Hypothalamic SOCS-3 expression and the effect of intracerebroventricular angiotensin II injection on water intake and renal sodium handling in SHR

Adriana Zapparoli · Vivian Calegari · Lício Augusto Velloso · Dioze Guadagnini · Patrícia Aline Boer · José Antonio Rocha Gontijo

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Abstract In rats, the acute central dipsogenic and natriuretic action of angiotensin II (AngII) seems to be independent of the hemodynamic effects of the peptide; however, in genetically hypertensive models, this relationship has not yet been investigated. It has been demonstrated that AngII induces the suppressor of cytokine signaling (SOCS-3) expression in the brain that, in turn, modulates further activation of the pathway, leading to desensitization to AngII stimuli with regard to its dipsogenic effect. This study investigates age-related Janus kinase (JAK-2) and SOCS-3 hypothalamic expression, by immunoblotting, and the involvement of SOCS-3 expression in urinary sodium handling and dipsogenic response in spontaneously hypertensive rats (SHR), compared with age-matched Wistar–Kyoto (WKy) rats. The intracerebroventricular (i.c.v.) application of AngII significantly enhanced the dipsogenic response, reduced CCr, and reciprocally promoted increased absolute and fractional rates of excretion of sodium in WKy rats. The central AngII-induced dipsogenic effect in WKy and SHR was significantly attenuated by prior i.c.v. administration of DUP753. In addition, the magnitude of the dipsogenic and renal response to AngII was significantly attenuated in age-matched SHR. Blocking of hypothalamic SOCS-3 expression by an antisense oligonucleotide resulted in partial reversal of the refractory nature of AngII in thirst responses in SHR. The altered centrally applied AngII response in SHR associated with increased hypothalamic JAK-2/SOCS-3 expression may suggest that abnormal regulation of the central angiotensin pathways may contribute to dysfunction of water–electrolyte homeostasis in SHR.

Keywords Arterial hypertension · Central nervous system · Angiotensin II · SOCS-3 · SHR · Kidney function · Natriuresis · Lithium clearance

Introduction

The role of the central nervous system (CNS) in the control of blood pressure and water–electrolyte homeostasis has been demonstrated by several studies [1, 2]. Angiotensin II (AngII) also plays an important role in the control of water–electrolyte and blood pressure. It acts specifically, activating at least two well characterized transmembrane G-protein-coupled receptors belonging to the seven-transmembrane-spanning receptor family, the angiotensin type 1 receptor (AT1R), and the angiotensin type 2 receptor (AT2R) [3]. Cell bodies at the subfornical organ (SFO), hypothalamic paraventricular nucleus (PVN), medial preoptic lateral nucleus (MPOL), anterodorsal preoptic nucleus (ADP), and organum vasculosum of the lamina terminalis (OVLT) express high levels of AT1R, which respond rapidly to an AngII stimulus [4]. Most studies conclude that centrally administered AngII induces thirst by activating AT1R in neurons of the OVLT areas [5]. In dogs, the acute central natriuretic action of AngII seems to be independent of the hemodynamic effects of the peptide [6], whereas in the genetically hypertensive rat models this relationship has not yet been investigated. It has been demonstrated that spontaneously hypertensive rats (SHR) have a hyperactive brain renin–angiotensin system (RAS).
compared with that of Wistar–Kyoto (WKy) rats [7]. Sodium homeostasis disturbances seem to be important in the pathogenic process in the SHR strain, because chronic consumption of excess sodium increases, whereas sodium restriction generally attenuates, hypertension in this species [8]. We have previously demonstrated that AngII induces the suppressor of cytokine signaling (SOCS-3) via activation of AT1R and Janus kinase (JAK-2) [9], an intracellular kinase commonly engaged by receptors belonging to class I and class II cytokine receptor families, which rapidly direct the signal towards the nucleus through the signal-transducer-and-activator-of-transcription (STAT) proteins [10]. In a recent study, AngII was found to be capable of inducing SOCS-3 expression in heart and brain, which, in turn, modulates AngII-induced c-jun expression, and blocks further activation of the pathway, consequently leading to desensitization to AngII stimuli with regard to its dipsogenic effect [9, 11]. Taking in account these findings, this study, first, evaluates the effect of central AngII injection, in increasing concentrations, on water intake and urinary sodium handling in 12-week-old SHR. Additionally, possible involvement of hypothalamic SOCS-3 expression in the control of SHR water ingestion responses was investigated; these data were compared with those from age-matched appropriate normotensive WKy controls.

