Roles of vasoconstrictor prostaglandins, COX-1 and -2, and TP receptors in a rat model of early 2K,1C hypertension

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Angiotensin (ANG) II activates phospholipase A2 (PLA2) to release arachidonate (AA), whose metabolism by cyclooxygenases (COXs) generates prostaglandins (PGs). PGs normally have an antihypertensive action (12). Thus nonsteroidal anti-inflammatory drugs (NSAIDs) exacerbate human hypertension (4). However, PGH2 (prostaglandin endoperoxide), thromboxane (Tx)A2 generated by TxA2 synthase (TxA2-S), and prostaglandin metabolites (TGF) responses (39, 40), potentiate central actions of ANG II on the sympathetic nervous system, vasopressin release, and drinking (5, 18, 45), and enhance renal NaCl reabsorption (41). Activation of TPRs has salt-retaining and other prohypertensive actions (37). Unlike in essential hypertension, NSAIDs such as aspirin or indomethacin reduce blood pressure (BP) in human renovascular hypertension (10) and two-kidney, one-clip (2K,1C) hypertension in the rat (32), suggesting a potentially important role for vasoconstrictor PGs in these conditions.1

The relative roles of COX-1 versus COX-2 products in ANG II-dependent hypertension are controversial. Thus, during short-term ANG II infusion in the mouse, blockade or genetic deletion of COX-1, but not COX-2, moderates hypertension and renal medullary vasoconstriction (28). In contrast, prolonged administration of a COX-2 antagonist moderates hypertension in a rat aortic coarctation model of renovascular hypertension (36) and in a prior study in early 2K,1C hypertension in the rat (26), whereas a COX-1 antagonist is not effective (19). This may relate to a reduction in plasma renin activity (PRA) accompanying prolonged COX-2 blockade reported in some (26, 36) but not all (6) studies on 2K,1C rats.

ANG II acting on type 1 receptors (AT1R) activates reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (2) that generates O2−•. Metabolism of O2−• by superoxide dismutase generates H2O2, which activates PLA2 (34) and COX (7) and enhances the membrane expression of TPRs (35). Therefore, ANG II may cause hypertension via generation of COX metabolites or by Iso that activate TPRs. In contrast, activation of ANG II type 2 receptors (AT2R) reduces hypertension in a renal wrap model (31).

There is increased renal generation of PGs including the prostacyclin (PGI2) metabolite 6-keto-PGF1α and the TxA2 metabolite TxB2 in the clipped kidneys or glomeruli of rats with 2K,1C hypertension (8, 32). AT1R and TPR maintain hypertension throughout 2K,1C hypertension (21, 22, 43). Studies implicate TxA2 (8, 46, 48) and/or PGH2 as the predominant vasoconstrictor PG in renovascular models (21, 22).

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The present studies were undertaken in the early (3 wk), ANG II-dependent phase of 2K,1C Goldblatt hypertension in the anesthetized rat. They were designed to test the hypothesis that ANG II acts on AT₁Rs or AT₂Rs to generate products of COX-1 or -2 that activate TPRs to maintain hypertension. The first aim was to contrast the generation of renin, vasoconstrictor PGs, and markers of oxidative stress in sham-operated and 2K,1C hypertensive rats. The second aim was to test the acute BP responses to graded doses, or short infusions, of drugs that inhibit the generation of ANG II by angiotensin-converting enzyme (ACE) or of PGs by COX-1 or -2 or that block AT1R, AT₂R, or TPR to assess the contributions of these pathways to the maintenance of hypertension. Studies were performed after acute drug administration to minimize the effects of renin secretion.

