg per minute) or vehicle added to 0.15 M NaCl solution and infused at 1 mL/h. Rats of Group 1 received an intra-aortic infusion of vehicle during Period 2. The remaining groups received U-46,619 (Upjohn Pharmaceuticals, Kalamazoo, MI) dissolved in 1% ethanol, diluted with 0.15 M NaCl solution, and infused during Period 2 at 1 µg/kg per minute intra-arterially. Rats of Groups 1 and 2 received an iv infusion of vehicle throughout. Rats of Group 3 received SQ-29,548 (Sigma, 5 mg/kg bolus at the completion of surgery and 5 mg/kg per hour iv throughout). SQ-29,548 (Bristol Myers Squibb, Princeton, NJ) was dissolved in ethanol and diluted in a 0.15 M NaCl solution. This dose was selected because it produced maximal blockade of the renal actions of endogenous TXA2 (24).

Series 2. Renal and Femoral Hemodynamic Response to U-46,619: Effect of Indomethacin, UK-38,485, and SQ-29,548. The aim of this series was to determine the effects of cyclooxygenase inhibition on the renal response to U-46,619. Rats were studied during a basal period and during an intra-aortic infusion of U-46,619 as in Series 1 above. Group 4 received a daily ip injection of the cyclooxygenase inhibitor indomethacin (2 mg/kg per day; Sigma) for 3 days before the study. Indomethacin was dissolved in a 1 M Na2CO3 solution at pH 12.5, titrated to pH 8.5 with HCl and diluted in 0.15 M saline. This dose reduces the excretion of PG and TXB2 by more than 70% (25). Group 5 received a daily ip injection of the TXA2 synthetase inhibitor UK-38,485 (50 mg/kg per day; Pfizer Central Research, Groton, CT) (26) for 3 days before the study. UK-38,485 was dissolved in 1 M NaOH at pH 12.5 and titrated with 1 N HCl to pH 8.5 at 100 mg/mL. This dose reduces the excretion of TXB2 in the rat by 50% without significant effects on the excretion of PGE2 or 6kPGF1α (21). Group 6 received the LTD4/E4 receptor antagonist LY-163,443 (5 mg/kg and 5 mg/kg per hour; Lilly Research, Indianapolis, IN) dissolved in 0.154 M NaCl. This is a specific competitive antagonist of LTD4/E4 receptors (27,28).

Chemical Methods and Calculations

Sodium concentrations in plasma and urine were measured by flame photometry (IL Flame Photometer, Lexington, MA). The GFR was estimated from the clearance of inulin and the RPF was estimated from the clearance of para-aminohippuric acid without correction for renal extraction. The RBF was calculated from RPF and hematocrit, and the RVR and femoral vascular resistance (FVR) were calculated from the MAP and the RBF or FBF. The fractional excretion of sodium (FeNa) was calculated from the urine-to-plasma concentration ratio for Na divided by that for insulin.

Statistics

In Series 1, the differences between Groups 1, 2, and 3 were compared by an unpaired t test. In Series 2, the effects of drug pretreatment on the response to U-46,619 were compared with Group 2 by analysis of variance in a 4-by-2 repeated-measures design. Differences were determined by post hoc t tests.

RESULTS

For Series 1, the hemodynamic and renal responses to U-46,619 and its antagonism by SQ-29,548 are shown in Table 1. In the time control group, which received an infusion of the vehicle for U-46,619 during the second period (Group 1), there was a small fall in MAP and a rise in urine flow (UV), but other parameters remained stable. In contrast, when U-46,619 was infused during Period 2 (Group 2), there were pronounced reductions in the GFR and RBF (averaging 48%), despite unchanged MAP and FVR. The calculated RVR more than doubled. All of these effects of U-46,619 were fully blocked by an infusion of SQ-29,548 given before and during the infusion of U-46,619.

For Series 2, pretreatment with indomethacin (Group 4) reduced the basal MAP and reversed the increase in UV seen during Period 2 but did not otherwise modify the response to U-46,619. Pretreatment with UK-38,485 (Group 5) reduced the basal FVR and increased the basal UV and FeNa. UK-38,485 blunted the reductions in GFR and RBF and the increase in RVR induced by U-46,619 by 50 to 75%. Pretreatment with LY-163,443 (Group 6) reduced the basal GFR and RBF and increased the RVR. This drug blunted or prevented the renal hemodynamic responses to U-46,619.

DISCUSSION

At the dose tested, the RVR and GFR were far more responsive to U-46,619 than were the MAP and FVR. Our previous studies showed that the infusion of U-46,619 at a dose of only 0.01 µg/kg per minute increased the tubuloglomerular feedback response (3) and reduced renin secretion (29). This highlights the extreme sensitivity and responsiveness of the kidney to infused U-46,619.

