Adenoviral inhibition of AT₁a receptors in the paraventricular nucleus inhibits acute increases in mean arterial blood pressure in the rat

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Northcott CA, Watts S, Chen Y, Morris M, Chen A, Haywood JR. Adenoviral inhibition of AT₁a receptors in the paraventricular nucleus inhibits acute increases in mean arterial blood pressure in the rat. Am J Physiol Regul Integr Comp Physiol 299: R1202–R1211, 2010.—Brain and peripheral renin-angiotensin systems are important in blood pressure maintenance. Circulating ANG II stimulates brain RAS to contribute to the increase mean arterial pressure (MAP). This mechanism has not been fully clarified, so it was hypothesized that reducing angiotensin type 1a (AT₁a) receptors (AT₁aRs) in the paraventricular nucleus (PVN) would diminish intravenous ANG II–induced increases in MAP. Adenoviruses (Ad) encoding AT₁a small hairpin RNA (shRNA) or Ad-LacZ (marker gene) were injected into the PVN [1 × 10⁹ plaque-forming units/ml, bilateral (200 nl/site)] of male Sprague-Dawley rats instrumented with radiotelemetry transmitters for MAP and heart rate measurements and with venous catheters for drug administration. No differences in weight gain or basal MAP were observed. ANG II (30 ng·kg⁻¹·min⁻¹ iv, 15 µl/min for 60 min) was administered 3, 7, 10, and 14 days after PVN Ad injection to increase blood pressure. ANG II–induced elevations in blood pressure were significantly reduced in PVN Ad-AT₁a shRNA rats compared with Ad-LacZ rats (32 ± 6 vs. 8 ± 9 mmHg at 7 days, 35 ± 6 vs. 10 ± 6 mmHg at 10 days, and 32 ± 2 vs. 1 ± 5 mmHg at 14 days; P < 0.05). These observations were confirmed by acute administration of losartan (20 nmol/l, 100 nl/site) in the PVN prior to short-term infusion of ANG II; the ANG II–pressor response was attenuated by 69%. In contrast, PVN Ad-AT₁a, shRNA treatment did not influence phenylephrine–induced increases in blood pressure (30 µg·kg⁻¹·min⁻¹ iv, 15 µl/min for 30 min). Importantly, PVN Ad-AT₁a shRNA did not alter superior mesenteric arterial contractility to ANG II or norepinephrine; ACh–induced arterial relaxation was also unaltered. β-Galactosidase staining revealed PVN Ad transduction, and Western blot analyses revealed significant reductions of PVN AT₁ protein. In conclusion, PVN–localized AT₁Rs are critical for short-term circulating ANG II–mediated elevations of blood pressure. A sustained suppression of AT₁aR expression by single administration of shRNA can interfere with short-term actions of ANG II.

angiotensin II; hypertension

ANGIOTENSIN II (ANG II) is an intensely investigated peptide with regard to its role in blood pressure regulation and the pathogenesis of cardiovascular disease. The importance of ANG II is highlighted by the effectiveness of drugs used clinically in the treatment of hypertension that target the ANG II pathway. Acute administration of ANG II increases blood pressure, largely by constricting vascular smooth muscle and increasing peripheral resistance. In addition, circulating ANG II increases blood pressure through neurogenically mediated mechanisms during acute and long-term ANG II exposure (9, 22, 28, 33, 43).

In order for ANG II to stimulate neurally mediated increases in blood pressure, ANG II must act at angiotensin type 1 (AT₁) receptors (AT₁Rs) in regions of the brain that recognize changes in circulating hormones, since ANG II does not cross the blood-brain barrier. However, circulating ANG II stimulates AT₁Rs in specific regions of the brain that are devoid of a blood-brain barrier, such as the subfornical organ (SFO) (20), organum vasculosum of the lamina terminalis (OVLT) (14), and area postrema (18). These structures, referred to as circumventricular organs (CVOs), are windows through the blood-brain barrier that allow molecules, such as peptides, to stimulate downstream pathways from these CVO brain regions. The forebrain CVOs send projections to the paraventricular nucleus (PVN), which in turn signals the spinal cord, rostral ventrolateral medulla (RVLM), and other brain regions that regulate blood pressure. Within the brain, ANG II also serves as a neurotransmitter and/or neuromodulator within the neural SFO/OVLT-PVN-RVLM/spinal cord pathway to regulate blood pressure.

