Thromboxane Is Required for Full Expression of Angiotensin Hypertension in Rats

Henry L. Keen, Michael W. Brands, Mans J. Smith, Jr, Eugene W. Shek, John E. Hall

Abstract Recent studies suggest that thromboxane (TX) mediates a significant component of angiotensin II (ANG II)-induced hypertension. However, there is little information to support the hypothesis that this relationship is important during chronic, physiological increases in ANG II, particularly while controlling for variation in endogenous ANG II levels induced by TX inhibition. This study tested that hypothesis in 27 chronically instrumented rats. After baseline measurement, suppression of endogenous TX was induced and maintained throughout the study in 13 rats by IV infusion of the thromboxane synthesis inhibitor (TSI) U63557A, the other 14 rats remained throughout the study in 13 rats by IV infusion of the received vehicle. Baseline mean arterial pressure (MAP) was not different between groups and was unchanged by TSI or vehicle. Continuous inhibition of ANG II production was then initiated in both groups of rats by IV infusion of the angiotensin-converting enzyme inhibitor (ACEI) benazepril. ACEI reduced blood pressure similarly in vehicle and TSI rats, from 105 ± 3 to 91 ± 2 mm Hg and 103 ± 1 to 89 ± 1 mm Hg, respectively. ANG II was then infused at 5 ng kg⁻¹ min⁻¹ IV for 7 days in six rats from each group to restore ANG II activity to baseline levels. This dose increased MAP to 103 ± 2 and 101 ± 1 mm Hg in vehicle and TSI rats, respectively, values not different from pre-ACEI levels. Seven TSI rats and eight vehicle rats received a higher dose of ANG II (20 ng kg⁻¹ min⁻¹ IV) After 7 days, MAP was higher in vehicle than in TSI rats (143 ± 5 versus 120 ± 4 mm Hg). These results suggest that endogenous TX is an important determinant of MAP in ANG II hypertension but may have a diminished role in blood pressure regulation when ANG II is at normal and subnormal levels (Hypertension. 1997; 29[part 2]:310-314.)

Key Words • angiotensin II • thromboxane • blood pressure • hypertension

Numerous interactions exist between thromboxane, a labile cyclooxygenase-dependent metabolite of arachidonic acid, and the renin-angiotensin system. Infusion of ANG II stimulates thromboxane production,1-5 and inhibition of thromboxane synthesis attenuates the acute renal and systemic actions of ANG II infusion.1,2 Furthermore, thromboxane has been suggested to be important in various forms of chronic ANG II-dependent hypertension such as two-kidney, one-clip hypertension and the spontaneously hypertensive rat.6 The hypertensive action of thromboxane may be due, in part, to alterations in renal hemodynamics. The increase in renal vascular resistance and the development of hypertension in the spontaneously hypertensive rat are almost totally prevented by chronic administration of a thromboxane synthesis inhibitor. Furthermore, Mistry et al.7 have shown that concurrent administration of a thromboxane receptor antagonist attenuated the increase in renal vascular resistance and the increase in blood pressure caused by the combination of high salt diet and minipump infusion of high dose ANG II.

In acute studies, thromboxane infusion inhibits renal release while blockade of thromboxane receptors or inhibition of thromboxane synthesis stimulates renal secretion.9 Variation in endogenous levels of ANG II secondary to thromboxane inhibition could make difficult interpretation of the role of thromboxane per se during ANG II infusion. Therefore, the objective of this study was to determine the role of endogenous thromboxane in regulation of renal and systemic hemodynamics when ANG II levels were clamped either at normal levels or at elevated levels within the physiological range.

Methods

Male Sprague-Dawley rats weighing 325 to 350 g were used for all experiments. Surgery and care of the rats were conducted in accordance with National Institutes of Health guidelines using protocols approved by the Animal Care and Use Committee of the University of Mississippi Medical Center. Under pentobarbital sodium anesthesia and aseptic conditions, a laparotomy was performed, and a nonocclusive polyvinyl catheter was inserted into the abdominal aorta, distal to the kidneys, through a puncture made with an 18-gauge needle up. The insertion point was sealed with cyanoacrylate adhesive, and the catheter was exteriorized through the lateral abdominal wall. A femoral vein catheter was implanted through a separate incision and the tip was maneuvered into the inferior vena cava distal to the kidneys. Incisions were infiltrated with penicillin G procaine and sensorcaine, and both catheters were routed subcutaneously to the scapular region and exteriorized through a stainless steel button that was implanted subcutaneously.