Materials and methods

Animals and surgical procedures

The general guidelines established by the Brazilian College of Animal Experimentation (COBEA) were followed throughout the investigation. Our local colonies originated from a breeding stock supplied by the University of Campinas Animal Breeding Center, Campinas, SP, Brazil. For AngII thirst and natriuresis induction evaluations, male 12-week-old WKy and SHR (250–320 g) were chronically instrumented with an intracerebroventricular (i.c.v.) guide cannula, and kept under controlled temperature (25°C) and light conditions (0700–1900 hours) in individual metabolic cannula, and kept under controlled temperature (25°C). Before experiments, the animals’ (WKy and SHR) water supply was removed from the home cage. The indwelling obturator was replaced by a 30-gauge stainless steel injector at the end of PE-10 tubing connected to a 10-µl Hamilton syringe entirely loaded with test solution. Immediately afterwards, AngII (at the doses stated in Fig. 1 for dose–response evaluation, in 3 µl saline for the remaining experiments) or a similar volume of saline (vehicle) was injected into the lateral ventricle in a different group of animals. To examine the thirst-induced effect after i.c.v. AngII administration, rats were randomly assigned to one of the following protocol groups:

1. i.c.v. 0.15 M NaCl injection: rats received 3 µl saline and the volume of water consumed was measured during the next 30 min;
2. i.c.v. dose–response AngII (at 40 pmol to 40 nmol) injection: rats received 3 µl AngII and the volume of water consumed was measured during the next 30 min;
3. i.c.v. SOCS-3 oligonucleotide injection for evaluation of the participation of AngII-thirst-induced behavior and SOCS-3 expression: WKy and SHR rats were treated with 4 nmol (i.c.v. in 3 µl TE (10 mM Tris/Cl, 1 mM EDTA, pH 7.6) buffer) of sense or antisense SOCS-3 oligonucleotide, based on the Rattus norvegicus SOCS-3 mRNA sequences (5'-CTG TGG GTG ACC ATG-3', accession no. AF075383 at NCBI Entrez Nucleotide), injected 30 min before 40 pmol AngII; and
4. i.c.v. administration of AT1R antagonist (DUP753): rats received 1 µl 10 nmol DUP753 10 min before i.c.v. injection of 40 pmol AngII and the volume of water consumed in 30 min was measured.

Renal function evaluation

In order to evaluate the effect of i.c.v. administration of AngII on tubular sodium handling, the 12-week-old rats were randomly assigned to one of two separate groups.
Fourteen hours before the renal test, 60 l mol LiCl per 100 g body weight was given by gavage. After an overnight fast, each animal received a load of tap water by gavage (5% of the body weight), followed by a second load of the same volume 1 h later. Thirty minutes after the second load (control period), 0.15 M NaCl or 40 pmol AngII was i.c.v. microinjected in a volume of 3 l with a 10-l Hamilton microsyringe and spontaneously voided urine was collected over four periods of 30 min into a graduated centrifuge tube. At the end of the experiment, blood samples were drawn from anesthetized rats by cardiac puncture, and urine and plasma samples were collected for analysis.

Western blot

Tissue extraction, immunoprecipitation and immunoblotting were performed as previously described [11]. Briefly, WKy (n = 5) and SHR (n = 5) rats (4, 8, and 12-weeks old), not chronically instrumented with an i.c.v. guide cannula, were anesthetized and subjected to craniotomy. Hypothalami were obtained and homogenized in freshly prepared ice-cold buffer (1% Triton X-100, 100 mM Tris, pH 7.4, 100 mM sodium pyrophosphate, 100 mM sodium fluoride, 10 mM EDTA, 10 mM sodium vanadate, 2 mM PMSF, and 0.01 mg aprotinin/ml). Insoluble material was removed by centrifugation (10,000g) for 25 min at 4°C. Aliquots of the resulting supernatants containing 2.0 mg total protein (protein determination by the Bradford method) [14] were used for immunoprecipitation with specific antibodies at 4°C overnight, followed by addition of Protein A Sepharose 6 MB for 2 h. The pellets were washed three times in ice-cold buffer (0.5% Triton X-100, 100 mM Tris, pH 7.4, 10 mM EDTA, and 2 mM sodium vanadate), and then resuspended in Laemmli sample buffer [15] and boiled for 5 min before SDS-PAGE in a miniature slab gel apparatus (Bio-Rad). Electrotransfer of proteins from the gel to nitrocellulose was performed for 90 min at 120 V (constant). The nitrocellulose transfers were probed with specific antibodies. The blots were subsequently incubated with 125I-protein A. Results were visualized by autoradiography using pre-ashed Kodak XAR film. Band intensities were quantified by optical densitometry of developed autoradiographs (Scion Image software; Scion-Corp, Frederick, MD, USA). For immunoblotting of total protein extracts, 0.2 mg total protein was suspended in Laemmli sample buffer, boiled for 5 min, and loaded on to the electrophoresis gel. SDS-PAGE, electrotransfer, and blot followed the same steps as described above for immunoprecipitation. To ensure equal loading, membranes were stained with Coomassie brilliant blue dye before blotting. As shown in Fig. 3, all membranes were also incubated with &beta-actin antibody to avoid possible inequalities in protein loading and/or transfer. Only homogeneously stained membranes were used in the study.