The balance of excitatory and inhibitory neurotransmitters/pathways in the PVN contributes to blood pressure maintenance and neuroendocrine responses involved in energy balance and fluid regulation. ANG II is an important excitatory neurotransmitter in the PVN, mediating cardiovascular function through AT₁Rs at pre- and postsynaptic sites (6, 7, 12, 14, 21). These responses are mediated in part through the sympathetic nervous system (8) and can be activated by circulating ANG II administered exogenously (12) or generated in pathophysiological states such as heart failure (42). In both situations, PVN angiotensinergic mechanisms contribute to blood pressure. Adenovirus encoding of AT₁a small hairpin RNA (shRNA) has been successfully used to interfere with the expression of AT₁aRs (8). The goals of the present study were 1) to determine if AT₁a shRNA administration into the PVN would interfere with short-term infusions of ANG II intravenously and 2) to assess the duration of effective suppression of expression of AT₁aR protein and physiological response.

MATERIALS AND METHODS

Animals

All animal procedures were carried out in accordance to and with the approval of the Institutional Animal Care and Use Committee at Michigan State University. Upon arrival at the facility, male Sprague-Dawley rats (275–300 g; Charles River Laboratories, Portage, MI) were housed in clear plastic boxes with wood chip bedding and
allowed ad libitum access to rat chow (Teklad) and tap water. For surgical procedures, anesthesia was administered by isoflurane gas (2% in O2) unless otherwise specified. All animals were treated with postsurgical analgesics, and all surgery was performed aseptically.

**Surgical Interventions**

_Vascular catheterization._ Animals were prepared with femoral arterial and/or femoral venous catheters, with tips in the abdominal aorta and vena cava. The catheters were tunneled subcutaneously to the nape of the neck and secured with sutures for direct conscious blood pressure measurements or drug delivery.

_Telemetry._ Radiotelemetry transmitter catheters (Data Sciences International, St. Paul, MN) were surgically implanted in the femoral artery for continuous chronic blood pressure and heart rate (HR) recording. The radiotransmitter unit was then placed subcutaneously. After a 1-wk recovery period, arterial pressure and HR were recorded for 1 wk to obtain baseline recordings (measurements were taken for 10 s every 10 min, 24 h/day).

_Viral microinjections._ After ≥1 wk of baseline blood pressure measurements, the rat was anesthetized with an intraperitoneal injection of ketamine (75 mg/kg) and xylazine (7.5 mg/kg) and placed in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA). The skull was exposed for bilateral microinjections of adenovirus into the PVN (target: 2.0 mm caudal to bregma, 1.2 mm lateral to midline, and 7.4 mm ventral from the skull, 10° angle). A small burr hole was hand-drilled in the skull for chronic placement of microinjectors [30-gauge stainless steel tubing attached to a 1-μl Hamilton syringe via polyethylene (PE-20) tubing]. Bilateral microinjections of adenovirus encoding AT1a shRNA (Ad-AT1a shRNA) to silence the AT1aR or Ad-LacZ (a marker gene) were introduced into the PVN (1 × 10^6 plaque-forming units/ml, 200 nl/site over 2 min). Preparation of Ad-AT1a shRNA and Ad-LacZ has been described previously (8). Microinjectors were removed 15 min after injection, the skin was sutured over the skull, and the animals were returned to their home cages.

_Chronic PVN cannulas._ The rats were placed in a stereotaxic apparatus, and the skull was exposed. A small burr hole was hand-drilled in the skull for chronic placement of a cannula into the PVN (target: 2.0 mm caudal to bregma, 1.2 mm lateral to midline, and 7.4 mm ventral from the skull, 10° angle). Stainless steel screws and dental acrylic were used to anchor the cannulas into the skull, and the skin was sutured over the acrylic skull cap.

