TP receptors regulate renal hemodynamics during angiotensin II slow pressor response

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METHODS

Animals. Pathogen-free male and female TP-R heterozygous mice (TP+/−; C57/black6J background, Jackson Lab) were generously provided by Dr. T. Coffman (Division of Nephrology, Duke Univ., Durham, NC) (33). These had been backcrossed for more than nine generations. They were inbred and genotyped in our laboratory. Male homozygous (TP−/−) and wild-type (TP+/+) mice were housed in a quiet room at 25°C with a 12:12-h light-dark cycle and free access to food and water. This study was approved by the Georgetown University Animal Care and Use Committee.

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Delivery of ANG II and AT1A receptor antagonist. The details of the mouse slow pressor response model have been published (16). ANG II (Peninsula Laboratory, San Carlos, CA) was infused at 0 [vehicle (V)] and 400 ng·kg⁻¹·min⁻¹ (ANG II 400) through subcutaneous (sc) osmotic minipumps (model 1002; Alza, Palo Alto, CA). One group of TP −/− mice infused with ANG II received the selective AT1A receptor antagonist candesartan (Cand; Astra Zeneca). This was dissolved in the drinking water at a dose of 25 mg/l. This is a fully effective dose in a rat model of renovascular hypertension (36).

Renal function studies. Twelve to 14 days after implantation of the minipumps, mice were prepared for clearance experiments, as described previously (16). The following groups were studied: TP +/+ Veh (n = 7), TP −/− Veh (n = 7), TP +/+ ANG II (n = 7), TP −/− ANG II (n = 9), and TP −/− ANG II with Cand (n = 6).

Renal excretion of aldosterone, 8-isoprostane, TXB2, 6-keto-PGF1α, TBARS, and NOx. On days 12 and 13, mice were housed in mouse metabolic cages (Nalgene Nunc International, Rochester, NY) and were fed a NOx-free synthetic diet. Urine was collected for 24 h into antibiotics (penicillin G: 0.8 mg, streptomycin: 2.6 mg, and amphotericin B: 5.0 mg). Aldosterone was measured by a RIA kit (Diagnostic Systems Lab). Total 8-isoprostane (8-iso-PGF2α), TXB2, and 6-keto-PGF1α in urine were purified, extracted, diluted, and assayed with an enzyme immunoassay (EIA) procedure (Cayman Chemical, Ann Arbor, MI) using a method that we validated (27). TBARS were measured by an OXItec TBARS assay kit (ZeoMetriC). Nitrate plus nitrite (NOx) was measured in a NO Chemiluminescence Analyzer (model 270B, Sievers Instrument). These values are factored by creatinine, which was measured in a Creatinine Analyzer2 (Beckman Instruments).

Plasma ANG I and TBARS. Blood was collected from the femoral artery into EDTA-containing tubes under anesthesia with 1% isoﬂurane and was centrifuged to obtain plasma. Plasma ANG I was measured by a RIA kit (Peninsula Laboratories). TBARS were measured by an OXItec TBARS assay kit (ZeoMetriC).

Statistics. Results are expressed as means ± SE. Statistical analyses of four groups were performed with two-way factorial ANOVA to determine the interaction of two factors. If there was a positive interaction (shown in the figures as Interaction ANG II × TP-R), a post hoc t-test was performed to assess the difference between groups (shown in the figures by bars). If there was no interaction, the individual effects of ANG II and TP-Rs were assessed by two-way factorial ANOVA (shown in the figures as ANG II or TP-R). Statistical analyses of two groups were performed with a Student’s t-test, and the differences are reported with a nominal P value. Statistical significance was defined as P < 0.05. Professional statistical advice was kindly provided by Franca Benedicty Barton M.S. (Dept. of Biostatistics, Georgetown University).