After recovery from surgery, the rats were placed in individual metabolic cages in a quiet, air-conditioned room with a 12-hour light cycle. The catheters were connected to a dual-channel infusion swivel (Instech) mounted above the cage and were protected by a stainless steel spring. The arterial catheter was filled with heparin solution (1000 USP U/mL) and connected, via the swivel, to a pressure transducer (Cobe) mounted on the cage exterior at the level of the rat. Pulsaatile arterial pressure signals were sent to an analog-to-digital converter and analyzed by computer using customized software. The analog signal was sampled 4 seconds each minute, 24 hours per day.

The rats received food and water ad libitum throughout the study. An intravenous infusion of 20.5 mL sterile 0.9% saline per day combined with sodium-deficient rat chow, allowed sodium intake to be clamped at 31 mmol/d independent of food intake. This infusion was started immediately after placement of the rats in the metabolic cages, and 5 to 7 days were allowed for accl-
Selected Abbreviations and Acronyms

ANG = angiotensin
tX = thromboxane
TSI = TX synthetase inhibitor
MAP = mean arterial pressure
ACE = angiotensin-converting enzyme
GFR = glomerular filtration rate
PRA = plasma renin activity
PG = prostaglandin
ACEI = ACE inhibitor
TGF = tubuloglomerular feedback

Results

Thromboxane Synthesis Inhibition

Baseline MAP and urinary sodium excretion were not different between the two groups and were unchanged by administration of TSI or vehicle. In contrast, GFR increased from 2.7±0.2 to 3.3±0.1 mL/min, renal plasma flow increased from 7.2±0.2 to 9.7±0.5 mL/min, and PRA increased from 2.7±0.2 to 3.1±0.2 ng ANG I per milliliter per hour during TSI administration. Chronic TSI caused a partial suppression of urinary thromboxane B2 excretion. Compared with vehicle rats, this inhibition averaged approximately 60% to 70% (Table 1). There was no difference in excretion of 6-keto-PGF1α between the two groups.

ACE Inhibition

ACE inhibition reduced MAP similarly in vehicle and TSI rats, from 105±2 to 91±2 mm Hg and 103±1 to 89±1 mm Hg, respectively, and PRA increased markedly from 2.9±0.1 to 3.1±0.2 to 55.0±4.7 and 57.7±7.2 ng ANG I mL⁻¹ hr⁻¹, respectively. There was no change in urinary sodium excretion or GFR in either group. Renal plasma flow was unchanged in the TSI rats, but tended to increase, although not significantly, from 8.0±0.5 to 9.1±0.5 mL/min in vehicle-treated rats. There was no significant change in urinary excretion of 6-keto-PGF1α in either group or of thromboxane B2 in TSI rats, however, there was a small but significant reduction in thromboxane B2 excretion in vehicle rats (Table 1).

Angiotensin II Infusion at 5 ng kg⁻¹ min⁻¹

As shown in Fig 1, ANG II infusion at 5 ng kg⁻¹ min⁻¹ increased MAP to 103±2 and 101±1 mm Hg in vehicle and TSI rats, respectively, values not different than pre-ACEI levels. GFR and renal plasma flow were not different between the two groups prior to the ANG II infusion, and both tended to decrease, although not significantly, during the ANG II infusion. PRA was restored to near normal levels, averaging 4.8±1.2 and 5.5±0.9 ng ANG I mL⁻¹ hr⁻¹ in vehicle and TSI rats, respectively. Urinary sodium excretion averaged 2.8±0.2 and 2.7±0.1 mmol/d in vehicle and TSI rats, respectively, and decreased during the first 2 to 3 days of the ANG II infusion in both groups before sodium balance was achieved. There was no change in urinary excretion of 6-keto-PGF1α in either group, but urinary thromboxane B2 excretion increased approximately 15% in the vehicle rats (Table 2).

Angiotensin II Infusion at 20 ng kg⁻¹ min⁻¹

ANG II infusion at 20 ng kg⁻¹ min⁻¹ significantly increased MAP in both groups of rats (Fig 3). However, the 19 mm Hg increase in the TSI rats was a significant attenuation compared with the 40-mm Hg increase measured in the vehicle rats. There were no differences in GFR or renal plasma flow between the two groups prior to the ANG II infusion, and GFR was not changed.