METHODS

All animal protocols were reviewed and approved by the Animal Use and Care Committee of Georgetown University. Male Sprague-Dawley rats (4–6 wk old; 125–150 g) were fed a standard rat chow (Na⁺ content of 0.35 g/100 g; Rodent Laboratory Chow 5001; Ralston Purina, St. Louis, MO). 2K,1C Goldblatt hypertension was induced under halothane anesthesia. The left renal artery was approached through a flank incision. It was cleared of supporting tissue. A 0.22-mm inner diameter silver clip was placed around it (2K,1C hypertension) or the artery was prepared but not clipped (sham operation). The wound was closed with sutures, and the animal was allowed to recover. Animals were housed in individual cages for 3 wk. Studies were undertaken in five series of rats.

All studies were undertaken under anesthesia with thiobutabarbital (Inactin; 100 mg/kg ip). Rats received 0.5 ml 0.154 M NaCl at 1 ml/h; 2K,1C hypertensive rats. The second group was placed in metabolism cages for collection of 24 h urine. In anesthetized rats and the excretion of vehicle (0.154 M NaCl solution; n = 8), SC-560 (COX-1 inhibitor; 0.003, 0.01, 0.03, 0.1, and 0.3 mg/kg; n = 8), valdecoxib (COX-2 inhibitor; 0.1, 0.3, 1, 3, and 10 mg/kg; n = 8), or indomethacin (noselective COX inhibitor; 0.03, 0.1, 0.3, 1, and 10 mg/kg; n = 8).

MAP was found to fall to a nadir within 20 min of the injection of ACEI. Therefore, data are presented as changes in MAP at this time after which rats received the next bolus dose of the test agent.

Another two groups of rats were prepared as above and given vehicle (n = 9) or a supramaximal dose of benazeprilat (100 mg/kg iv; n = 8). Urine was collected from both kidneys via a cannula in the bladder for 45 min after vehicle or ACEI administration for analysis of TxB₂.

Series III tested the hypothesis that the maintenance of hypertension depends on the generation of COX-1 or -2 products. Four groups of anesthetized 2K,1C rats received graded intravenous injections of vehicle (0.154 M NaCl solution; n = 8), SC-560 (COX-1 inhibitor; 0.003, 0.01, 0.03, 0.1, and 0.3 mg/kg; n = 8), valdecoxib (COX-2 inhibitor; 0.1, 0.3, 1, 3, and 10 mg/kg; n = 8), or indomethacin (noselective COX inhibitor; 0.03, 0.1, 0.3, 1, and 10 mg/kg; n = 8).

These doses of SC-560 and valdecoxib were selected to be equivalent to those of indomethacin when adjusted for relative blockade of COX-1 or COX-2, respectively, based on the molar IC₅₀ value of each drug for inhibition of each COX isofrom (4, 24). Valdecoxib is the active principal of paracoxib (33).

Additional rats (n = 3) were studied by using the Na₂CO₃ solution required to solubilize indomethacin (42) as a further vehicle control group. This small quantity of Na₂CO₃ did not produce changes in BP different from those with the saline vehicle (data not presented).

Series IV tested the hypothesis that hypertension is maintained by AT₁R, AT₂R, or TPR. Four groups of 2K,1C rats received a bolus followed by a constant intravenous infusion over 150 min of vehicle (0.154 M NaCl at 1 ml/h; n = 8), losartan (AT₁R antagonist; 10 mg/kg and 10 mg·kg⁻¹·h⁻¹; n = 6), PD-123319 (AT₂R antagonist; 3 mg/kg and 3 mg·kg⁻¹·h⁻¹; n = 5), or SQ-29548 (TPR antagonist; 10 mg/kg and 10 mg·kg⁻¹·h⁻¹; n = 10).

This dose of SQ-29548 was selected from pilot studies (n = 6) that showed that 3 and 30 mg/kg doses of SQ-29548 produced equivalent and apparently maximal reductions in MAP. The doses of losartan and PD-123319 were selected from studies of ANG II-infused rats (20, 40).

Bolus injections were given over 1 min. MAP was recorded as a maximum reduction within 20 min, which encompassed the full early effects for bolus injections when assessed in pilot studies over 30-min periods. All groups had similar starting values for MAP. Therefore, data are presented as changes in MAP from baseline.

