Possible role for brain prostanoid pathways in the development of angiotensin II-salt hypertension in rats

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Asirvatham-Jeyaraj N, Fink GD. Possible role for brain prostanoid pathways in the development of angiotensin II-salt hypertension in rats. Am J Physiol Regul Integr Comp Physiol 311: R232–R242, 2016. First published May 25, 2016; doi:10.1152/ajpregu.00535.2015.—Prostanoids generated by the cyclooxygenase (COX) pathway appear to contribute to the neurogenic hypertension (HTN) in rats. The first goal of this study was to establish the time frame during which prostanoids participate in ANG II-salt HTN. We induced HTN using ANG II (150 ng·kg⁻¹·min⁻¹·sc) infusion for 14 days in rats on a high-salt (2% NaCl) diet. When ketoprofen pretreatment was combined with treatment during the first 7 days of ANG II infusion, development of HTN and increased neurogenic pressor activity (indexed by the depressor response to ganglion blockade) were significantly attenuated for the entire ANG II infusion period. This suggests that prostanoid generation caused by administration of ANG II and salt leads to an increase in neurogenic pressor activity and blood pressure (BP) via a mechanism that persists without the need for continuing prostanoid input. The second goal of this study was to determine whether prostanoid products specifically in the brain contribute to HTN development. Expression of prostanoid pathway genes was measured in brain regions known to affect neurogenic BP regulation. ANG II-treated rats exhibited changes in gene expression of phospholipase A2 (upregulated in organum vasculosum of the lamina terminalis, paraventricular nucleus, nucleus of the solitary tract, and midline cerebral artery) and lipocalin-type prostaglandin D synthase (upregulated in the organum vasculosum of the lamina terminalis). On the basis of our results, we propose that activation of the brain prostanoid synthesis pathway both upstream and downstream from COX at early stages plays an important role in the development of the neurogenic component of ANG II-salt HTN.

Prostanoids are lipid mediators that act as cellular messengers (12). They have physiological and pathophysiological roles in fever, inflammation, pain, sleep, gastrointestinal function, bone remodeling, allergic asthma, luteolysis, and parturition. They also contribute to the pathophysiology of hypertension (HTN) (1, 12, 14, 16, 27, 28), but because they exhibit both prohypertensive and antihypertensive effects (27), a complete understanding of their specific roles in HTN remains elusive. In human patients with established HTN, whole body inhibition of prostanoid synthesis with cyclooxygenase (COX) inhibitors, if anything, tends to slightly increase blood pressure (BP) (4, 19, 25). This suggests a neutral or slightly antihypertensive "net" effect of COX-derived prostanoids on BP regulation once HTN is established. Nevertheless, given the complexity and scope of prostanoid influences on cardiovascular regulation (42), this does not rule out the possibility that specific prohypertensive prostanoids play a causal role in the development of HTN.

We recently investigated the contribution of prostanoids to a partially neurogenic experimental model of HTN produced by subcutaneous administration of ANG II to rats ingesting a high-salt (2% NaCl) diet (ANG II-salt HTN) (3). We found that continuous blockade of prostanoid generation with a nonselective COX inhibitor, or a COX-1 selective inhibitor, only modestly reduced BP in the early phase (1–4 days) of ANG II-salt HTN, but markedly lowered BP during the later phase (5–14 days). The later phase of HTN in this model is substantially dependent on increased sympathetically mediated effects on BP (i.e., neurogenic pressor activity) (23), and we also showed that COX inhibition reduced this neurogenic pressor activity (3). Furthermore, Cao et al. (7) have reported that COX-1 inhibition in the brain subfornical organ (SFO), a circumventricular organ with leaky blood-brain barrier and sympathetic neurons, reduces ANG II-induced sympathoexcitation and HTN in mice. Collectively, these observations suggest that ANG II HTN is caused, in part, by sympathoexcitatory mechanisms initiated by COX-1-derived prostanoids in the brain.