Rat glomerular membranes contain a high-affinity binding site for radiolabeled TXA2 ligands that is displaced by U-46,619 (16,17). The observation that the fall in RBF and GFR with U-46,619 was antagonized in full by SQ-29,548 suggests that U-46,619 activates TXA2/PGF2 receptors in vivo to initiate renal vasoconstriction.
Renal Vasoconstriction With U-46,619

TABLE 1. Responses of rats of Series 1 to a vehicle or a TxA2/PGH2 mimetic and effects of pretreatment with a TxA2/PGH2 receptor antagonist

<table>
<thead>
<tr>
<th>Group</th>
<th>MAP (mm Hg)</th>
<th>FVR (mm Hg/mL per min)</th>
<th>GFR (mL/min)</th>
<th>RBF (mm Hg/mL per min)</th>
<th>RVR (mm Hg/mL per min)</th>
<th>UV (Hg/mL)</th>
<th>FeNa (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (N = 7)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Basal</td>
<td>128 ± 2</td>
<td>29 ± 4</td>
<td>2.69 ± 0.18</td>
<td>12.8 ± 0.7</td>
<td>10 ± 1</td>
<td>9 ± 3</td>
<td>0.4 ± 0.3</td>
</tr>
<tr>
<td>2. Vehicle</td>
<td>124 ± 2</td>
<td>28 ± 4</td>
<td>2.64 ± 0.27</td>
<td>13.2 ± 1.1</td>
<td>10 ± 1</td>
<td>21 ± 6</td>
<td>1.0 ± 0.4</td>
</tr>
<tr>
<td>Change</td>
<td>-4 ± 1</td>
<td>-1 ± 1</td>
<td>-0.05 ± 0.15</td>
<td>+0.3 ± 0.8</td>
<td>0 ± 0</td>
<td>+11 ± 4</td>
<td>+0.6 ± 0.3</td>
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<tr>
<td>Group 2 (N = 10)</td>
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<td></td>
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</tr>
<tr>
<td>1. Basal</td>
<td>126 ± 4</td>
<td>35 ± 8</td>
<td>2.61 ± 0.17</td>
<td>12.7 ± 1.0</td>
<td>10 ± 1</td>
<td>9 ± 2</td>
<td>0.1 ± 0.1</td>
</tr>
<tr>
<td>2. U-46,619</td>
<td>132 ± 5</td>
<td>41 ± 6</td>
<td>1.35 ± 0.17</td>
<td>6.5 ± 0.8</td>
<td>23 ± 3</td>
<td>21 ± 6</td>
<td>0.7 ± 0.2</td>
</tr>
<tr>
<td>Change</td>
<td>+6 ± 4</td>
<td>+7 ± 3</td>
<td>-1.25 ± 0.13</td>
<td>-6.2 ± 0.8</td>
<td>+13 ± 3</td>
<td>+12 ± 5</td>
<td>+0.7 ± 0.2</td>
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<tr>
<td>Group 3 (N = 8)</td>
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<td></td>
</tr>
<tr>
<td>1. SQ-29,548</td>
<td>109 ± 4b</td>
<td>29 ± 2</td>
<td>2.25 ± 0.21</td>
<td>12.1 ± 1.2</td>
<td>10 ± 1</td>
<td>4 ± 1</td>
<td>0.1 ± 0.1</td>
</tr>
<tr>
<td>2. SQ + U-46,619</td>
<td></td>
<td>108 ± 6</td>
<td>28 ± 2</td>
<td>2.40 ± 0.21</td>
<td>11.8 ± 1.4</td>
<td>10 ± 1</td>
<td>13 ± 3</td>
</tr>
<tr>
<td>Change</td>
<td>-1 ± 3</td>
<td>-1 ± 1</td>
<td>+0.15 ± 0.23</td>
<td>-0.5 ± 1.5</td>
<td>+1 ± 1</td>
<td>+9 ± 3</td>
<td>+0.4 ± 0.2</td>
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<td>By t-test</td>
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<tr>
<td>Effects of U-46,619</td>
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<tr>
<td>(1 versus 2)</td>
<td>P &lt; 0.05</td>
<td>NS</td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.001</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Effects of SQ on response to U-46,619</td>
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<tr>
<td>(2 versus 3)</td>
<td>P &lt; 0.05</td>
<td>NS</td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.001</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

a Mean ± SE; data from rats of Series 1 for MAP, FVR, GFR, RBF, RVR, UV, and FeNa. The TxA2/PGH2 mimetic U-46,619 (1 µg/kg per min) or equivalent vehicle was infused during Period 2. For Group 3, the TxA2/PGH2 receptor antagonist SQ-29,548 (SQ) (Group 3) or vehicle (other groups) was infused throughout. Change: mean ± SE differences between Periods 1 and 2. NS, not significant.

b Compared with Group 1 in the basal period. P < 0.05.