Series V tested the hypothesis that the reductions in MAP seen with SQ-29548 and SC-560 (but not with valdecoxib) could be ascribed to reductions in PRA or TxA₂ generation in the postclip kidneys. Groups of 2K,1C rats were prepared as in series III and IV and given vehicle,
SQ-29548, SC-560, or valdecoxib. Thereafter, blood was drawn for PRA, and the postclip kidney was removed and frozen at 

\[-80^\circ C\]

before extraction and assay for TxB2 (39).

**Twenty-four-hour urine collections.** A 24-h urine was collected from rats of series I into containers with indomethacin and antibiotics to prevent ex vivo COX metabolism or bacterial overgrowth (2, 39). It was filtered and centrifuged, and the supernatant was frozen at 

\[-70^\circ C\]

until analysis.

**Chemical methods.** For assay of TxB2, each sample was spiked with \(^{3}H\)-labeled TxB2 to assess individual recovery. Samples were purified and extracted with organic extraction, thin-layer chromatography and C18 column separation before analysis with a radioimmunoassay for TxB2. The details of the method, together with normal values, recovery, and intra- and interassay coefficients of variation and validation against GC-MS were published previously (39).

Plasma for MDA was measured as described previously (2) (Oxi-Tek, Zeptometrix, Buffalo, NY). Blood for PRA was taken into cooled EDTA-containing tubes, and the plasma was frozen at 

\[-70^\circ C\]

PRA was assayed from the generation of ANG I (42).

**Statistical analysis.** Data are presented as means ± SE. Within each series, data were analyzed at each time point for changes in MAP relative to baseline with analysis of variance (ANOVA) for repeat observations. Where appropriate, a post hoc Dunnett’s \(t\)-test was used to assess differences from vehicle between groups. Statistical significance is taken at \(P < 0.05\).

## RESULTS

Data are shown in Fig. 1 for sham-operated control and 2K,1C rats 2–3 wk after operation. Compared with control rats, anesthetized 2K,1C rats had significant elevations of MAP that averaged 35 mmHg and twofold elevations of PRA and plasma MDA. TxB2 excretion by conscious 2K,1C rats during a 24-h urine collection from both kidneys also was increased twofold.

Changes in MAP at 20 min after administration of vehicle or drugs to rats of series II are shown in Fig. 2. There was a small reduction in MAP with vehicle. Administration of benazeprilat (ACEI) produced graded reductions of MAP with a maximal effect of \(-36 ± 5\) mmHg (\(P < 0.001\)). Renal excretion of TxB2 from both kidneys was significantly lower over 45 min after administration of benazeprilat than after vehicle (\(26 ± 4\) vs. \(40 ± 5\) pg/min; \(P < 0.05\)).

The results of series III are shown in Fig. 3. The COX-1 antagonist SC-560 (Fig. 3A) reduced MAP significantly, with a maximum effect at 20 min of \(-28 ± 7\) mmHg (\(P < 0.001\) vs. vehicle). In contrast, administration of the COX-2 antagonist valdecoxib (Fig. 3B) produced no significant changes in MAP (\(-9 ± 7\) mmHg; nonsignificant vs. vehicle). The nonselective COX inhibitor indomethacin reduced MAP by \(-25 ± 5\) mmHg (\(P < 0.001\) vs. vehicle). When plotted at equivalent doses based on relative ID50 values for inhibition of COX-1 (Fig. 3A), the responses to indomethacin were closely similar.
to those of SC-560. In contrast, for equivalent COX-2 inhibitory doses, the responses to indomethacin were quite distinct from those of valdecoxib (Fig. 3B).

Results of series IV are shown in Fig. 4. There were no significant changes with vehicle. Infusion of the AT\textsubscript{1}R antagonist losartan reduced MAP by 24 ± 6 mmHg ($P < 0.001$) at 150 min. This was similar to the reduction achieved with the TPR antagonist SQ-29548 of 22 ± 3 mmHg ($P < 0.001$). In contrast, the AT\textsubscript{2}R antagonist PD-123319 failed to change MAP.