An interesting and notable aspect of both studies discussed above was that the generation of prostanoids could be dissociated in time from their effects on BP. In the Cao study, for example, prostanoid synthesis in the SFO peaked at 3 days of ANG II infusion and had returned to normal by 14 days, whereas the effects of COX-1 inhibition on BP persisted for 14 days. This is particularly relevant because Johnson and colleagues (10) have demonstrated that short-term (over several days) exposure to ANG II in rats can produce long-term neuroplastic changes in the brain that promote increased neurogenic pressor activity and BP on subsequent exposure of the animals to ANG II, salt, or aldosterone. Because COX-1-derived prostanoids are capable of inducing neuroplastic changes in the brain (5, 9, 40), together, these observations indicate that the timing of prostanoid generation in the brain may be a critical element in their ability to cause HTN.

Prostanoids were first described in the central nervous system by Samuelsson in 1964 (37), but relatively little research has focused on how brain prostanoids affect BP regulation or HTN development. Studies involving acute intracerebroventricular administration of various prostanoids into the brain ventricles showed that both prostaglandin (PG) E₂ and PGD₂ cause pressor responses that require sympathetic nervous system activation (13, 18). Similarly, stimulation of thromboxane (Tx) A₂ or PGH₂ receptors in the brain increases BP (15). Interestingly, the pressor response to intracerebroventricularly administered PGE₂ was abolished by lesion of the median eminence, an important link between brain regions such as the subfornical organ (SFO) and the organum vasculosum of the
lamina terminalis (OVLT), brain regions known to participate in salt sensing (18). Prostanoid-induced neuroplasticity in the central autonomic nuclei as discussed by Clayton et al. (10) in ANG II hypertensive rats is possibly initiated by a pathway distinct from the one shown to operate in the hypothalamus. Importantly, considering these findings and the studies of Cao et al. (7) discussed above, lesions of both SFO and OVLT have been found to impair ANG II-salt HTN development (11, 34). Therefore, in the present work, we tested the hypotheses that 1) a major effect of prostanoids in HTN development is to “prime” a later engagement of neurogenic pressor mechanisms, and 2) early changes in the expression of COX pathway enzymes and/or receptors in key brain regions could be the cause of this “priming” of ANG II-salt HTN.

**METHODS**

**Animals**

The experiments were performed after approval from the Institutional Animal Care and Use Committee at Michigan State University and were in compliance with the National Institutes of Health’s laboratory animal care and use guidelines. Male Sprague-Dawley rats from Charles River Laboratories, weighing 225 to 250 g, were allowed free access to a 2% NaCl diet (Research Diets) and distilled water starting 7 days prior to telemeter implantation and throughout the remainder of the experiment.

**Telemeter Implantation Surgery and Hemodynamic Measurements**

Telemeter implantation and postoperative procedures were similar to those previously described (24). Briefly, radio-telemeters (Data Sciences International) were implanted in the descending aorta via the femoral artery. The telemeter body was placed inside the pocket made subcutaneously in the abdominal region. Carprofen (5 mg/kg sc) was used to provide postsurgical analgesia, and enrofloxacin (5 mg/kg ip) was used for antimicrobial prophylaxis. Hemodynamic measurements were obtained for at least 10 s every 10 min for the entire experimental protocol using a commercial radio-telemetry data acquisition program (Dataquest ART 4.1; Data Sciences International).

**Experimental Protocols**

**Nonselective COX inhibition in established ANG II-salt hypertensive rats.** Rats were allowed a 5-day recovery period after telemeter implantation and postoperative procedures were similar to those previously described (24). Briefly, radio-telemeters (Data Sciences International) were implanted in the descending aorta via the femoral artery. The telemeter body was placed inside the pocket made subcutaneously in the abdominal region. Carprofen (5 mg/kg sc) was used to provide postsurgical analgesia, and enrofloxacin (5 mg/kg ip) was used for antimicrobial prophylaxis. Hemodynamic measurements were obtained for at least 10 s every 10 min for the entire experimental protocol using a commercial radio-telemetry data acquisition program (Dataquest ART 4.1; Data Sciences International).