_Viral Studies Experimental Protocol_  

At 3, 7, 10, and 14 days after viral injection, ANG II (Sigma, St. Louis, MO; 30 ng·kg^-1·min^-1 iv, 15 μl/min) was infused for 1 h. At 1 h prior to the infusion (for baseline recordings) and throughout the ANG II infusion, blood pressure and HR were monitored continuously. At 16 days after viral injection, phenylephrine (PE, 30 μg·kg^-1·min^-1, 15 μl/min; Sigma) was infused for 30 min. Similar to the previous ANG II

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**Fig. 1.** Neither the adenovirus encoding the angiotensin type 1a (AT1a) short hairpin RNA (shRNA) nor LacZ had an effect on basal mean arterial blood pressure (A) or heart rate [B: beats/min (BPM)]. Time points are 24-h averages. No significant change in weight gain between groups of animals was observed throughout the study (C). Values are means ± SE.
protocol, blood pressure and HR were monitored continuously for 30 min prior to and throughout the intravenous infusion.

**Acute Microinjections**

Losartan (10 nmol/l, 100 nl/site) or artificial cerebrospinal fluid (aCSF; in mmol/l: 130 NaCl, 2.5 KCl, 1.0 MgSO4, 0.5 NaH2PO4, 0.5 NaHPO4, 1.25 CaCl2, 3.3 glucose, and 20 NaHCO3; filtered, pH 7.4) was microinjected bilaterally into the PVN of conscious male Sprague-Dawley rats 10 min prior to an acute 60-min intravenous ANG II (30 mg·kg⁻¹·min⁻¹, 15 μl/min) or saline infusion. Animals were previously prepared with femoral arterial catheters (see above) for direct measurement of blood pressure using Power Lab software (AD Instruments, Colorado Springs, CO). MAP and HR were monitored throughout the experimental protocol. The rat was allowed to recover for ≥2 days; then the same animal was subjected to the other treatment (losartan or aCSF). Throughout all studies, MAP and HR were continuously monitored. At the conclusion of the studies, histological analyses were performed to ensure PVN cannula placement.

**Contractility Studies**

In another series of experiments, the adenovirus was microinjected into the PVN as stated above. At 7 and 10 days after viral injections, the animals were anesthetized using pentobarbital sodium (50 mg/kg ip). The superior mesenteric artery (SMA) was removed, cleaned of all connective tissue, and cut into helical strips for isometric contractility recordings. One strip from each set of animals was placed in a heated, aerated (95% O₂-5% CO₂) tissue bath containing physiological salt solution (PSS; in mmol/l: 103 NaCl, 4.7 KCl, 1.18 KH₂PO₄, 1.17 MgSO₄·7H₂O, 1.6 CaCl₂·2H₂O, 14.9 NaHCO₃, 5.5 dextrose, and 0.03 CaNa₂-EDTA, 37°C). Strips were placed under optimum resting tension (600 mg), equilibrated for 1 h, and challenged initially to PE (10⁻⁵ mol/l). Tissues were washed with PSS to allow tone to return to baseline. Relaxation of the SMA (whereby strips were contracted to half-maximal concentration of PE) by exposure to norepinephrine (NE, 10⁻⁹–10⁻⁵ mol/l), ANG II (10⁻¹⁰–10⁻⁷ mol/l), and Ach (10⁻⁹–10⁻⁵ mol/l) was used to examine any differences in responses between the SMA removed from animals treated with Ad-AT1 shRNA and animals treated with Ad-LacZ.

**Histology**

Rats were anesthetized with pentobarbital sodium (50 mg/kg ip) and perfused with 4% paraformaldehyde in 0.1 mol/l phosphate buffer. The brains were postfixed in 4% paraformaldehyde for 24 h at 4°C, transferred to 20% sucrose in 0.1 mol/l phosphate buffer, and stored at 4°C until analysis. Paraformaldehyde-fixed brains were cut into serial sections (40 μm), placed onto slides, and allowed to dry.
overnight. On day 2, the slides were rinsed in 95% alcohol, 70% alcohol, distilled H2O, cresyl violet stain, distilled H2O, 70% alcohol, 95% alcohol, 100% alcohol, and xylene (3 min each step). Slides were mounted and viewed under a microscope for staining.