Table 1. Urinary Excretion of 6-Keto-PGF1α and TXB2 During the Baseline Period and in Response to TSI or ACE Inhibition

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline</th>
<th>TSI/Vehicle</th>
<th>TSI/Vehicle + ACEI</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-Keto-PGF1α, ng/d</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle (n=14)</td>
<td>8.7±0.8</td>
<td>8.9±0.6</td>
<td>8.4±0.8</td>
</tr>
<tr>
<td>TSI (n=13)</td>
<td>8.8±0.6</td>
<td>8.7±0.4</td>
<td>8.7±0.6</td>
</tr>
<tr>
<td>TXB2, ng/d</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle (n=14)</td>
<td>21.1±0.0</td>
<td>19.7±0.5</td>
<td>17.5±0.6*</td>
</tr>
<tr>
<td>TSI (n=13)</td>
<td>20.5±0.4</td>
<td>7.1±0.2†</td>
<td>6.6±0.4†</td>
</tr>
</tbody>
</table>

*P<0.05 compared with baseline
†P<0.05 compared with vehicle
with this dose of ANG II in either group (Fig 4). Renal plasma flow decreased in both groups of rats; however, the reduction was statistically significant only in the vehicle rats. PRA was suppressed to 0.39±0.12 and 0.47±0.11 ng ANG 1·mL⁻¹·h⁻¹ in vehicle and TSI rats, respectively. Urinary sodium excretion fell on day 1 of the ANG II infusion in both groups and sodium balance was restored by day 2. Urinary excretion of 6-keto PGF₁α increased significantly in both groups, but excretion of thromboxane B₂ increased only in the vehicle rats (Table 2).

Discussion

The main findings from this study are that thromboxane is an important mediator of the hypertensive response to chronic, physiological increases in ANG II, but may play a lesser role in regulation of blood pressure when ANG II is at normal or reduced levels.

Previous studies have shown that thromboxane synthesis inhibitors or receptor antagonists do not affect blood pressure under basal conditions. However, interpretation of those studies is complicated by the fact that thromboxane synthesis inhibition increases PRA and, presumably, endogenous levels of ANG II. This increase in ANG II then might offset any potential blood pressure–lowering action of thromboxane inhibition. To address this issue, ANG II levels were clamped at normal in the present study by chronic converting enzyme inhibition and IV infusion of ANG II at a rate that returned ANG II activity (assessed by steady state MAP) to control levels. Under these conditions, with ANG II held constant, there still was no effect of chronic TSI on blood pressure. This evidence suggests that thromboxane is not a major regulator of blood pressure under basal conditions. In addition, the similar blood pressure reduction in TSI and vehicle rats given ACEI alone suggests that TX does not have an important effect on blood pressure when ANG II is low. However, since chronic TSI only partially reduced urinary thromboxane

### Table 2. Urinary Excretion of 6-Keto-PGF₁α and TXB₂ Before, During, and After Infusion of ANG II at 5 or 20 ng·kg⁻¹·min⁻¹

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>ANG II</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANG II at 5 ng·kg⁻¹·min⁻¹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6-Keto-PGF₁α, ng/d</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle (n=6)</td>
<td>9.9±0.9</td>
<td>7.8±0.5</td>
<td>8.1±1.0</td>
</tr>
<tr>
<td>TSI (n=6)</td>
<td>0.6±0.6</td>
<td>4.5±1.0</td>
<td>0.2±0.6</td>
</tr>
<tr>
<td>TXB₂, ng/d</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle (n=6)</td>
<td>173±12</td>
<td>22.2±11</td>
<td>18.2±15</td>
</tr>
<tr>
<td>TSI (n=6)</td>
<td>69±0.6</td>
<td>6±8±11</td>
<td>5.8±0.7</td>
</tr>
<tr>
<td>ANG II at 20 ng·kg⁻¹·min⁻¹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6-Keto-PGF₁α, ng/d</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle (n=8)</td>
<td>7.6±0.8</td>
<td>13.7±1.6</td>
<td>7.6±0.6</td>
</tr>
<tr>
<td>TSI (n=7)</td>
<td>8.5±0.7</td>
<td>12.0±0.9</td>
<td>7.7±0.7</td>
</tr>
<tr>
<td>TXB₂, ng/d</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle (n=8)</td>
<td>17.6±1.1</td>
<td>34.0±3.2</td>
<td>19.6±1.6</td>
</tr>
<tr>
<td>TSI (n=7)</td>
<td>6.4±0.6</td>
<td>6.0±0.9</td>
<td>6.0±0.5</td>
</tr>
</tbody>
</table>

*Control* values are the *TSI/vehicle+ACEI* values from Table 1 segregated according to the appropriate ANG II infusion dose

*P<0.05 compared with control

†P<0.05 compared with vehicle.
excretion (60% to 70%), it is possible that the remaining thromboxane may have had an effect on blood pressure which would have been revealed by more complete inhibition of thromboxane synthesis.