**Nonselective COX inhibition prior to and during early stages of ANG II-salt HTN.** Two sets of experiments were performed to investigate the effect of nonselective cyclooxygenase inhibition at different time points on the development of ANG II-salt HTN. In the first set of experiments, radiotelemeter-implanted rats received either saline vehicle (n = 3) or ketoprofen (2 mg/kg; n = 3) subcutaneously for 4 days. Then all of the rats received a subcutaneous osmotic minipump infusing 150 ng·kg⁻¹·min⁻¹ of ANG II. Neurogenic pressor activity was evaluated on day 17, as described previously. In the second set of experiments, after three control days of BP measurement, ketoprofen (2 mg/kg sc) or saline was administered once daily for 4 days and continued for one more week (starting day 3 and ending on day 14 of the protocol). ANG II (150 ng·kg⁻¹·min⁻¹ sc) containing osmotic minipumps were implanted on day 7. Neurogenic pressor activity was assessed on day 17 of the protocol.

**Transcriptional and translational regulation of central prostanoid pathways in early stages of ANG II-salt hypertension.** For performing PCR and Western blot analyses, a total of 22 radiotelemeter-implanted rats were used. After 3 days of control recording, ANG II (150 ng·kg⁻¹·min⁻¹ sc; n = 12) or physiological saline (n = 10) was delivered using miniosmotic pumps (Alzet, 2ML2) for 4 days. Then, the rats were killed, brains were collected, snap frozen in dry ice, and kept at −80°C for the PCR array and Western blot analysis.

**PCR Array**

Rats brains were from 4-day ANG II (n = 5) and saline (n = 5) treatment groups were sectioned using a cryostat (Microm) at −15°C. Tissues collected from control and ANG II-treated rats were blood vessels that could act as a source of enzymes in the prostanoid pathway, the middle cerebral artery (MCA) (35, 41); brain regions that contain neurogenic pressor activity and with a leaky blood-brain barrier: the subfornical organ (SFO), paraventricular nucleus (PVN), organum vasculosum lamina terminalis (OVLT), rostral ventrolateral medulla (RVLM), nucleus tractus solitarius (NTS), and choroid plexus from the third ventricle (3VCV), and fourth ventricle (4VCV) (32, 33). Punches were obtained using the Palkovits microdissection technique. RNA was extracted using GenElute Mammalian Total RNA Miniprep Kit (Sigma Aldrich). To test the quality of extracted RNA, a Nanodrop Spectrophotometer (Thermo Scientific) was used, and samples with low-quality RNA (assessed by OD 260/280 ratio outside the range of 1.8 and 2.1) were excluded from further analysis. Reverse transcription for cDNA synthesis was performed with RT² first-strand kit (SABiosciences) using 100 ng of RNA from the samples. A custom Rat RT² profiler PCR array (SABiosciences) with 21 gene primers and 3 housekeeping genes (Table 1) in a 96-well plate format were used. The PCR reaction mixture for the 96-well plate included 12 μl diluted cDNA synthesis reaction, 1048 μl RNA grade water, and 1150 μl RT² SYBR Green master mix. PCR reactions were carried out on ABI 7500 Fast real-time PCR system (Applied Biosystems) using RT² SYBR Green master mix (SABiosciences). The PCR conditions included a holding stage (95°C for 10 min) and cycling stage (95°C for 15 s, 60°C for 1 min, and 72°C for 35 s) followed by melt curve to confirm the specificity of the amplified products. To control for DNA contamination, no template control was included. The Ct values were normalized to the average of three stable housekeeping genes. The housekeeping genes were lactate dehydrogenase (ldha), Ribosomal protein13a (Rpl13a), and β-actin. The fold change was calculated by 2ΔΔCT method.

**Western Blot Analysis**

Brains extracted from 4-day ANG II- (n = 7) and saline- (n = 5) treated rats anesthetized with pentobarbital sodium (50 mg/kg ip) were snap frozen on dry ice. Cryostat sections (each 500 μm) containing the PVN, SFO, NTS, RVLM, CP, and OVLT were made and punched using the Palkovits technique. Tissues were lysed using
**RESULTS**

Within-group differences were assessed by a one-way repeated-measures ANOVA with post hoc multiple comparisons using Dunnett’s procedure (GraphPad Instat 3). Between-group differences were assessed by a two-way mixed-design ANOVA, and post hoc testing at each time point was performed using Bonferroni’s procedure to assess the secondary antibody for 1 h at 4°C. An Odyssey imager was used to measure ANOVA with post hoc multiple comparisons using Dun-.