**β-Galactosidase Staining**

Serial sections were stained for β-galactosidase activity using 5-bromo-4-chloro-3-indoyl-β-D-galactopyranoside (X-Gal, Boehringer Mannheim). Briefly, sections were rehydrated in 1× PBS, transferred to X-Gal working solution, incubated at 37°C for 2 h, and rinsed in distilled H2O, 50% alcohol, 95% alcohol, 100% alcohol, and xylene. Slides were mounted and viewed under a microscope for staining.

**Immunocytochemistry**

Serial brain sections were blocked with 1.5% blocking serum in PBS and then incubated with a rabbit anti-AT1R antibody (1:500 dilution; Santa Cruz Biotechnology, Santa Cruz, CA) or no antibody (blocker alone, as a control) overnight at 4°C. Sections were then washed three times with PBS, incubated with secondary antibody for 30 min, washed with PBS, and incubated with ABC Elite Reagent (Vector Laboratories, Burlingame, CA). For detection of antibody binding, sections were incubated with 3,3-diaminobenzidine solution (NiDAB, Vector Laboratories). Staining was shown as a dark brown coloring.

**Protein Isolation and Western Blot Analyses**

Rats were anesthetized using pentobarbital sodium (50 mg/kg ip). The brains were quickly removed, immediately frozen on dry ice, and sectioned on a cryostat (PVN and supraoptic nucleus (SON) at 600 μm, SFO at 300 μm). A microscope was used to localize the PVN, SON, and SFO, which were isolated using a tissue punch (17-gauge needle). The tissue was solubilized in 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/μl aprotinin, and 10 μg/μl leupeptin). An ultrasonic processor was used to homogenize punches (1–2 s pulses, with intermediate vortexing), which were centrifuged for 10 min at 5,000 rpm at 4°C. Supernatant was collected, and protein concentration was determined using a bicinchoninic acid protein assay (Sigma).

Proteins (4:1 dilution in denaturing sample buffer, boiled for 5 min) were separated on SDS-polyacrylamide gels and transferred to Immobilon-P membranes. Membranes were blocked for 3 h [Tris-buffered saline (TBS)-Tween (TBS-T), 4% chick egg ovalbumin, and 2.5% sodium azide]. Blots were probed overnight at 4°C with primary antibody [AT1 (1:200 dilution; Santa Cruz Biotechnology) and tubulin (1:5,000 dilution; Millipore; Temecula, CA)], rinsed in TBS-T with a final rinse in...
TBS, and incubated with the appropriate secondary antibody for 1 h at 4°C. The AT1 antibody had been tested previously with appropriate positive controls in the laboratory (data not shown). Blots were then incubated with ECL reagents for visualization of the bands.

Data Analysis and Statistics

Values are means ± SE. For comparison of two groups, the appropriate Student’s t-test was used. For multiple comparisons, an ANOVA followed by a Student-Newman-Keuls post hoc test was used. For telemetry experiments using repeated measurements from the same animal, Proc Mixed and repeated-measures statistical analyses were performed using SAS statistical software (version 9.1). In all cases, P ≤ 0.05 was considered statistically significant.

RESULTS

Baseline Blood Pressure and HR

Blood pressure and HR were monitored with radiotelemeters prior to the viral injection and throughout the experimental protocol to determine if AT1a shRNA administered into the PVN altered resting parameters. To eliminate possible influences on baseline blood pressure during the days of agonist infusions, these time frames were not included in the overall basal blood pressure and HR data analysis. No observable differences in blood pressure and HR throughout the studies illustrate that AT1a shRNA had no basal effects on these parameters (Fig. 1, A and B). There were also no changes in weight gain between the control (LacZ) and AT1a shRNA PVN-microinjected animals throughout the study (Fig. 1C).

