Role of Pressor Prostanoids in Rats with Angiotensin II-Salt-Induced Hypertension

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SUMMARY This study was designed to assess the contribution of thromboxane A₂ to high blood pressure in rats with angiotensin II (Ang II)-salt hypertension. Hypertension was induced in rats drinking 0.15 M NaCl by infusion of Ang II (125 ng/min i.p.) for 12 days. Relative to values in water-drinking rats without Ang II infusion, Ang II-salt hypertensive rats exhibited augmentation (p < 0.05) of blood pressure (from 129 ± 3 to 217 ± 12 mm Hg), urinary thromboxane B₂ excretion (from 5.4 ± 0.9 to 25.4 ± 2.1 ng/day), and thromboxane B₂ release from renal cortex slices (from 28.8 ± 2.9 to 115.8 ± 12.8 pg/mg). Treatment with an inhibitor of thromboxane A₂ synthetase, UK 38485, had no effect on blood pressure in normotensive and Ang II-salt hypertensive rats. Treatment with a thromboxane A₂ receptor blocker, SQ 29548, decreased blood pressure in Ang II-salt hypertensive rats from 191 ± 9 to 152 ± 9 mm Hg after 3 hours, but it had no effect on blood pressure in normotensive rats. Since SQ 29548 interfered with the pressor effects of the prostaglandin endoperoxide analogue U-46619, prostaglandin F₂₁, and 9α,11β-prostaglandin F₂, we suggest that the SQ 29548-induced blood pressure reduction in Ang II-salt hypertensive rats is the manifestation of blockade of the vascular actions of one or more endogenous prostanoids including thromboxane A₂ and prostaglandin endoperoxides. If so, pressor prostanoids may be contributory factors in the pathogenesis of severe Ang II-salt hypertension in rats.

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KEY WORDS • hypertension • angiotensin II • salt • thromboxane • UK 38485 • SQ 29548

INCREASED urinary thromboxane B₂ (TXB₂) excretion and TXB₂ synthesis by platelet and renal structures accompany the development of hypertension in spontaneously hypertensive rats (SHR). The development of hypertension in rats with subtotal renal ablation and in saline-drinking rats infused with angiotensin II (Ang II) also is accompanied by increased TXB₂ urinary excretion. Since thromboxane A₂ (TXA₂), the biologically active precursor of TXB₂, produces vasoconstriction and platelet aggregation, the possibility arises that TXA₂ contributes to the pathogenesis of hypertension and associated vascular and renal disturbances in SHR and in other models of hypertension featuring augmentation of TXA₂ synthesis.

Participation of TXA₂ in the mechanisms of hypertension also is suggested by reports that treatment with inhibitors of TXA₂ synthetase lowers blood pressure in SHR and in rats with subtotal renal ablation hypertension, in association with improvement of renal hemodynamic and excretory functions and reduction of hypertension-related structural lesions of the renal microvasculature and glomeruli. However, in a recent study, blood pressure in SHR was affected by neither a TXA₂ synthesis inhibitor nor a TXA₂ receptor antagonist, indicating that it is premature to assign to TXA₂ a role in the pathogenesis of hypertension.

In saline-drinking rats, chronic infusion of Ang II causes severe hypertension associated with increased urinary excretion and glomerular synthesis of TXB₂. The present study was designed to assess the contribution of TXA₂ to high blood pressure in rats with Ang II-salt hypertension. To this end, we investigated the effect of a TXA₂ synthesis inhibitor and of a TXA₂ receptor antagonist on blood pressure in normotensive rats and in rats with Ang II-salt hypertension of 12 days' duration.

Materials and Methods

Studies were conducted on male Sprague-Dawley rats (Harlan Industries, Indianapolis, IN, USA) weighing 200 to 250 g. The animals were housed in individual metabolism cages or in group cages and were kept in a temperature-controlled (24°C) and humidity-controlled (50%) room that was illuminated between 600
and 1800; they were fed ad libitum a standard chow (Ralston Purina, St. Louis, MO, USA).