Results of series V are shown in Fig. 5. At the time that MAP was reduced by SQ-29548 or SC-560 (but not by valdecoxib) in series III and IV, there were no significant changes in PRA (Fig. 5A). However, the generation of TxB\textsubscript{2} by the postclip kidney was reduced by SC-560 and was increased by valdecoxib (Fig. 5B). We conclude that the falls in MAP cannot be ascribed to reductions in PRA, and that the doses of SC-560 and valdecoxib are effective in changing renal TxA\textsubscript{2} generation.

**DISCUSSION**

This study was undertaken during the early phase of 2K,1C hypertension in the rat, characterized by a doubling of PRA, lipid peroxidation products, and renal excretion of TxB\textsubscript{2} and a 35-mmHg increase in MAP assessed under anesthesia. We confirm that blockade of the generation of ANG II reduces MAP substantially and report that this is accompanied by a 35% reduction in renal excretion of TxB\textsubscript{2}. The acute administration of a COX-1 antagonist in this model reduces BP similarly to an NSAID and reduces renal TxB\textsubscript{2} generation, whereas a COX-2 antagonist does not change MAP and increases renal TxB\textsubscript{2} generation. The role of vasoconstrictor PGs was shown further by the similar reductions in MAP following inhibition of COX-1 or blockade of TPR or AT\textsubscript{1}R. At the time that these measurements of BP were undertaken, there were no significant effects of the COX-1 or -2 antagonists or the TPR antagonist on PRA.

One limitation is that rats were studied under anesthesia. However, prior studies in conscious 2K,1C rats studied 3 wk after clipping have shown similar increases in BP and PRA and similar reductions in MAP with AT\textsubscript{1}R or TPR antagonists (43). A second limitation is that urine was collected from the clipped and nonclipped kidneys together. However, enhanced TxB\textsubscript{2} release has been shown directly in studies of isolated glomeruli from the postclipped kidneys of 2K,1C rats (32).

We confirm that an ACEI or AT\textsubscript{1}R antagonist reverses hypertension in early 2K,1C hypertension (43). The AT\textsubscript{2}R apparently is not implicated, since PD-123319 failed to moderate hypertension, consistent with the failure of an AT\textsubscript{2}R antagonist to change the BP in ANG II-infused rats (20) or the 2K,1C hypertensive mouse (9). In contrast, blockade of AT\textsubscript{2}Rs enhances hypertension in renal wrap hypertension (31). The prohypertensive actions of ANG II in early 2K,1C hypertension apparently are expressed predominantly via AT\textsubscript{1}Rs.

Stahl et al. (32) demonstrated that the clipped kidneys of 2K,1C hypertensive rats release excessive thromboxane, similar to the findings from excised kidney of patients with renovascular hypertension (1). Himmelstein and Klotman (8) demonstrated that infusion of a TxA\textsubscript{2}-S inhibitor or a TPR antagonist reduces BP in the 2K,1C rat model. The present study, which also was at an early phase of 2K,1C hypertension, confirms a substantial increase in the excretion of TxB\textsubscript{2} and a substantial fall in MAP with a TPR antagonist.
NSAIDs normally increase BP in hypertension patients, indicating a net antihypertensive effect of COX products (4). In contrast, indomethacin given to animal models of the late phase of renovascular hypertension reduces MAP (13, 32). Acute aspirin administration to patients with renovascular hypertension reduces BP, PRA, and release of PGE2 from the poststenotic kidney (10, 11). Renin release in the rat is inhibited by indomethacin (13, 42) and by prolonged COX-2 blockade in the aortic coarctation model (3) and in two reports in the early 2K,1C rat model (26, 36) of renovascular hypertension in which the COX-2 blocker also reduces the MAP. However, a third study of prolonged COX-2 blockade did not detect a reduction in PRA or BP in this model (6). COX-1 blockade is reported not to modulate renin release (3). Blockade of TPRs increases PRA (42). Thus studies were undertaken with acute dosing of drugs to obviate the effects of large changes in renin secretion seen during prolonged administration in rat models of renovascular hypertension (3, 13, 26, 32, 36). The role of PRA in the changes in BP reported in this study was examined in a further series of 2K,1C rats dosed as in the MAP studies. At the time corresponding to the changes in MAP, there were no significant changes in PRA in 2K,1C rats after SC-560, SQ-29548, or valdecoxib, consistent with the half-life of renin in the rat of 65 min (17). We conclude that the reductions in MAP produced by SQ-29548 and SC-560 in this protocol occur without reductions in PRA.