In contrast to those results, when ANG II levels were increased above normal, blood pressure was significantly higher in vehicle rats compared with TSI rats. This suggests that full expression of ANG II hypertension requires an intact ability to synthesize thromboxane. However, because inhibition of thromboxane synthetase has been suggested to redirect prostaglandin endoperoxides into enzymatic pathways for vasodilatory PGs, another explanation is that these vasodilatory PGs were responsible for the attenuated increase in blood pressure associated with TSI. In support of this possibility, furosemide-stimulated renal prostaglandin production is enhanced by pretreatment with a thromboxane synthetase inhibitor. In the present study, there was a significant increase in urinary excretion of 6-keto-PGF1α, the stable hydrolysis product of PG12, during the high dose ANG II infusion. However, there was no difference in urinary excretion of 6-keto-PGF1α between the two groups, suggesting that PG12 levels were not affected by TSI in this study. In addition, because the TSI dose was constant throughout the study, any potential stimulatory effect on synthesis of vasodilator PGs that might influence blood pressure also should have been present throughout. However, there was no indication that this potential mechanism had any effect on blood pressure.

Another potential factor that could have influenced these results is an alteration in bradykinin metabolism secondary to ACE inhibition. However, since both groups of rats were treated with the same fixed dose of ACE inhibitor throughout the study, it is unlikely that the differential blood pressure response between vehicle and TSI rats was due to a difference in bradykinin metabolism, although this possibility cannot be completely ruled out. Moreover, there was a clear effect of urinary thromboxane B2 levels to vary directly with ANG II levels, but only in the vehicle-treated rats and only markedly at the ANG II infusion dose that increased ANG II activity above normal. This suggests, therefore, that the attenuated ANG II hypertension in the TSI rats was due to a reduction in ANG II-stimulated thromboxane synthesis.

The mechanism through which thromboxane potentiates the hypertensive action of ANG II is not known. Thromboxane is known to have both vascular and tubular actions. One possibility is that thromboxane interacts with ANG II at a tubular site. However, the tubular actions of ANG II predominate in the lowest effective concentration range, and the similar changes in blood pressure in vehicle and TSI rats in response to ACEI and to restoration of normal ANG II activity suggests that thromboxane did not have a significant effect on the tubular actions of ANG II. With increased ANG II levels, the renal vascular actions are of increased importance, and this was confirmed by the significant decrease in renal plasma flow measured with ANG II hypertension. However, this effect was significantly greater in the vehicle rats. Thus, the effect of TSI to attenuate ANG II-induced renal vasoconstriction and the increase in blood pressure suggests that throm-
boxane potentiated the hypertensive action of ANG II via interaction primarily at a vascular site. ANG II is known to constrict the efferent arteriole indirectly by activation of the TGF mechanism. Furthermore, thromboxane also has been shown to potentiate TGF while thromboxane synthesis inhibition markedly attenuates this response. It is possible, therefore, that thromboxane could interact with ANG II at the macula densa to activate TGF and constrict the efferent arteriole. However, in the present study, the maintenance of GFR with a significant fall in renal plasma flow suggests that the predominant site of vasoconstriction is the efferent arteriole. Although most studies have shown the predominant site of constriction for exogenously administered thromboxane to be the afferent arteriole, Tucker et al. reported that inhibition of endogenous thromboxane synthesis caused a reduction in both afferent and efferent arteriolar resistance. This provides evidence that the direct effect of ANG II to constrict the efferent arteriole could be influenced by thromboxane.

Thus, these results suggest that ANG II levels increased within the physiological range cause an increase in blood pressure that is mediated significantly through the effect of ANG II to increase thromboxane synthesis. However, the importance of thromboxane in determining the blood pressure and renal hemodynamic response to ANG II may be diminished when ANG II is at normal or reduced levels. In addition, these results suggest that the effect of thromboxane to potentiate the hypertensive action of ANG II may be due to interaction with ANG II in the control of renal vascular resistance, possibly at the level of the efferent arteriole.

Acknowledgments

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