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

MAHESH MISTRY AND ALBERTO NASJLETTI

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 71.3 ± 6.7 to 121.1 ± 14.4 pg/mg) and aortic rings (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 F₂, 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.

(Hypertension 11: 758-762, 1988)

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 (III)–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 methoxyflurane, 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 radioimmunoassay12 after prostanoids were purified according to a published method.13 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, measured to 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 normotensive rats with and without SQ 29548 pretreatment was calculated as described in Protocol 1.

Briefly, slices of renal cortex and rings of thoracic aorta and superior mesenteric artery were placed in 20-ml flasks containing Krebs-Ringer solution (2.0 ml) and were incubated for 20 minutes at 37°C in an atmosphere of 95% O2, 5% CO2 with 100 cycle/min agitation; the TXB2 released into the medium was measured by radioimmunoassay,12 and the results are expressed as picograms of immunoreactive TXB2 released during the 20-minute incubation period per milligram dry tissue.

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 38485 (1-cm 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 radioimmunoassay12 after prostanoids were purified according to a published method.13 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, measured to 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 normotensive rats with and without SQ 29548 pretreatment was calculated as described in Protocol 1.

Briefly, slices of renal cortex and rings of thoracic aorta and superior mesenteric artery were placed in 20-ml flasks containing Krebs-Ringer solution (2.0 ml) and were incubated for 20 minutes at 37°C in an atmosphere of 95% O2, 5% CO2 with 100 cycle/min agitation; the TXB2 released into the medium was measured by radioimmunoassay,12 and the results are expressed as picograms of immunoreactive TXB2 released during the 20-minute incubation period per milligram dry tissue.

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-SZ-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 (mmHg)</th>
<th>Urinary TXB₂ (ng/day)</th>
<th>Renal TXB₂ release (pg/mg)</th>
<th>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>
</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>
</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 F₂α (PGF₂α) 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 38458 or vehicle. Treatment with UK 38458 for 3 hours did not affect the blood pressure of normotensive or Ang II–salt hypertensive rats. In confirmation of the effectiveness of UK 38458 to inhibit TXA₂ synthetase, the concentration of serum TXB₂ in Ang II–salt hypertensive rats treated with UK 38458 (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 38458 (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 38458 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 38458.

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
Figure 3. Pressor effects of Ang II, norepinephrine, PGF$_2$,$\alpha$, 9$_\alpha$, 11$_\beta$-prostaglandin F$_2$, and U-46619 in normotensive rats with (closed bars) and without (open bars) SQ 29548 pretreatment (2 mg/kg i. v. bolus plus 2 mg/kg/hr infusion). All results are the means ± SE of six experiments. Asterisk (p < 0.001) and dagger (p < 0.005) denote significant difference between groups.

Discussion

This study demonstrates that saline-drinking rats infused with Ang II exhibit severe hypertension associated with increased urinary TXB$_2$ excretion and TXB$_2$ release from aortic rings, mesenteric artery rings, and renal cortex slices. The association of hypertension and increased TXB$_2$, urinary excretion or production (or both) by renal structures was reported first in SHR$^{1, 2, 5, 6}$ 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 TXA$_2$. Hence, augmentation of vascular and renal TXB$_2$ production by vascular and renal cortical tissues in Ang II–salt hypertensive rats may be a secondary event reflecting vascular injury.

Inhibitors of TXA$_2$ synthetase have been reported to reduce arterial blood pressure both in the developmental and the established phases of hypertension in SHR$^{1, 2, 10, 11}$ and to interfere with the development of renal ablation hypertension.$^7$ In the present study, treatment with the TXA$_2$ synthetase inhibitor UK 38485 greatly reduced the serum TXB$_2$ concentration and the release of TXB$_2$ 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 TXA$_2$, as a contributory factor to Ang II–salt hypertension, as the functional consequence of a reduction in TXA$_2$ due to inhibition of TXA$_2$ 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 receptor, 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 F2, 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 prostanoïd-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 prostanoïds, including TXA2 and prostaglandin endoperoxides. If so, pressor prostanoïds 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 F2, 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 prostanoïds including TXA2 and prostaglandin endoperoxides.
Role of pressor prostanoids in rats with angiotensin II-salt-induced hypertension.

M Mistry and A Nasjletti

*Hypertension*. 1988;11:758-762
doi: 10.1161/01.HYP.11.6.758

*Hypertension* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1988 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/11/6_Pt_2/758