Short-Term ANG II and PE-Induced Increases in Blood Pressure

During the experimental period, ANG II was infused intravenously for 1 h to acutely increase blood pressure. The infusions occurred 3, 7, 10, and 14 days after viral injections. At the end of the study, PE was infused for 30 min for evaluation of a different noncentrally mediated pressure response. At 3 days after PVN microinjection of AT1a shRNA or LacZ, a separation in the responsiveness to ANG II was evident (Fig. 2). However, the overall one-way analysis of variance was not different between the groups of rats. Despite no significant difference in the peak change in blood pressure, a trend had begun for a reduced response to ANG II intravenous infusion in the AT1a shRNA PVN-microinjected animals compared with the LacZ-treated animals: 29 ± 6 vs. 21 ± 4 mmHg peak change in MAP (Fig. 2F). The difference in ANG II-induced increases in blood pressure were statistically significant by 7 days after viral injection. Throughout the ANG II intravenous infusion period, there was a significantly greater increase in acute ANG II-induced blood pressure in the control LacZ- than the AT1a shRNA-treated rats (Fig. 2B): 32 ± 6 vs. 26 ± 6 mmHg.
8 ± 9 mmHg peak change in MAP at 7 days (Fig. 2F). These differences in acute ANG II-induced increases in blood pressure continued 10 and 14 days after viral injection (Fig. 2, C and D): 35 ± 6 vs. 10 ± 6 mmHg and 32 ± 2 vs. 1 ± 5 mmHg peak change in MAP at 10 and 14 days, respectively (Fig. 2F).

The pressor response to ANG II in the LacZ-treated rats was not different. No significant changes in HR were observed during the ANG II infusions (Fig. 3, A–D).

To further confirm that a reduction in AT1R would alter acute ANG II-mediated elevations in blood pressure, bilateral cannulas were placed in the PVN for microinjections of losartan, an AT1 antagonist, into conscious male Sprague-Dawley rats prior to an acute ANG II-induced increase in blood pressure. The ANG II-induced elevation (69%) of blood pressure was significantly inhibited in animals pretreated with those treated with the aCSF vehicle (Fig. 4A). In addition, losartan alone had no effect on basal blood pressure levels (data not shown). No overall differences in HR were observed; however, there was a trend for the reduction in HR to be greater in the losartan-treated animals than in those infused with aCSF (Fig. 4B). At the conclusion of the study, histological examination confirmed cannula placement localized to the PVN (Fig. 4C). All injections that fell outside the PVN were excluded from the analyses.

For further investigation of acute pressor responses, PE was infused for 30 min at the conclusion of the adenoviral study to evaluate a different pressor stimulus. There were no statistical differences in PE-induced changes in MAP between the animals treated with PVN-directed AT1a shRNA and those treated with LacZ, suggesting that the change in pressure was an ANG II-mediated response (Fig. 2E). In addition, no changes in HR were observed during the PE infusion (Fig. 3E).

**Isometric Contractility**

Nearly complete suppression of the intravenous ANG II-induced increases in blood pressure following the inhibition of the PVN AT1aR was an unexpected result, since it is generally accepted that at least some component of the intravenous ANG II-induced increase in blood pressure is mediated through its vasoconstrictive effects (41). Therefore, even though the virus was specifically targeted to the PVN, it was important to determine that peripheral smooth muscle reactivity was not altered. In a separate group of animals, the SMA was isolated 7 and 10 days after virus injection to evaluate alterations in peripheral contractile responses to NE- and ANG II-induced relaxation, as well as ACh-induced relaxation. SMA isolated 7 or 10 days after viral injections showed no significant differences in contraction to NE or ANG II or any differences in ACh-induced relaxation (Fig. 5). These data confirm that the virus had no effect on SMA vascular reactivity and that, most likely, it was a centrally mediated event that was observed in prior experiments.

**Confirmation of PVN Injections**

At the conclusion of the studies, animals were perfused with 4% paraformaldehyde, and the brains were removed for immunocytochemical analyses of AT1Rs, histological staining,
and staining for β-galactosidase (to identify the LacZ component of the adenovirus). Figure 6 demonstrates that the virus was targeted to the PVN, as indicated by blue staining throughout the PVN (Fig. 6, A and B). Histological analysis confirmed that this was the PVN region. Finally, immunocytochemistry showed less punctate staining qualitatively in the PVN region of the animals treated with AT1α shRNA [Fig. 6, D and F (representative section)] than in animals microinjected with LacZ (Fig. 6, C and E).