Protocol 1

The purpose of the study using Protocol 1 was to contrast normotensive rats and rats with Ang II–salt hypertension in terms of urinary TXB₂ excretion and rate of TXB₂ production by vascular and renal tissues in vitro.

An Alzet osmotic minipump (Model 2002, Alza, Palo Alto, CA, USA) filled with Ang II ([IIe⁵]-Ang II, Sigma Chemical, St. Louis, MO, USA) or with vehicle only (0.01 N acetic acid) was placed through a 1-cm midline incision in the abdominal cavity of rats anesthetized with methoxyflurane; the calculated infusion rate of Ang II was 125 ng/min. Animals infused with Ang II drank saline (0.15 M NaCl) throughout the study, whereas animals infused with vehicle drank tap water only. On the 12th day of Ang II or vehicle infusion, a 24-hour urine sample for measurement of TXB₂ was collected into containers kept in dry ice. Once urine sampling was completed, systolic blood pressure was determined by tail sphygmography (Narco Bio-Systems, Houston, TX, USA) after warming the rats at 37°C for 10 minutes. Subsequently, the animals were anesthetized with methoxyfluran, the abdomen and the thorax were exposed through a midline incision, all blood was removed from the animal by exsanguination coupled to transcardiac perfusion with Krebs-Ringer solution, and the thoracic aorta, the superior mesenteric artery, and one kidney were excised for estimation of in vitro TXB₂ release.

The concentration of TXB₂ in urine was measured by radioimmunoassay after prostanoids were purified according to a published method. The urinary excretion of TXB₂ was calculated as the product of a 24-hour urine volume and urinary TXB₂ concentration and was expressed as nanograms per 24 hours. In six normal rats, urinary TXB₂ excretion measured at 4-day intervals over a 20-day period ranged between 4.3 ± 0.6 and 7.5 ± 0.8 ng/day. The rate of TXB₂ release from vascular and renal tissues in vitro was estimated as described previously for other prostanoids. Briefly, slices of renal cortex and rings of thoracic aorta and superior mesenteric artery were placed in 20-ml flasks containing Krebs-Ringer solution, and the thoracic aorta, the superior mesenteric artery, and one kidney were excised for estimation of in vitro TXB₂ release.

The purpose of the study using Protocol 1 was to contrast normotensive rats and rats with Ang II–salt hypertension in terms of urinary TXB₂ excretion and rate of TXB₂ production by vascular and renal tissues in vitro.

Protocol 2

The purpose of the study using Protocol 2 was to investigate in normotensive and in Ang II–salt hypertensive rats the effect on blood pressure of treatment with UK 38458 (1S-[1α,2β(5Z),3β,4α]-7-[3-{[(phenylamino)carboxyl]hydrazino methyl]-7-oxabicyclo(2.2.1)hept-2-yl}-5-heptenoic acid; Squibb Institute for Medical Research) to block TXA₂ receptors. Saline-drinking rats infused with Ang II and water-drinking rats infused with vehicle only were prepared as described in Protocol 1. Eleven days after the onset of Ang II or vehicle infusion, the rats were anesthetized with an i.m. injection of xylazine (13 mg/kg) and ketamine hydrochloride (87 mg/kg) and the left femoral artery and vein were cannulated with polyethylene tubing for blood pressure recording and drug administration, respectively. The indwelling catheters, filled with saline containing heparin (100 IU/ml) and plugged with metal rod pins, were exteriorized at the nape of the neck. One day later, the awake rats were placed in plastic holders and the arterial cannula was connected to a pressure transducer (Model P23ID, Statham, Oxnard, CA, USA) connected to a polygraph (Model 7D, Grass, Quincy, MA, USA).