Antibodies and chemicals

SDS/PAGE and immunoblotting reagents were obtained from Bio-Rad (Richmond, CA, USA). Hepes, PMSF, aprotinin, dithiothreitol, Triton X-100, Tween 20, glycerol, AngII, and BSA (fraction V) were from Sigma Chemical (St Louis, MO, USA). Protein A-Sepharose 6 MB was from Pharmacia (Uppsala, Sweden) and 125I-Protein A and nitrocellulose membranes were from Amersham (Aylesbury, Bucks, UK). Antibodies against JAK-2 (rabbit polyclonal, sc-7229, for immunoprecipitation), SOCS-3 (rabbit polyclonal, sc-9023, for immunoprecipitation), and anti-&beta-actin were from Santa Cruz Biotechnology (CA, USA). Secondary antibodies and conjugated complexes utilized in immunohistochemistry were from Vector...
Laboratories (Burlingame, CA, USA). Sodium pentobarbital was from Cristália (São Paulo, Brazil).

Data presentation and statistical analysis

All numerical results are expressed as the mean ± SD of the indicated number of experiments. Plasma and urine sodium, potassium, and lithium concentration were measured by flame photometry (Micronal, B262, São Paulo, Brazil), and creatinine concentrations were determined spectrophotometrically (Instruments Laboratory, Genesys V, USA). Creatinine clearance was used to estimate glomerular filtration rate (GFR) and lithium clearance (CLi) measured by flame photometry (Micronal, B262, São Paulo, Brazil), and creatinine concentrations were determined according to the method of Neilson (Instruments Laboratory, Genesys V, USA). Creatinine clearance was used to estimate glomerular filtration rate (GFR) and lithium clearance (CLi).

Fractional sodium excretion (FE Na) was calculated as

\[ \text{C_{Na}/C_{Cr} \times 100} \]

where \( C_{Na} \) is sodium clearance and \( C_{Cr} \) is creatinine clearance. Fractional proximal (FEPNa) and post-proximal (FEPP Na) sodium excretion were calculated as

\[ \frac{\text{FE}_{\text{Na}}}{\text{C}_{\text{Cr}}} \times 100 \]

where \( \text{FE}_{\text{Na}} \) is sodium excretion and \( C_{Cr} \) is creatinine clearance. Fractional sodium handling (Fig. 2). This is further highlighted by the significant differences in FE Na during the 30th to 90th experimental minute in WKy rats, but just transiently at 30 min in the genetic hypertensive strain. This increased natriuresis was followed by pronounced enhancement of the FEK in WKy, compared with a transient and smaller increase in SHR. The increased AngII-induced FE Na in WKy rats was also accompanied by enhanced proximal and post-proximal sodium excretion and absolute sodium excretion (UNaV) in WKy animals, compared with the SHR-injected rats (Fig. 2). This effect occurred despite a parallel fall in \( C_{Cr} \). Likewise, the \( C_{Cr} \), higher natriuresis and kaliuresis responses to i.v. 40 pmol AngII injections in WKy rats were blunted in SHR \(( P \leq 0.025)\) (Fig. 2). This blunted urinary ion excretion response to AngII in SHR, compared with normotensive rats, was associated with a significantly changed proximal tubule and post-proximal sodium handling (Fig. 2). This is further highlighted by the significant differences in \( \text{FE}_{\text{Na}} \) during the same concentration–response stimuli.