### Statistical Analysis

Within-group differences were assessed by a one-way repeated-measures ANOVA with post hoc multiple comparisons using Dunnett’s procedure (GraphPad Instat 3). Between-group differences were assessed by a two-way mixed-design ANOVA, and post hoc testing at each time point was performed using Bonferroni’s procedure to assess for multiple comparisons (GraphPad Prism 4). For the PCR array, between-group differences were analyzed by one sample t-test with the hypothesis mean as 1. Student’s t-test was used to analyze the Western blot data to compare the control and ANG II-treated rats. A P value of <0.05 was considered significant. All results are presented as means ± SE.

### RESULTS

**COX Inhibition in Established ANG II-Salt Hypertensive Rats**

The baseline MAP of both groups of rats (randomly assigned later to receive saline vehicle or ketoprofen) was identical from control day 1 to control day 3 (Fig. 1A). After starting ANG II infusion, MAP increased from 101 ± 1 mmHg on control day 3 to 125 ± 7 mmHg on day 8 of ANG II infusion in the designated vehicle group, and from 102 ± 1 mmHg on day 3 to 122 ± 3 mmHg on day 8 in the designated ketoprofen group (Fig. 1A). Rats then received either saline vehicle or ketoprofen (2 mg·kg⁻¹·day⁻¹ ip) for three consecutive days. The plasma half-life of ketoprofen in rats is ~10.5 h, and 97% steady-state plasma concentration is reached after five half-lives (38). Therefore, ketoprofen concentrations reached closer to steady-state by the second day of ketoprofen dosing in our study. Arterial pressure was not affected by ketoprofen treatment in these animals with established hypertension: being 126 ± 8 mmHg in the vehicle group and 128 ± 3 mmHg in the ketoprofen group, respectively, on the last day of treatment (Fig. 1A). Ganglionic blockade with hexamethonium on the second day of ketoprofen or vehicle treatment caused similar depressor responses in the two groups of rats (Fig. 1B). After the 14-day ANG II infusion period, the osmotic pumps were removed, and MAP fell over several days to 103 ± 1 mmHg and 103 ± 2 mmHg in the vehicle and ketoprofen groups, respectively. At this point, we again administered ketoprofen or vehicle for 3 days and saw no change in MAP in either group (Fig. 1A).

**Figure 2** and **Figure 3** show data from rats treated with ketoprofen for different lengths of time prior to, or during the early days of, chronic ANG II infusion. Figure 2A shows MAP in rats pretreated with vehicle (n = 3) or ketoprofen (2 mg/kg, sc; n = 3) for 5 days prior to ANG II infusion, with treatment stopped before ANG II infusion was started. Ketoprofen treatment alone did not affect MAP. After 14 days of ANG II infusion, MAP rose to 139 ± 15 mmHg in vehicle-treated rats and to only 122 ± 9 mmHg in ketoprofen-treated rats; however, there was not a statistically significant difference in MAP between the two groups (P = 0.3). Acute depressor responses to hexamethonium on day 10 of ANG II infusion were similar in vehicle (−57 ± 5 mmHg) and ketoprofen (−56 ± 14 mmHg) treated groups (Fig. 2B).

Figure 3 shows results from studies in which ketoprofen administration was started 4 days prior to beginning ANG II infusion and then extended for 7 more days (during the initial week of ANG II infusion). Another group of animals received only saline vehicle treatment over the same time course. MAP in these animals with established hypertension: being 126 ± 8 mmHg on day 1, 123 ± 7 mmHg on day 3, 121 ± 3 mmHg on day 8 of ANG II infusion and was 141 ± 10 mmHg by the end of the infusion period. In rats treated with ketoprofen, however, MAP rose initially up to day 4 of ANG II infusion (125 ± 3) but was only 109 ± 7 mmHg at the end of the infusion period (significantly lower than MAP in vehicle-treated animals). The depressor response to acute ganglionic blockade on day 10 of ANG II infusion (Fig. 3B) was significantly less in ketoprofen-treated rats (−35 ± 7 mmHg) than in vehicle-treated rats (−92 ± 12 mmHg).