Normotensive and Ang II–salt hypertensive rats were left undisturbed for 60–90 minutes before being treated with UK 38458 dissolved in 0.1 M NaOH and adjusted to pH 8.5 with 0.1 M HCl, with SQ 29548 dissolved in 0.15 M NaCl containing 15 mM Na₂CO₃, or with the appropriate drug vehicle. UK 38458 treatment was initiated by i.v. injection of a 30 mg/kg bolus followed by i.v. infusion at 15 mg/kg/hr for 3 hours. SQ 29548 treatment was initiated by i.v. injection of a 2 mg/kg bolus followed by i.v. infusion at 2 mg/kg/hr for 3 hours. Three hours after the onset of treatment with UK 38458 or with its vehicle, blood was sampled from the femoral artery cannula of Ang II–salt hypertensive rats and was left to clot in a glass tube at 37°C for 1 hour; after centrifugation, the serum was frozen until the concentration of TXB₂ was measured by radioimmunoassay. The abdomen and the thorax were exposed through a midline incision, all blood was removed from the animal by exsanguination coupled to transcardiac perfusion with Krebs-Ringer solution, and the thoracic aorta, the superior mesenteric artery, and one kidney were excised for estimation of in vitro TXB₂ release.

Protocol 3

The purpose of the study using Protocol 3 was to ascertain the effectiveness and specificity of SQ 29548 as a TXA₂ receptor blocker. Normal rats were prepared with indwelling femoral arterial and venous catheters as described in Protocol 2. One day later, the effect on blood pressure of prostanoid and nonprostanoid pressor agents injected as an i.v. bolus was investigated in normotensive rats with and without SQ 29548 pretreatment (2 mg/kg i.v. bolus injection followed by 2 mg/kg/hr i.v. infusion). The pressor agents included in the study were U-46619, 15(S)-hydroxy-11α,9α (epoxy-methano)prosta-S₂-dienoic acid (0.5–5.0 μg; Cayman Chemical, Ann Arbor, MI, USA), a prosta-
Table 1. Systolic Blood Pressure, Urinary Excretion of Immunoreactive Thromboxane B₂, and Net Release of Thromboxane B₂ from Rings of Aorta and Superior Mesenteric Artery and from Slices of Renal Cortex, Incubated in Krebs-Ringer Solution for 20 Minutes

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Blood pressure (mm Hg)</th>
<th>Urinary TXB₂ (ng/day)</th>
<th>Renal cortex TXB₂ release (pg/mg)</th>
<th>Aorta TXB₂ release (pg/mg)</th>
<th>Superior mesenteric artery TXB₂ release (pg/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water-drinking, vehicle-infused</td>
<td>129 ± 3</td>
<td>5.4 ± 0.9</td>
<td>71.3 ± 6.7</td>
<td>28.8 ± 2.9</td>
<td>150.0 ± 29.4</td>
</tr>
<tr>
<td>Saline-drinking, Ang II-infused</td>
<td>217 ± 12*</td>
<td>25.4 ± 2.1*</td>
<td>121.1 ± 14.4*</td>
<td>115.8 ± 12.8*</td>
<td>374.5 ± 59.4*</td>
</tr>
</tbody>
</table>

Values are the means ± SE of observations in eight saline-drinking rats infused with Ang II (125 ng/min i.p.) and in eight water-drinking rats infused with vehicle only for 12 days. TXB₂ = thromboxane B₂.

*p < 0.05, compared with corresponding value in water-drinking, vehicle-infused rats.

glandin endoperoxide analogue that is an agonist for TXA₂ receptors; prostaglandin E₂ (PGE₂) and 9α,11β-prostaglandin F₂ (0.5–5.0 µg; Biomol Research Laboratories, Philadelphia, PA, USA), norepinephrine bitartrate (5–500 ng; Winthrop-Breon, New York, NY, USA), and Ang II (2.5–250 ng; Sigma).