Results

Dose–response curve to AngII-induced water-intake response

Intracerebroventricular AngII injections (40 and 400 pmol and 4 and 40 nmol) dose-dependently increased water consumption over 30 min in 12-week-old WKy and SHR rats (Fig. 1). The effects of AngII on increasing water consumption were significantly higher in WKy rats than in SHR animals \(( P < 0.001)\). After dose–response experiments, a dose of 40 pmol AngII was selected as optimum for the rest of the study. A single dose of saline injected i.c.v. (the protocol is given in the section “Protocols for AngII-thirst induction evaluation”) led to mean volumes of 0.35 ± 0.18 and 0.16 ± 0.10 ml per 100 g b.w. \(( n = 5 \) for each group) of water consumption over 30 min for WKy and SHR, respectively, while a single dose of AngII (40 pmol) promoted the consumption of 2.26 ± 0.3 and 0.9 ± 0.2 ml per 100 g b.w. of water in 30 min in WKy and SHR, respectively \(( n = 5, P < 0.05 \) for each group).

In addition, the i.c.v. 10 nmol DUP753 (AT1R antagonist) injection, caused sustained attenuation of intake of water after an i.c.v. dipsogenic dose (40 pmol) of AngII (Fig. 1). i.c.v. AngII-induced changes in renal function in 12-week-old WKy rats and SHR

The data for renal function (expressed as mean ± SD) in 12-week-old SHR and WKy rats are summarized in Fig. 2 and Table 1. The tail arterial blood pressure in 12-week-old SHR averaged 180.6 ± 8 mmHg in SHR and 117 ± 10 mmHg in WKy \(( P < 0.01)\). There were no significant differences between serum sodium, potassium, and lithium levels (Table 1) in the groups. The GFR, estimated by \( C_{Cr} \) immediately after AngII injection, decreased significantly in SHR and WKy rats. These decreases were significantly greater in the WKy group, when compared with SHR (Fig. 2). The i.c.v. microinjection of 40 pmol AngII time-dependently increased the \( \text{FE}_{\text{Na}} \) from the 30th to 90th experimental minute in WKy rats, but just transiently at 30 min in the genetic hypertensive strain. This increased natriuresis was followed by pronounced enhancement of the FEK in WKy, compared with a transient and smaller increase in SHR. The increased AngII-induced \( \text{FE}_{\text{Na}} \) in WKy rats was also accompanied by enhanced proximal and post-proximal sodium excretion and absolute sodium excretion (UNaV) in WKy animals, compared with the SHR-injected rats (Fig. 2). This effect occurred despite a parallel fall in \( C_{Cr} \). Likewise, the \( C_{Cr} \), higher natriuresis and kaliuresis responses to i.v. 40 pmol AngII injections in WKy rats were blunted in SHR \(( P \leq 0.025)\) (Fig. 2). This blunted urinary ion excretion response to AngII in SHR, compared with normotensive rats, was associated with a significantly changed proximal tubule and post-proximal sodium handling (Fig. 2). This is further highlighted by the significant differences in \( \text{FE}_{\text{Na}} \) during the same concentration–response stimuli.

Hypothalamic JAK-2 and SOCS-3 expression in WKy and SHR animals

Expression of the JAK-2 and SOCS-3 proteins by the WKy and SHR hypothalami were determined at 4, 8, and 12 weeks of age in unanesthetized, unrestrained rats. Evaluation of age-dependent JAK-2 and SOCS-3 expression in extracts of hypothalami resulted, respectively, in a significant time-dependent decrease in JAK-2 protein, accompanied by enhancement of detectable SOCS-3 bands in SHR with no appreciable difference, for both proteins, for the WKy strain during the same time period (Fig. 3). Additionally, as shown in Fig. 3, the SOCS-3/JAK-2 association increased significantly from 4 to 12 weeks of age only in the SHR strain \(( P \leq 0.05)\).
AngII-induced thirst response desensitization in WKy and SHR is abrogated by SOCS-3 antisense oligonucleotide treatment