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**Table 1. 96-well custom PCR array template for prostanoid-related genes**

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<th>Number</th>
<th>Gene</th>
<th>Symbols</th>
<th>Gene RefSeq No.</th>
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<tr>
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<td>PG-endoperoxide synthase 2 (COX-2)</td>
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<td>NM_017232</td>
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<td>Alox5</td>
<td>NM_012822</td>
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<td>6</td>
<td>Arachidonate 15-lipoxygenase</td>
<td>Alox15</td>
<td>NM_031010</td>
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<td>Tbaa1</td>
<td>NM_012687</td>
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<tr>
<td>8</td>
<td>PG2 (prostacyclin) synthase</td>
<td>Pgis</td>
<td>NM_031557</td>
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<tr>
<td>9</td>
<td>PGE synthase 1 (microsomal)</td>
<td>Pgts1</td>
<td>NM_021583</td>
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<tr>
<td>10</td>
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<td>11</td>
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<td>12</td>
<td>PGD2 synthase (brain)</td>
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<td>Lactate dehydrogenase</td>
<td>Ldha</td>
<td>NM_012583</td>
</tr>
<tr>
<td>24</td>
<td>Ribosomal protein13a</td>
<td>Rpl13a</td>
<td>NM_017008</td>
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lysis buffer (0.5 mmol/l Tris-HCl (pH 6.8), 10% SDS, and 10% glycerol) with protease inhibitors (0.5 mmol/l PMSF, 10 µg/ml aprotinin, and 10 µg/ml leupeptin). Protein concentration was measured using a bicinchoninic acid protein assay (Sigma), which was run on an SDS-PAGE 10% gel and transferred to nitrocellulose mem-

brane, and later blocked for 1 h with Odyssey Blocking Buffer. Blots prepared were then incubated overnight (4°C) with primary antibody anti-COX-1 (1:500; Millipore) or anti-tubulin (1:2,000 dilution; Mil-

lipore) or PGDs (Cayman Chemicals) or phospholipase A2 (PLA2) (Novus Biologicals). After rinsing the membrane 4 times for 5 min with TBS-T and final rinse in TBS, the blots were incubated using a secondary antibody for 1 h at 4°C. An Odyssey imager was used to visualize the bands. For a positive control, one lane in the membrane had the purified form of the protein of interest.
Prostanoid Gene Expression in the Brain of Rats in the Early Stage of ANG II-Salt HTN

PCR array analysis was used to measure transcriptional regulation of genes in brain samples obtained from rats on day 4 of ANG II-salt administration. MAP on day 4 was 105 \pm 2 mmHg in control rats and 125 \pm 5 mmHg in ANG II-salt-treated rats. The results from the PCR were analyzed after normalizing the values to vehicle-infused rats. The data are shown in Fig. 4. There were relatively few changes in prostanoid pathway gene expression in most brain regions. For example, gene expression was unaffected in the subfornical organ (SFO; n = 5), middle cerebral artery (MCA; n = 5), paraventricular nucleus (PVN; n = 5), third ventricle choroid plexus (3VCP; n = 5), and fourth ventricle choroid plexus (4VCP; n = 5). However, the preliminary PCR array result in the organum vasculosum lamina terminalis (OVLT; n = 2) suggested that LPGD-synthase (Ptgds) expression was increased (4.26-fold, which was confirmed with RT-PCR with n = 5 samples), whereas preliminary PCR array results from OVLT brain punches also revealed that cyclooxygenase-1 (Ptgs1; 0.17-fold) and PGD2 receptor (Ptgdr; 0.61-fold) expression were downregulated. RVLM of ANG II-treated rats (n = 4) showed a significant upregulation of PGE3-synthase (Ptges3; 1.16-fold) and arachidonate 5-lipoxygenase (Alox5; 2.02-fold) along with downregulation of PGI2 synthase (Ptgis; 1.37-fold) and cyclooxygenase-1 (Ptgs1; 1.56-fold). In the NTS (n = 5), PGD2-synthase 2 (Ptgds2; 0.19-fold) was significantly downregulated.