Table 1. Total body and kidney weight, heart rate, hematocrit, plasma electrolytes, and urine volume of TP-R-deficient mice infused with ANG II

<table>
<thead>
<tr>
<th></th>
<th>TP-R (+/+)</th>
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<tr>
<td></td>
<td>Day 14</td>
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<tr>
<td>n</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td>Total body weight, g</td>
<td>25.2 ± 1.3</td>
<td>25.9 ± 1.1</td>
<td>24.1 ± 1.0</td>
<td>25.0 ± 1.2</td>
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<tr>
<td>ΔBW, %</td>
<td>3.4 ± 0.7</td>
<td>3.0 ± 1.3</td>
<td>8.9 ± 0.9</td>
<td>2.9 ± 1.3</td>
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<tr>
<td>Kidney weight, g</td>
<td>0.29 ± 0.01</td>
<td>0.35 ± 0.02</td>
<td>0.32 ± 0.02</td>
<td>0.35 ± 0.02</td>
</tr>
<tr>
<td>%Kidney weight, g BW</td>
<td>1.21 ± 0.04</td>
<td>1.27 ± 0.03</td>
<td>1.16 ± 0.04</td>
<td>1.25 ± 0.03</td>
</tr>
<tr>
<td>Heart rate, min</td>
<td>440 ± 100</td>
<td>419 ± 20</td>
<td>428 ± 16</td>
<td>431 ± 13</td>
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<tr>
<td>Hematocrit, %</td>
<td>38 ± 1</td>
<td>38 ± 1</td>
<td>41 ± 1</td>
<td>37 ± 1</td>
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<tr>
<td>Plasma Na⁺, meq/l</td>
<td>146 ± 1</td>
<td>148 ± 1</td>
<td>146 ± 1</td>
<td>149 ± 1</td>
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<tr>
<td>Plasma K⁺, meq/l</td>
<td>3.8 ± 0.2</td>
<td>3.9 ± 0.2</td>
<td>3.6 ± 0.3</td>
<td>3.6 ± 0.2</td>
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<tr>
<td>Plasma Cl⁻, meq/l</td>
<td>113 ± 2</td>
<td>114 ± 1</td>
<td>112 ± 3</td>
<td>117 ± 1</td>
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<tr>
<td>n</td>
<td>6</td>
<td>6</td>
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<tr>
<td>24-H urine volume, ml</td>
<td>1.2 ± 0.2</td>
<td>1.5 ± 0.2</td>
<td>2.0 ± 0.3</td>
<td>1.8 ± 0.2</td>
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<th>ANG II</th>
<th>TP-R</th>
<th>Interaction</th>
</tr>
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Values are means ± SE, n, No. of mice; BW, body weight; TP-R, thromboxane A2–prostaglandin H2 receptor; n.s., not significant; N/A, not available.
ANG I and increased the aldosterone (TP-R: P < 0.001). This effect was not affected by TP-R deletion. AT1 receptor blockade of TP −/− mice infused with ANG II reduced the renal excretion of aldosterone (P < 0.001).

**Plasma TBARS and renal excretion of TBARS and 8-iso-PGF2α.** Figure 4 shows the plasma TBARS and the renal excretion of TBARS and 8-iso-PGF2α in TP −/− and +/+ mice after 12–14 days of Veh or ANG II infusion. TP-R deletion increased basal TBARS and 8-iso-PGF2α excretion (P < 0.05). There was a significant interaction between TP-Rs and ANG II for plasma TBARS (P < 0.05) and the excretion of TBARS and 8-iso-PGF2α (TBARS: P < 0.05, 8-iso-PGF2α: P < 0.05). ANG II infusion in TP +/+ mice increased plasma TBARS (P < 0.05) and excretion of TBARS (P < 0.05) and 8-iso-PGF2α (P < 0.01). In contrast, a similar infusion into TP −/− mice did not change excretion of TBARS and 8-iso-PGF2α.

**Renal excretion of NOx.** Figure 5 shows the renal excretions of NOx in TP −/− and +/+ mice during 12 days of Veh or ANG II infusion. TP-R deletion increased basal NOx excretion (P < 0.05). There was a significant interaction between TP-Rs and ANG II for NOx (P < 0.05). As anticipated, infusion of ANG II into TP +/+ mice did not change the excretion of NOx (P < 0.05). In contrast, a similar infusion into TP −/− mice increased excretion of NOx (P < 0.01). This implies that TP-Rs counteract NO production during ANG II infusion.