Statistical Analysis

Results are expressed as the means ± SE. The data were analyzed by Student’s t tests (Protocols 1 and 3) or by analyses of variance for repeated measurements (Protocol 2). Probability values (p) less than 0.05 were considered significant.

Results

As shown in Table 1, the systolic blood pressure of saline-drinking rats infused with Ang II (125 ng/min i.p.) for 12 days was about 88 mm Hg higher (p < 0.01) than the blood pressure of water-drinking animals without Ang II infusion. Relative to values in water-drinking normotensive rats, rats with Ang II–salt hypertension of 12 days’ duration exhibited augmentation (p < 0.05) in the urinary excretion of immunoreactive TXB₂ and in the net release of immunoreactive TXB₂ from renal cortex slices and from rings of aorta and superior mesenteric artery during incubation in Krebs-Ringer solution for 20 minutes (see Table 1).

Figure 1 illustrates the blood pressure of water-drinking normotensive rats and of rats with Ang II–salt hypertension of 12 days’ duration before and during the administration of UK 38485 or vehicle. Treatment with UK 38485 for 3 hours did not affect the blood pressure of normotensive or Ang II–salt hypertensive rats. In confirmation of the effectiveness of UK 38485 to inhibit TXA₂ synthetase, the concentration of serum TXB₂ in Ang II–salt hypertensive rats treated with UK 38485 (2.8 ± 1.5 ng/ml) was only 1.4% (p < 0.01) of the serum TXB₂ concentration in hypertensive animals not treated with UK 38485 (200.3 ± 19.9 ng/ml). Similarly, the net release of TXB₂ from renal cortex slices (6.8 ± 1.2 pg/mg) and aortic rings (13.2 ± 2.0 pg/mg) obtained from Ang II–salt hypertensive rats receiving UK 38485 treatment was diminished (p < 0.01) relative to the values of TXB₂ release from renal cortical slices (155.4 ± 13.3 pg/mg) and aortic rings (130.0 ± 6.7 pg/mg) obtained from hypertensive animals not treated with UK 38485.

Figure 2 illustrates the blood pressure of water-drinking normotensive rats and of rats with Ang II–salt hypertension of 12 days’ duration before and during the administration of SQ 29548 or vehicle. Mean arterial pressure in awake rats with Ang II–salt hypertension fell from 191 ± 9 mm Hg before SQ 29548 treatment to 165 ± 11 (p < 0.01), 149 ± 12 (p < 0.01), and 152 ± 9 mm Hg (p < 0.01) after 1, 2, and 3 hours of treatment, respectively. In contrast, treatment with SQ...
29548 for 3 hours did not affect the blood pressure of water-drinking normotensive rats.

Figure 3 depicts the pressor responses to i.v. bolus injections of prostanoid and nonprostanoid agonists in awake normotensive rats with and without pretreatment with SQ 29548 to block TXA2 receptors. The prostaglandin endoperoxide analogue U-46619, a known agonist for TXA2 receptors, caused a dose-related elevation of mean blood pressure; that this effect was greatly reduced in rats receiving SQ 29548 is evidence of the ability of the latter to block vascular TXA2 receptors in vivo. Treatment with SQ 29548 also attenuated the pressor effect of PGF2α and of 9α,11β-prostaglandin F2, albeit not as much as it attenuated the pressor effect of U-46619. In contrast, the administration of SQ 29548 did not modify the pressor effect of bolus injections of Ang II and norepinephrine.

**Discussion**

This study demonstrates that saline-drinking rats infused with Ang II exhibit severe hypertension associated with increased urinary TXB2 excretion and TXB2 release from aortic rings, mesenteric artery rings, and renal cortex slices. The association of hypertension and increased TXB2 urinary excretion or production (or both) by renal structures was reported first in SHR1,2,5,6 and subsequently in rats with renal ablation hypertension,7 Dahl salt-sensitive hypertensive rats,16 and two-kidney, one clip hypertensive rats.17 Rats with severe hypertension and vascular disease may feature infiltration of the arterial vascular wall by blood-formed elements that are capable of synthesizing TXA2. Hence, augmentation of vascular and renal TXB2 production by vascular and renal cortical tissues in Ang II–salt hypertensive rats may be a secondary event reflecting vascular injury.