Quantitative RT-PCR

RT-PCR was performed to confirm the result of gene expression changes found in OVLT with PCR array. In rats (n = 5) treated with ANG II for 4 days, there was a significant three-fold increase in L-PGD-synthase gene expression com-
pared with vehicle-treated rats (Fig. 5B). RT-PCR was also performed for another rate-limiting enzyme in prostanoid synthesis, i.e., cytosolic phospholipase A2 (cPLA2), since it was inadvertently not included in the customized prostanoid pathway PCR array. cPLA2 expression was significantly higher in OVLT (2.2-fold; \( n = 5 \)), PVN (2.4-fold; \( n = 5 \)), NTS (9-fold; \( n = 5 \)), and MCA (22-fold; \( n = 5 \)) of ANG II-treated rats compared with vehicle-treated rats (Fig. 5A). There was no change in cPLA2 expression in RVLM or SFO.

Western Blot Analysis

Western blot analysis showed a significant overexpression of cPLA2 protein in the MCA and L-PGDS protein in the 3VCP (Fig. 6). There were no changes in protein levels of COX-1 in ANG II-treated rats in MCA, SFO, OVLT, PVN, or RVLM (Table 2).

DISCUSSION

Our laboratory and others have used the ANG II-salt HTN model in rats to understand sympathetic mechanisms contributing to hypertension development (23). In this model, an initial increase in BP occurs during the first few days of ANG II infusion that is followed by a substantial secondary increase in BP; the latter, but not the former, appears to be largely neurogenic in origin (30, 31). On the basis of an earlier study from our laboratory (3) and findings by others (7, 8), here, we sought to test the hypothesis that prostanoids contribute to the neurogenic phase of ANG II-salt HTN. Prostanoids could regulate sympathetic activity and BP in ANG II-salt HTN in a variety of ways, but our studies focused on a possible role of prostanoids that are generated and act in the brain. The main findings were 1) COX inhibition (to reduce prostanoid synthesis) does not reverse established ANG II-salt HTN during the
neurogenic phase of the model; 2) transient COX inhibition during the first phase of ANG II-salt HTN development impairs the ultimate development of HTN in the second, neurogenic phase; 3) ANG II-salt HTN (and increased sympathetic support of BP) is associated with early changes in transcription of prostanoid pathway genes in some brain regions (e.g., the OVLT and cerebral vasculature) but not others; and 4) altered expression of PLA2 protein in the cerebral vasculature and brain nuclei, and lipocalin prostaglandin D synthase (L-PGDS) in the choroid plexus, occurs during the early phase of ANG II-salt HTN. We conclude that in rats on a high-salt diet, exposure to ANG II causes an increase in the activity of specific prostanoid pathways in the forebrain. This may lead to long-lasting changes in neural function in brain regions that regulate sympathetic activity and BP.

In our previous studies, we found that continuous treatment with ketoprofen, a nonselective COX inhibitor, markedly impaired ANG II-salt HTN development and sympathetic overactivity (3). In the first experiment in the current study, we showed that in rats with already well-established ANG II-salt HTN, ketoprofen treatment failed to decrease BP or sympathetic support of BP. We also confirmed that ketoprofen treatment does not affect BP in normotensive rats on a high-salt diet (but not receiving ANG II infusion). These findings are consistent with the observation that most normotensive humans and humans with long-standing HTN, show little or no change in BP when they take COX inhibitors (19, 29, 36). We conclude from these findings that ongoing production of COX-derived prostanoids is not required to maintain elevated BP and sympathetic support for BP in the neurogenic phase of ANG II-salt HTN.
II-salt HTN. A caveat is that we did not confirm that the dose of ketoprofen that we used in this study was sufficient to fully block prostanoid production in the brain. However, we note that the same dose was effective in preventing ANG II-salt HTN development.