**Renal excretion of TxB2 and 6-keto-PGF1α.** Figure 6 shows the renal excretion of TxB2 and 6-keto-PGF1α in mice after 12 days of Veh or ANG II infusion. TP-R deletion increased basal TxB2 excretion (P < 0.01). There was a significant interaction between TP-Rs and ANG II for TxB2 (P < 0.05) and 6-keto-PGF1α (P < 0.05). ANG II infusion in TP +/+ mice increased the excretion of TxB2 (P < 0.01) and 6-keto-PGF1α (P < 0.05). In contrast, a similar infusion into TP −/− mice did not change excretion of TxB2 or 6-keto-PGF1α. This implies that TP-Rs mediate ANG II-induced increases in TxB2 and 6-keto-PGF1α.

**DISCUSSION**

We confirmed that ANG II increases MAP, FF, and RVR and increases parameters of oxidative stress (16) in wild-type mice and that the pressor response to ANG II is diminished in the TP −/− mouse (12). The main new findings are that TP −/− mice infused with ANG II fail to increase oxidative stress or PGs but have an enhanced NO production with a paradoxical reduction in RVR. The RVR and oxidative stress in TP −/− mice infused with ANG II are not responsive to AT1 receptor blockade.

As in the study by Coffman et al. (12), using a higher dose of ANG II, TP-R deletion blunted the rise in MAP with ANG II. This cannot be ascribed to a failure to stimulate aldosterone (Fig. 3). RVR normally accounts for 20% of the total peripheral vascular resistance. Whereas ANG II increased RVR by +19% in TP +/+ mice, it reduced the RVR by −12% in TP −/− mice. Therefore, this paradoxical reduction in RVR may account for a part of the blunted rise in MAP with ANG II in the TP −/− mice. The blunted pressor response and the paradoxical reduction in renal vasoconstriction to ANG II in TFα2 and oxidative stress (as indicated by reduced plasma TBARS and unchanged excretion of 8-iso and TBARS) and an increase in the NO (as indicated by increased excretion of NOx). Thus mice infused with the permeant nitroxide superoxide dismutase mimetic tempol at a dose that prevents oxidative stress do not have a rise in MAP and have blunted renal vasoconstriction with ANG II, as in the TP −/− mice in this study (16). We concluded that the principal site of vasoconstriction of O2·− generated during ANG II was on the preglomerular arterioles (6, 16, 31). The present finding that ANG II failed to increase RVR or FF in TP −/− mice suggests that activation of TP-Rs by ANG II enhances the pre- and postglomerular vascular resistance in the mouse kidney. Afferent arterioles from ANG II-infused rabbit have an enhanced vasoconstriction to ANG II that depends on O2·− and TP-Rs (26, 34, 35). Activation of TP-Rs can also cause renal vasoconstriction by promotion of tubuloglomerular feedback (TGF) (37). O2·− and TXα2 generated in response to prolonged ANG II potentiate vasoconstriction (38). Thus the effect of TP-Rs to mediate renal vasoconstriction during oxidative stress accompanying prolonged ANG II infusion likely entails direct effects in the renal microvessels and enhanced TGF responses.

The renal circulation is uniquely sensitive to TP-R activation during infusion of a TP-R agonist, U-46,619, perhaps reflecting activation of TGF (37). Indeed, Yamaguchi et al. (44) showed...
that renal vasoconstriction during TP-R activation is prevented by blockade of TGF by a loop diuretic. TGF causes vasoconstriction of the afferent arteriole that is enhanced during ANG II infusion (3, 23) or TP-R activation (37). Therefore, the failure of TP−/− mice to decrease RVR during ANG II infusion could be a consequence of a failure to enhance TGF-induced preglomerular vasoconstriction. Schnermann et al. (28) reported that whereas TP−/− mice reduce glomerular capillary pressure with increased delivery to NaCl to the macula densa, they have a blunted macula densa regulation of single-nephron GFR. This implies a blunted role for TGF in the regulation of glomerular hemodynamics.

ANG II increases the FF in normal mice. This effect is not prevented by tempol (16), but, in the present study, was prevented by TP-R deletion. This suggests that ANG II induces vasoconstriction of the postglomerular vessels through activation of TP-Rs and that, in contrast to the preglomerular effect, this is likely independent of O₂· formation. Arima et al. (4) and Ren et al. (25) proposed that prostanoids and 20-HETE released from the glomeruli by ANG II regulate postglomerular vascular resistance. Because metabolites of 20-HETE and PGH₂ both provide ligands for the TP-R (9, 14, 29), those findings are consistent with our present study.