Because SOCS-3 is a well-known controller of cytokine and hormone signaling, we decided to test the hypothesis that AngII-induced SOCS-3 expression may participate in the mechanisms of hormone desensitization in the SHR hypothalamus response by blocking SOCS-3 expression, utilizing a SOCS-3 antisense phosphorothioate-modified oligonucleotide. The i.c.v. injections of 4 nmol antisense or respective sense oligonucleotide (in 3 μl) were designed and tested for their ability to block SOCS-3 synthesis by measuring SOCS-3 associated with JAK-2 in immunoprecipitates of WKy hypothalamic protein extracts (n = 3). The antisense oligonucleotide sequence was capable of desensitizing the thirst response to AngII in WKy and SHR. The data are reported as the mean ± SD. *P ≤ 0.05 versus WKy (ANOVA and Bonferroni’s contrast test, n = 10). See “Results” for details of statistical analysis.

Table 1  Body weight as related to age, sodium intake, serum sodium, potassium, and lithium levels in spontaneously hypertensive (SHR) and strain normotensive (WKy) rats fed a standard diet

| Groups | Na⁺ (mM) | K⁺ (mM) | Li⁺ (μM) | Body weight (g) 12 weeks | Sodium intake (mmol/weeks/100 g) |
|--------|---------|---------|----------|---------------------------|---------------------------------|
| WKy (n = 10) | 138 ± 2.6 | 4.3 ± 0.6 | 85 ± 19 | 262 ± 15 | 12.7 ± 2.3 |
| SHR (n = 11) | 142 ± 3.1 | 4.1 ± 0.4 | 79 ± 15 | 180 ± 13* | 11.8 ± 1.9 |

Data are reported as mean ± SD
* P ≤ 0.05 versus WKy (Student’s t test)
reducing basal or AngII-stimulated SOCS-3 association to JAK-2 by 80% ($P < 0.05$) and was utilized in all experiments in parallel with its respective sense sequence as a control (Fig. 4a). All the protocols described in the section “Protocols for AngII-thirst induction evaluation” were repeated in the presence of sense or antisense SOCS-3 oligonucleotide (i.c.v. injected 30 min before 40 pmol AngII) and drinking water volume was measured. As depicted in Fig. 4b, pretreatment with SOCS-3 antisense (2.17 ± 0.4 ml per 100 g b.w. of water in 30 min, $n = 5$) but not with sense oligonucleotide (1.07 ± 0.2 ml 100 g b.w. of water in 30 min, $n = 5$) significantly reversed the SHR desensitization response of AngII as an inducer of the water-drinking response (Fig. 4).

**Discussion**

Many investigators studying mammalian species have demonstrated that administration of AngII into the cerebral ventricles elicits enhancement in renal sodium excretion and water intake [18, 19]. Previously, we and others have shown that i.c.v. administration of hypertonic saline promotes natriuresis and that losartan inhibits responses to the central administration of AngII, and to vasopressin secretion, natriuresis, and the pressor response to i.c.v. hypertonic saline [17, 20, 21]. This study shows the effect of central AngII administration on spontaneous water consumption in a concentration-dependent fashion, but also demonstrated that the water-intake response to graded AngII concentrations was strikingly attenuated in 12-week-old SHR, compared with age-matched WKy controls. This study confirmed that AngII, when microinjected into the lateral ventricle of conscious rats, produced a marked absolute and fractional natriuretic response in normotensive rats (Fig. 2), associated with a rise in proximal and post-proximal sodium excretion, despite a transient decrease in creatinine clearance in both strains. In addition, the study confirms previous data [22] obtained with Wistar rats showing that water intake effects in WKy and SHR were significantly attenuated by prior i.c.v. administration of DUP753 (10 nmol), as shown in Fig. 1b. Similar to the dipsogenic effect of AngII, which was lower in the hypertensive strain than in normotensive rats, our results clearly demonstrate that the magnitude of the urinary sodium excretion response to centrally injected AngII was significantly attenuated in SHR compared with age-matched WKy. Taking these findings into account, we may hypothesize that these AngII-mediated pathways, in the CNS, participate in the regulation of fluid and electrolyte balance in both strains.