To determine more precisely when an increase in prostanoid production is required for full development of ANG II-salt HTN, in our second experiment, we performed COX inhibition prior to and early during the development of HTN. In the former case (prior to ANG II infusion only), a small (but not statistically significant) effect on HTN development was observed. However, COX inhibition that continued throughout the first week (early phase) of the ANG II-salt HTN, but not beyond that point, significantly attenuated the usual secondary
blockade, even compared with data obtained in the first two studies. However, comparing the control and ketoprofen-treated rats in individual studies, we conclude that COX products generated during the first few days of ANG II infusion (in rats eating a high-salt diet) in some manner are critical for causing a later increase in both BP and sympathetic support of BP in ANG II-salt HTN.

Johnson and colleagues (10) have reported that short-term (several days) increases in circulating ANG II in rats produce prolonged “sensitization” to the hypertensive effects of subsequent administration of the peptide (a week or more later). Sensitization appears to occur through an action of ANG II on the brain that results in increased sympathetic control of BP (10, 46). Furthermore, they have evidence that this apparent long-lasting alteration in brain function is due to neuroplastic changes in the forebrain, based on measured increases in putative markers of neuroplasticity, such as brain-derived neurotrophic factor, p38 MAPK, and cAMP response element-binding protein (10). There are obvious similarities with the results we report here, although we have no evidence that the sensitizing effect of ANG II shown by Johnson et al. (19) requires brain prostanoids. Nevertheless, we speculate that an early prostanoid-dependent sensitizing (or priming) action of ANG II could account for the more pronounced later rise in BP in our animals that received continuous infusions of ANG II. On the basis of the findings here and on the work of others, we speculate that in rats on a high-salt diet, exposure to ANG II could increase the activity of specific prostanoid pathways in the forebrain; if so, this could lead to long-lasting changes in neural function in brain regions that regulate sympathetic activity and BP. Further studies are needed to establish a clear link and provide evidence for the role of prostanoids in the priming action of ANG II in this model, as we have not addressed this question in our study. Our studies certainly do not exclude the possibility that other factors (e.g., the immune system) could play a key role in the priming effect.

Prostanoid products of arachidonic acid are produced throughout the brain, including the cerebral vasculature and the choroid plexus, in response to numerous physiological and pathophysiological stimuli (14, 22). Blood vessels in the brain are a particularly rich source of the first enzyme in the PG-
compared to vehicle-treated rats changes in prostanoid gene expression in the SFO, since other enzymes or receptors.

Multiple brain sites known to control sympathetic activity were investigated. Surprisingly, few changes in prostanoid pathway expression were found early in ANG II-salt HTN. Table 2. Cytosolic phospholipase A2, cyclooxygenase-1, prostaglandin D synthase protein expression in 4-day ANG II compared to vehicle-treated rats

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Groups</th>
<th>MCA</th>
<th>OVLTV</th>
<th>PVN</th>
<th>SFO</th>
<th>RVLM</th>
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<td>cPLA2</td>
<td>Vehicle</td>
<td>6.6 ± 2.4</td>
<td>3.3 ± 0.7</td>
<td>4.9 ± 1.1$</td>
<td>3.3 ± 0.5</td>
<td>2.1 ± 0.2</td>
<td>4.0 ± 0.2</td>
<td>4.2 ± 0.9</td>
<td>20 ± 3</td>
</tr>
<tr>
<td></td>
<td>ANG II</td>
<td>36.4 ± 8.2$</td>
<td>2.2 ± 0.8</td>
<td>4.2 ± 0.4S</td>
<td>4.2 ± 0.5</td>
<td>2.2 ± 0.4</td>
<td>3.3 ± 0.2</td>
<td>5.4 ± 1.1</td>
<td>27 ± 5</td>
</tr>
<tr>
<td>COX-1</td>
<td>Vehicle</td>
<td>62.5 ± 15.4</td>
<td>0.077 ± 0.02</td>
<td>0.075 ± 0.01</td>
<td>0.17 ± 0.05</td>
<td>0.08 ± 0.07</td>
<td>0.01 ± 0.03</td>
<td>1.04 ± 0.2</td>
<td>4.8 ± 2</td>
</tr>
<tr>
<td></td>
<td>ANG II</td>
<td>4.7 ± 9.2</td>
<td>0.078 ± 0.02</td>
<td>0.085 ± 0.01</td>
<td>0.16 ± 0.1</td>
<td>0.07 ± 0.05</td>
<td>0.06 ± 0.03</td>
<td>0.8 ± 0.1</td>
<td>2.6 ± 0.5</td>
</tr>
<tr>
<td>L-PGDS</td>
<td>Vehicle</td>
<td>0.4 ± 0.03</td>
<td>0.2 ± 0.08</td>
<td>0.05 ± 0.2</td>
<td>0.02 ± 0.003</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ANG II</td>
<td>0.2 ± 0.03</td>
<td>0.3 ± 0.04</td>
<td>0.6 ± 0.1</td>
<td>0.01</td>
<td></td>
<td></td>
<td>1.1 ± 0.2$</td>
<td>0.3 ± 0.2</td>
</tr>
</tbody>
</table>