Breyer and coworkers (28) reported that genetic deletion or inhibition of COX-1, but not COX-2, moderates the rise in BP and renal medullary vasoconstriction during acute infusion of ANG II in mice. Our results in this ANG II-dependent hypertensive model confirm the importance of COX-1 in maintaining MAP.

These studies have not identified the predominant PG activating TPRs in this model. Prolonged infusions of ANG II increase the excretion of PGs and TxB2 (23). Acute blockade of TXa2-S does not reduce BP in ANG-infused rats (25), but more prolonged blockade does lower BP (47) and reduces the renal vasoconstriction (48). However, a TPR antagonist is consistently effective in reversing hypertension and renal vasoconstriction in ANG II-infused rats (25, 47, 48) and in the early 2K,1C model in this and a previous study (43). We now find that a COX-1 antagonist, given acutely to anesthetized 2K,1C rats, reduces BP and TxB2 generation in the postclip kidney, whereas a COX-2 antagonist does not reduce BP and actually increases renal TxB2 generation. The fall in MAP of 28 ± 7 mmHg in response to the COX-1 antagonist was quite similar to the fall of 22 ± 3 mmHg in response to the TPR antagonist or of 36 ± 5 mmHg in response to the ACEI, which was also shown to reduce renal TxB2 excretion. These results are consistent with the reduction in TxB2 excretion reported in the COX-1 knockout mouse (16) and the increase in TxB2 excretion reported after COX-2 inhibition (27), and they confirm that the doses of these COX antagonists were effective at the time that the measurements of MAP were made. Since the antihypertensive responses to blockade of COX-1 and TPRs were quite similar in this study, it is not likely that Iso [which are generated by oxidative stress (29) but not by COX] are implicated. The results are consistent with the conclusion that hypertension is maintained in this model by activation of TPRs by TXa2 or, as suggested by Mistry and Nasjletti (25), by a prostaglandin endoperoxide that also activates TPRs. The finding of increased plasma MDA in this study confirms our prior observation of increased renal excretion of 8-isoprostane PGF2α in early 2K,1C rats (38), which we found was normalized by prolonged administration of an AT1R antagonist (38). AT1R activation of oxidative stress in this model may contribute to COX-dependent hypertension since COX is activated by peroxides and H2O2 (7). Mistry and Nasjletti (25) have proposed that PGH2 is the major COX-dependent TPR ligand maintaining BP in ANG II-induced hypertension. Our data are consistent with their conclusion.

**Perspectives**

Activation of TPRs leads to a “slow pressor” response that is broadly similar to that with ANG II (15, 37). Both responses generate O2•− (2, 30), cause renal vasoconstriction (44, 48), enhance TGF (40, 41), activate the sympathetic nervous system (5, 23), release arginine vasopressin (45), and potentiate drinking (18). Therefore, the TPR could mediate many of the prohypertensive actions of ANG II in this model of early, ANG II-dependent renovascular hypertension. Indeed, TPR-knockout mice have a blunted rise in BP and no increase in renal vascular resistance during a prolonged slow pressor infusion of ANG II (14). H2O2 stabilizes the TPR in the cell membrane, effectively enhancing its action (35), which may help to explain why the TPR is so important in maintaining hypertension in this model in which oxidative stress is prominent (38).

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