While circulating AngII tends to retain sodium by a direct renal action [23] and by aldosterone release from the adrenal gland, stimulation of brain AngII receptors has been reported to induce natriuresis [19, 20]. The mechanism by which central AngII induces its natriuretic effects remains to be elucidated. Several possibilities may be considered. First, the CNS may directly affect renal sodium excretion through neural routes. Second, hemodynamic factors may be responsible for the alterations in electrolyte excretion. Third, the natriuresis may result from fluctuations in the level of neural-borne factors which affect tubular sodium handling. There is substantial evidence supporting a role of the sympathetic nervous system in the control of urinary sodium excretion [1, 2]. A previous study reported that, in conscious rats, i.c.v. injection of...
AngII at doses of between 1 and 100 pmol induce an immediate reduction in efferent renal nerve and plasma renin activity [24]. A reduction in sympathetic nerve traffic to the kidney could be involved, because tubule sodium reabsorption can be increased by stimulation of renal nerves. Although the precise mechanism by which blood pressure rises in the SHR strain remains to be elucidated, renal control of the fluid and electrolyte balance is thought to play a dominant role in the long-term control of arterial blood pressure. Several reports indicate that SHR kidneys require higher arterial pressure than kidneys of normotensive rats to excrete the same amount of salt under basal conditions [16, 25]. Thus, efferent renal adrenergic overexcitability in SHR could be less depressed after i.c.v. AngII stimuli, promoting attenuated urinary excretion of salt. In addition, the observed central attenuated natriuretic responses to AngII injection in SHR may imply that the rise in renal perfusion pressure did not occur through (or may not exert a predominant role in) electrolyte excretion in hypertensive rats. Although the data presented here do not offer any support for the humoral hypothesis, we cannot rule out the possibility that several humoral factors, induced by central RAS activity, may be involved in mediating the natriuresis observed in this study.

It has been suggested that dysfunction of the brain RAS in SHR contributes to the pathogenesis of hypertension in
this strain [26, 27]. Our findings demonstrate in SHR, but not in WKy rats, a sustained age-related increase in the level of SOCS-3 expression, and in the JAK-2/SOCS-3 association, accompanied by reduced JAK-2 activity in the hypothalamus.

Several systems may participate in the control of signal transduction. Serine and threonine kinases or phosphatases act to enhance and suppress signal transduction [28]. In contrast with enzymatic interference, some systems may be regulated by physical blockade of the signal transducers’ functional sites. This seems to be true for the eight members of the SOCS family. SOCS proteins act by targeting members of the JAK family and interfering with downstream steps of their signaling cascade [29, 30]. Taking these data together with our current results, we may suppose that, acting through AT1R on the hypothalamus, the AngII overactivity in SHR may induce gradual and increased expression of SOCS-3 that, in turn, blocks further activation of the pathway and consequently leads to desensitization to the dipsogenic effect of AngII. This hypothesis was confirmed by blocking hypothalamic SOCS-3 expression by i.c.v. injection of an antisense oligonucleotide specific for SOCS-3, resulting in partial (but significant) reversal of the refractory nature of AngII in thirst responses in SHR.

As previously shown in rat hypothalamus and hearts [9, 11], and confirmed in this study, the expression of SOCS-3 is induced by AngII through AT1R, and blockade of SOCS-3 expression by antisense oligonucleotide treatment, i.c.v. injected 30 min before AngII, restores the capacity of AngII to stimulate water intake in SHR (Fig. 1). We have also demonstrated that, following AngII stimulus, an impressive change in the staining pattern was observed with a strong and compact labeling of MPOL and ADP neurons [9]. According to previous studies [31], neuron bodies of the anterior hypothalamic preoptic area, together with OVLT neurons, may act as primary sites for AngII action in the control of water balance and induction of drinking behavior. In our current experiments, i.c.v. injections of AngII provoked a smaller water volume intake in SHR than in age-matched WKy rats. Therefore, it seems that the refractory response to AngII in the hypothalamus could be mediated by an AngII inducible factor that may block thirst stimulus through AT1R. The i.c.v. administration of the AT1R antagonist significantly prevented the AngII dipsogenic effect and the JAK-2/SOCS-3 association, a fact that, taken together with the pattern of histological distribution of SOCS-3 and AT1R, strongly suggests that the effect of AngII upon SOCS-3 expression is mediated by AT1R [9].

In conclusion, these findings lend further support to the idea that AngII in the CNS is crucial in the regulation of body fluid homeostasis. The i.c.v. application of AngII significantly enhanced dipsogenic response, and reduced Ccr, and reciprocally promoted increased absolute and fractional excretion rates of sodium in WKy rats. The magnitude of the dipsogenic and renal response to AngII was significantly attenuated in age-matched SHR. The altered centrally applied AngII response in SHR, associated with increased hypothalamic JAK-2 and SOCS-3 expression, may suggest that abnormal function of central angiotensin pathways activity can contribute to dysregulated water–electrolyte balance in SHR.