Lower expression of proteins after ANG II infusion compared to control rats on day 4 of HTN.

Synthesizing pathway, PLA2 (41). PLA2 is upstream of the COX pathway and could increase brain availability of the substrate arachidonic acid on which COX acts to increase the generation of eicosanoids. Cyclooxygenase products are further metabolized by prostaglandin and Tx synthases to form PGs and Tx (12). Some evidence exists that prostanoids products can act in the brain to increase sympathetic activity and BP (28). In addition, there is evidence that prostanoids can cause adaptive changes in brain pathways that could lead to prolonged alterations in brain function (5, 9). Therefore, we tested the hypothesis that transcriptional and translational products of the prostanoid pathway could be transiently activated early during the development of ANG II-salt HTN, with possible consequences for long-term BP regulation.

Four days after starting ANG II-salt treatment in rats, we found an increase in mRNA levels of a cytosolic PLA2 (cPLA2), in various brain regions and the MCA. Since protein levels of cPLA2 also were increased in the MCA, this could indicate accelerated conversion of membrane phospholipids into arachidonic acid and then into the COX product PGH2, the main substrate for production of numerous prostanoids. This finding is particularly significant since it was recently reported that mice with homozygous deletion of cPLA2 are resistant to ANG II-induced hypertension (21). Downstream to cPLA2, COX-1, cyclooxygenase-1; L-PGDS, lipocalin prostaglandin D synthase. *Overexpression of proteins (P < 0.05) in ANG II infusion day 4 compared to control rats.

We found that the transcript levels of L-PGDS, which is primarily localized to meningeoepithelial cells and oligodendrocytes in the brain (44), were upregulated, and expression of the receptor for PGD2, the main enzymatic product of L-PGDS, was downregulated in the OVLTV early in ANG II-salt HTN development. Although these observations need to be confirmed at the protein level, it is noteworthy that PGD2 is the most abundant prostaglandin in the brain and previously has been implicated in the regulation of physiological and pathophysiological processes like sleep induction and fever (44). Studies from other laboratories have found the leptomeninges, arachnoid trabecular cells, and choroid plexus epithelial cells as the primary sources of L-PGDS secretion into the cerebrospinal fluid (6). Therefore, it is interesting that overexpression of L-PGDS protein in choroid plexus also was observed in ANG II-salt hypertensive rats. There is evidence that direct injection of PGD2 in the brain increases BP (13). Altogether then, our results suggest the novel hypothesis that increased PGD2 levels in the brain may contribute to increased sympathetic activity and BP in ANG II-salt HTN. Additional studies are necessary to test this hypothesis directly.

Perspectives and Significance

This study allowed us to advance two novel, albeit tentative, hypotheses. First, we propose that a short period of increased prostanoid generation may cause sustained changes in the neural pathways regulating arterial pressure in a way that leads to sustained hypertension. Second, analyses of genes (and a few proteins) in prostanoid synthesis and signaling pathways in
the brain during the development of ANG II-salt HTN revealed the possibility of a, heretofore, unsuspected role for PGD2 and PGDS in the pathophysiology of neurogenic hypertension. If these ideas can be confirmed, the availability of many small-molecule drugs affecting the prostanoic pathway could allow discovery of new pharmacological approaches to managing hypertension and other clinical conditions driven, in part, by excessive sympathetic activity.

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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

REFERENCES