To quantify any changes in AT1 expression and to ensure that the virus affected only the PVN, protein was isolated from the PVN, SFO, and SON from an additional set of animals 16–18 days after viral treatment, and AT1 protein was evaluated using Western blot analyses. AT1 protein expression in the PVN was reduced by 54% in animals subjected to PVN-targeted AT1α shRNA microinjection (Fig. 7A) compared with animals injected with LacZ. Two other brain regions, the SFO and SON, were also examined for AT1 protein expression. The SFO and SON showed no difference in AT1 protein expression, suggesting that the virus had no effect in these brain regions. To ensure that an equal amount of protein was loaded, tubulin was used as a comparative measure, and no differences in tubulin expression were found between the experimental groups (Fig. 7).

**DISCUSSION**

While circulating and brain-derived ANG II are individually involved in blood pressure regulation, studies are limited with regard to examination of the link between blood-borne and brain-derived ANG II in blood pressure regulation. Earlier studies support the concept of an ANG II-stimulated ANG II-mediated pathway through the PVN (9, 28, 33, 43). The present series of experiments sought to demonstrate that circulating ANG II activated a brain ANG II-mediated pathway through the PVN to alter the physiological end point of blood pressure by decreasing ANG II receptor function in the PVN with use of an adenovirus that silenced the AT1αR in the PVN. The studies presented here demonstrate a direct relationship between increasing circulating ANG II and the PVN AT1αR in short-term ANG II-mediated increases in blood pressure. Suppressed expression of the receptor did not affect increases in...
MAP mediated by vascular α-adrenergic stimulation. Importantly, vascular reactivity experiments confirmed that acute increases in circulating ANG II-mediated blood pressure were not due to a direct ANG II-elicited change in vascular resistance. Finally, there was a delay in the onset of the inhibition of the response by the virus; however, AT1a expression was reduced through ≈2 wk after administration.

Circulating ANG II acts at AT1Rs in the forebrain and hindbrain CVOs to modulate arterial pressure (14, 18, 20). Rostrally, CVOs that lie along the anterior third ventricle include the OVLT and SFO (1, 25). Ablation of the anteroventral third ventricle, which includes the OVLT and the median preoptic area surrounding the anterior commissure, interferes with ANG II-mediated increases in blood pressure and renin-dependent hypertension (4, 5, 23). Similar observations have been made regarding the role of the SFO (29, 31). Neurons from these ANG II-sensitive areas have been shown to converge on the median preoptic area and synapse with secondary angiotensinergic neurons that project to the PVN (32, 34, 39).

In mediating the central responses to peripheral ANG II stimulation, ANG II is a potent stimulator of magnocellular and parvocellular neurons in the PVN (6, 16). Electrophysiological studies have confirmed that circulating ANG II affects sympathetic premotor neurons in the PVN, some of which are associated with cardiovascular changes induced by ANG II locally (15). These PVN premotor neurons project to the RVLM, an area that is involved in cardiovascular regulation (11, 23, 26, 30, 35, 40). This pathway also appears to be mediated in part by ANG II (11, 26, 40). In addition, there are direct projections to premotor neurons in the spinal cord, where they synapse, with preganglionic effects in the intermediolateral cell column (3, 7, 11, 36). There is also evidence that an efferent projection from the PVN extends to the lateral parabrachial nucleus, and ANG II may also participate in the transmission of this pathway (19). The lateral parabrachial nucleus subsequently projects to the RVLM. Finally, there are projections from the PVN to the nucleus tractus solitarii (NTS), but there does not appear to be a role for ANG II in this pathway. In addition to these potential interactions with neurons affecting the sympathetic nervous system, ANG II can modulate neuroendocrine neurons in the PVN to affect hormone balance. Consequently, AT1Rs are located on neurons throughout the PVN that can stimulate several pathways activating sympathetic premotor neurons, as well as neurons not associated with the sympathetic nervous system.

In addition to the PVN-NTS projection, ANG II stimulates the area postrema to modulate NTS activity. There is also evidence that ANG II acts directly in the NTS to modulate blood pressure. The resulting effects are to suppress baroreflex activity contributing to elevated sympathetic outflow and blood pressure. In rats and humans, baroreflex responses of HR and muscle sympathetic nerve activity were attenuated by ANG II–relative to PE-induced responses. Further importance of these pathways is demonstrated by the impact of sinoaortic deafferentation on the pressor response induced by ANG II. After baroreceptor denervation, ANG II increased lumbar sympathetic nerve activity and HR (43).