Our result with an AT₁ receptor antagonist confirms the conclusion of Coffman et al. (5, 7) that AT₁ receptors mediate the increase in aldosterone with ANG II but shows further that this is independent of TP-Rs. In contrast, an AT₁ receptor antagonist did not change renal hemodynamics or excretion of oxidative stress markers in TP−/− mice infused with ANG II. This indicates that, during the ANG II slow pressor response,
AT₁ receptors mediate the vasoconstriction and O₂⁻ formation indirectly through activation of TP-Rs. Because NADPH oxidase is the major source for O₂⁻ generation in the blood vessels and kidneys and is activated by AT₁ receptors (6, 15), induction of NADPH oxidase during ANG II infusion apparently may require TP-Rs.

AT₁ receptors activate TP-Rs, but the precise mechanisms are not explored in this study. Whether this relates to an increase in the amount or the sensitivity of TP-Rs or simply to an increase in the production of eicosanoid agonists, which activate TP-Rs, requires further study.

Mice with AT₂ receptor gene deletion have an enhanced pressor response to ANG II (30). Thus the renal vasodilator response in TP⁻/⁻ mice during ANG II infusion could be an unopposed vasodilator effect mediated via AT₂ receptors (1).

Activation of TP-R in the aorta or kidneys releases endogenous TxA₂ (11, 41). The present study demonstrates that ANG II fails to increase excretion of TxB₂ or 6-keto-PGF₁α in TP⁻/⁻ mice. This indicates that TP-Rs mediate the effect of ANG II to activate phospholipase or COXs.

The present study confirms that prolonged ANG II infusion does not increase NOx excretion (8), despite an increase in NO synthase isoform expression in the kidney (24). The present study implicates TP-Rs in preventing NO generation, because NOx excretion increased with ANG II only in TP⁻/⁻ mice. The failure of NOx to increase normally with ANG II may be

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**Fig. 3.** Neither ANG I nor aldosterone was not affected by TP-R deletion. See Fig. 1 legend.

**Fig. 4.** TP-Rs mediate ANG II-induced O₂⁻ formation and AT₁ receptor blockade has no effect on O₂⁻ formation in TP⁻/⁻ mice infused with ANG II. See Fig. 1 legend. TBARS, thiobarbituric acid-reactive substances.
a consequence of increased O$_2^\cdot$ generation. Accordingly, it may be a failure to generate O$_2^\cdot$ with ANG II in TP $^{-/-}$ mice that promotes increased NOx excretion. Whether the paradoxical reduction in RVR with ANG II in TP $^{-/-}$ mice relates to a failure to enhance O$_2^\cdot$ generation or to an accompanying increase in NO generation requires further study.

The higher baseline FF and RVR in TP $^{-/-}$ mice in the present study are not consistent with TP-Rs being a vasoconstrictor signaling pathway. This could indicate compensatory activation of other vasoconstrictive signals in TP $^{-/-}$ mice. The FF and RVR commonly increase during activation of the renin-angiotensin-aldosterone system, and we reported that inhibition of TP-Rs or TxA$_2$ synthase increases plasma renin activity (39). However, TP $^{-/-}$ mice had a normal level of ANG I and aldosterone and a normal regulation during ANG II infusion. Moreover, TP $^{-/-}$ mice had no change in FF or RVR during AT$_1$ receptor blockade with candesartan. It is possible that an increased oxidative stress may account for the renal vasoconstriction, but the cause for the oxidative stress is obscure. Rats infused with TP-R antagonists do not have an increase in RVR or FF (37, 39, 41). Therefore, the increase in RVR and FF in TP $^{-/-}$ mice may be a consequence of a developmental defect in these mice. TP $^{-/-}$ mice in this study had consistently enlarged kidneys.

In conclusion, a slow pressor dose of ANG II increases generation of PGs including TxA$_2$ and causes oxidative stress. AT$_1$ receptors activate TP-Rs to mediate the increase in RVR, FF, O$_2^\cdot$ formation, and a part of the pressor response, but suppression of ANG I and stimulation of aldosterone secretion are independent of TP-Rs. The paradoxical reduction in RVR with ANG II in the absence of TP-Rs may relate to the absence of an increase in oxidative stress or to a rise in NO generation.

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GRANTS

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