Inhibitors of TXA2 synthetase have been reported to reduce arterial blood pressure both in the developmental and the established phases of hypertension in SHR1,2,10,11 and to interfere with the development of renal ablation hypertension.7 In the present study, treatment with the TXA2 synthetase inhibitor UK 38485 greatly reduced the serum TXB2 concentration and the release of TXB2 from aortic rings and renal cortical slices, but it did not reduce the arterial blood pressure in rats with Ang II–salt hypertension of 12 days' duration. Yet, based on these findings, one cannot exclude a role for TXA2 as a contributory factor to Ang II–salt hypertension, as the functional consequence of a reduction in TXA2 due to inhibition of TXA2 synthetase may be obscured by the functional consequence of increased levels of prostaglandin en-
doperoxide, the precursor of TXA2, which is known to cause contraction of vascular smooth muscle and platelet aggregation by interacting with the TXA2 receptor. Furthermore, UK 38485 treatment did not completely inhibit tissue TXA2 synthesis.

In contrast to the lack of effect of the TXA2 synthetase inhibitor UK 38485 on blood pressure, our present study demonstrates that the administration of SQ 29548 causes blood pressure to fall in rats with Ang II–salt hypertension of 12 days' duration. That the blood pressure of normotensive rats was not reduced by treatment with SQ 29548 suggests selectivity in the blood pressure–lowering effect of the drug in Ang II–salt hypertensive rats. Such a selectivity would be expected if the hypotensive effect of SQ 29548 is the manifestation of interference with a mechanism contributing to set the level of arterial blood pressure in Ang II–salt hypertensive rats but not in normotensive rats.

SQ 29548 is known to block the response of platelets and vascular smooth muscle to TXA2 and TXA2 mimetic agents by preventing the agonist–TXA2 receptor interaction. Confirming that SQ 29548 blocks TXA2 receptors in vivo, we found that the pressor effect of U-46619, a stable analogue of prostaglandin H2 that interacts with the TXA2 receptors, was greatly reduced in SQ 29548–treated animals. We also found that SQ 29548 reduced the pressor effect of PGF2α and of 9α,11β-prostaglandin F2α, a metabolite of prostaglandin D2. Inasmuch as SQ 29548 pretreatment did not affect the pressor response to bolus injections of Ang II or norepinephrine, it may be concluded that the agent interferes only with prostanoid–induced pressor responses.

Based on the preceding discussion, the blood pressure–lowering effect of SQ 29548 in Ang II–salt hypertensive rats may be the expression of blockade of the vascular actions of one or more endogenous pressor prostanoids, including TXA2, and prostaglandin endoperoxides. If so, pressor prostanoids may be contributory elements in the pathogenesis of severe Ang II–salt hypertension in rats.

In summary, urinary TXB2 excretion and the release of TXB2 from vascular and renal cortical tissues in vitro are increased in rats with severe Ang II–salt hypertension of 12 days' duration. Treatment with an inhibitor of TXA2 synthetase, UK 38485, did not change the blood pressure of normotensive or of Ang II–salt hypertensive rats. Treatment with a TXA2 receptor blocker, SQ 29548, lowered blood pressure in Ang II–salt hypertensive but not in normotensive rats. Since SQ 29548 interfered with the rise in blood pressure elicited by a prostaglandin endoperoxide analogue, PGF2α, and 9α,11β-prostaglandin F2α, we suggest that the SQ 29548–induced blood pressure reduction in Ang II–salt hypertensive rats is the result of blockade of the vascular actions of one or more endogenous pressor prostanoids including TXA2 and prostaglandin endoperoxides.

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