In the present studies, reduced expression of the AT1R in the PVN by microinjection of shRNA resulted in a significantly reduced pressor response to a 60-min short-term infusion of
ANG II between 7 and 14 days after administration. On first glance, it appears that the attenuated response resulted from an interference with excitatory pathways through the PVN to the RVLM and spinal cord. These pathways are the most direct means of interfering with excitatory pressor function. However, recognition that peripheral vascular responses are intact suggested that the sustained direct vasoconstriction by ANG II may be offset by a mechanism that would result in an active reduction in vascular tone. One possible enhanced depressor mechanism would be the removal of an ANG II-stimulated pathway from the PVN to the NTS that inhibits the baroreflex. The result would be a permissive activation of baroreflex-mediated vasodilation. Hence, interference with expression of ANG II receptors in the PVN may attenuate the pressor action of ANG II-mediated pathways through the PVN by a combination of limiting excitatory pathways and restoring full function of the baroreflex to peripheral vasoconstriction. The remaining pressor response suggests that the baroreflex does not completely offset the vasoconstrictor action of peripheral ANG II.

The effectiveness of the shRNA to suppress the expression of the AT1R was demonstrated using immunohistochemistry, which showed reduced appearance of AT1Rs, and using Western blot analysis, which showed a 54% reduction of AT1Rs. Together with the significantly attenuated pressor response, these data suggest that a complete attenuation of receptor expression is not necessary to significantly interfere with the pressor response to short-term infusion of ANG II. The importance of AT1Rs in the PVN during short-term ANG II intravenous infusion was further confirmed by blockade of the pressor actions of the short-term infusion of ANG II following acute administration of the AT1 antagonist losartan in the PVN. These studies indicate that reduced expression of the AT1R or blockade of the receptor resulted in an attenuated response during short-term infusion of ANG II. The greater inhibition of the ANG II response may be related to the dose of losartan or the incomplete conversion of the drug to the active metabolite.

While the source of the ANG II in the PVN remains controversial since angiotensinergic neurons have not been identified, it appears that neurons and glia work together to produce the active ANG II peptide by providing substrate and enzyme. This may be accomplished through production of ANG II by renin and angiotensinogen, creating ANG I, and its subsequent breakdown by converting enzyme. Alternatively, ANG II may be produced through a renin-independent pathway with use of ANG-(1–12) as an intermediary (2). Regardless of the source of ANG II, AT1Rs are important in mediating its response.

A variety of dosing regimens have been used to study the central actions of ANG II. Bolus injections (13), short-term infusions (9, 28, 43), and long-term infusions (17, 24, 27, 28), as well as the effects, of endogenous ANG II (37, 38, 39) have been shown to be at least partially mediated by an activation of the sympathetic nervous system through a stimulation of the brain. In the present studies, a short-term infusion of ANG II was chosen to study the role of PVN AT1Rs to initially investigate the effectiveness of the shRNA. The rationale for this approach was that the ability to assess the actions of ANG II over time would provide a time course of the duration of the suppression of the AT1R. In fact, the data indicate that the receptor expression and function are reduced for ≥2 wk.

Perspectives and Significance

Results of the present study indicate that acute increases in circulating ANG II elicit the increases in blood pressure via a PVN AT1R-mediated mechanism, as demonstrated by the significant attenuation of blood pressure during the acute ANG II infusion. The reduction in AT1 protein did not affect basal blood pressure, implying that this pathway is not intrinsically active. AT1a shRNA did not alter the vascular responsiveness of the SMA, eliminating the possibility that the virus affected ANG II-mediated vascular responsiveness. It is understood that the neural mechanisms involved in blood pressure regulation are complex, and these studies add to the knowledge base by further demonstrating that AT1Rs in the PVN are important in the regulation of acute changes in blood pressure. Further studies examining chronic models of hypertension will provide further insight into the mechanisms by which this pathway may be involved in hypertension.

DISCLOSURES

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

REFERENCES