As in a previous study in the anesthetized rat (25), the administration of SQ-29,548 reduced the MAP without altering renal hemodynamics, urine flow, or Na excretion. Relative to a small decline in the MAP of vehicle-infused control rats, U-46,619 led to a modest increase in MAP. This confirms the findings in a previous study in which U-46,619 increased the MAP but only in doses of 1 µg/kg per minute or above, whereas lower doses of 1 µg/kg per minute reduced the GFR and RBF (3). In the conscious rat, lower doses of U-46,619 of 0.1 µg/kg per minute increase the systolic blood pressure by about 20 mm Hg over a 3-day period of infusion, but after anesthesia, the blood pressure returned to normal levels (30). Therefore, some of the insensitivity of blood pressure to U-46,619 in these studies may relate to the effects of anesthesia. The local microperfusion of U-46,619 into the loop of Henle of the rat enhances net chloride absorption (3), but other effects on tubular transport have not been studied. It is possible that the modest rise in MAP during the infusion of U-46,619 was sufficient to maintain the urine flow rate and FeNa, despite a 50% reduction in the GFR. Previous studies with infusion of low doses of angiotensin II or its antagonists have shown that changes in blood pressure of only 10 mm Hg can have predominant effects in governing renal sodium excretion (29).

U-46,619 can promote the generation of TxA2 and prostacyclin (PGI2) in blood vessels and endothelial cells (19,20). The infusion of U-46,619 in the rat at a dose below that required to change renal hemodynamics increases the excretion of PG and TxB2 (29). Therefore, we investigated the functional consequences of the release of arachidonic metabolites by using drugs that inhibit the production of PG and TxA2 or that block LT receptors.

The MAP of rats that had received indomethacin pretreatment was significantly lower than that of the control group. This effect cannot be ascribed to the blockade of TxA2 generation because it was not seen with UK-38,485 pretreatment. However, it might relate in part to the blockade of the action of PGH2 because the MAP was rather lower after SQ-29,548 pretreatment in this, as in a previous study (25). Moreover, indomethacin has profound effects in reducing plasma renin activity in the anesthetized rat (31), and MAP is strongly dependent on angiotensin II in this model (25). Indomethacin pretreatment, in a dose that reduces TxA2 and PGI2 metabolite excretion substantially (25), did not modify the renal hemodynamic response to U-46,619. In one previous study, indomethacin led to a moderate potentiation of the renal vasoconstrictor response to a PG endoperoxide analogue (12). However, in other studies, indomethacin did not modify the vasoconstrictor action of U-46,619 on the mesenteric vessels of the cat (32) or the renal vessels of the dog (13) or pig (11). The conclusion that PG have little effect on the response to U-46,619 conflicts with the evidence of PG release. Therefore, we investigated an alternative hypothesis...
that there was a balanced release of vasodilator and vasoconstrictor cyclo-oxygenase products. The administration of a TxA2 synthase inhibitor indeed markedly blunted the renal vasoconstrictor actions of U-46,619. This extends the findings in an in vitro preparation of rat thoracic aortic rings where U-46,619 increased the local vascular synthesis of PGI2 and TxA2; the contractile response to U-46,619 increased the local vascular synthesis of PGI2 metabolites. However, our data in the intact kidney (21) or isolated blood vessel wall (19) do not suggest that UK-38,485 increases the release of PGI2 metabolites.

U-46,619-induced arachidonate release could generate other vasoactive metabolites besides TxA2 and PGI2. Indeed, TxA2 and LTB4 are released together from isolated rat lungs challenged with hypoxia (33). There are specific receptors for LTC4 in rat glomeruli (34), and the infusion of LTD4 reduces the RBF in the rat (35). Both LTC4 and LTD4 are vasoconstrictors in the isolated rat kidney (36) and can contract glomerular mesangial cells in culture (37). Our findings that the specific LTD4/E4 receptor antagonist LY-163,443, blunted the renal vasoconstrictor response to U-46,619 suggests that LT formation contributes to the TxA2-induced renal vasoconstriction in the intact rat kidney. However, the source of LT for this response is uncertain. Some synthesis of LTB4 by 5-lipoxygenase has been reported in isolated glomeruli (38), which also possess the enzymes required to process LTA4 to LTC4 and LTE4 (39). Previous studies have shown that TxA2 and LT contribute to increased RVR in rabbits with hydronephrosis (40).

This study provides pharmacologic evidence for a promotion of U-46,619-induced renal vasoconstriction by the generation of TxA2 and leukotrienes. Because the kidney is a major site for the production of arachidonate metabolites, this may be one reason why the kidney is so responsive to infused U-46,619. Moreover, the finding of widespread recruitment of vasoconstrictor pathways by U-46,619 may help to explain why TxA2 has been implicated in many models of renal vasoconstriction.

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REFERENCES