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Conflict of interest None.

References

1. DiBona GF (2000) Nervous kidney. Interaction between renal sympathetic nerves and renin-angiotensin system in the control of renal function. Hypertension 36:1083–1088
2. Gontijo JAR, Garcia WE, Figueiredo JF, Silva-Netto CR, Furtado MRF (1992) Renal sodium handling after noradrenergic stimulation of the lateral hypothalamic area in rats. Braz J Med Biol Res 25:937–942
3. Inagami T, Kambayashi Y, Ichiki T, Tsuzuki S, Eguchi S, Yamakawa T (1999) Angiotensin receptors: molecular biology and signalling. Clin Exp Pharmacol Physiol 26:544–549
4. Bunnemann B, Iwai N, Metzger R, Fuxe K, Inagami T, Ganten D (1992) The distribution of angiotensin II AT1 receptor subtype mRNA in the rat brain. Neurosci Lett 142:155–158
5. Fitzsimons JT (1998) Angiotensin, thirst, and sodium appetite. Physiol Rev 78:583–686
6. Shoji M, Kimura T, Ota K, Inoue M, Sato K, Ohta M, Yamamoto T, Abe K, Yoshinaga K (1991) Responses of atrial natriuretic peptide, vasopressin, aldosterone and renal function to intracerebroventricular infusion of angiotensin II in dogs. Tohoku J Exp Med 163:187–197
7. Saavedra JM (1999) Emerging features of brain angiotensin receptors. Regul Pept 85:31–45
8. Aoki K, Yamori Y, Ooshima A, Okamoto K (1972) Effects of high or low sodium intake in spontaneously hypertensive rats. Jpn Circ J 36:539–545
9. Torsoni MA, Carvalheira JB, Calegari VC, Bezerra RM, Saad MJ, Gontijo JA, Velloso LA (2004) Angiotensin II (AngII) induces the expression of suppressor of cytokine signaling (SOCS)-3 in rat hypothalamus—a mechanism for desensitization of AngII signaling. J Endocrinol 181:117–128
10. Marrero MB, Schieffer B, Paxton WG, Heerdt L, Berk BC, Delafontaine P, Bernstein KE (1995) Direct stimulation of JAK/STAT pathway by the angiotensin II AT1 receptor. Nature 375:247–250
11. Calegari VC, Bezerra RM, Torsoni MA, Torsoni AS, Franchini KG, Saad MJ, Velloso LA (2003) Suppressor of cytokine signaling 3 is induced by angiotensin II in heart and isolated cardiomyocytes, and participates in desensitization. Endocrinology 144:4586–4596
12. Michelotto JB, Carvalheira JB, Saad MJ, Gontijo JA (2002) Effects of intracerebroventricular insulin microinjection on renal
sodium handling in kidney-denervated rats. Brain Res Bull 57:613–618
13. Lovenberg W (1987) Techniques for measurements of blood pressure. Hypertension 9:15–16
14. Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254
15. Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680–685
16. Boer PA, Morelli JM, Figueiredo JF, Gontijo JA (2005) Early altered renal sodium handling determined by lithium clearance in spontaneously hypertensive rats (SHR): role of renal nerves. Life Sci 76:1805–1815
17. Guadagnini D, Gontijo JA (2006) Altered renal sodium handling in spontaneously hypertensive rats (SHR) after hypertonic saline intracerebroventricular injection: role of renal nerves. Life Sci 79:1666–1673
18. Ferguson AV, Washburn DL, Latchford KJ (2001) Hormonal and neurotransmitter roles for angiotensin in the regulation of central autonomic function. Exp Biol Med 226:85–96
19. Unger T, Horst PJ, Bauer M, Demmert G, Rettig R (1989) Natriuretic action of central angiotensin II in conscious rats. Brain Res 486:33–38
20. Mathai ML, Evered MD, McKinley M (1998) Central losartan blocks natriuretic, vasopressin, and pressor responses to central hypertonic NaCl in sheep. Am J Physiol 275:R548–R554
21. Seltzer A, Bregozzo C, Armando I, Baiardi G, Saavedra JM (2004) Oral administration of an AT1 receptor antagonist prevents the central effects of angiotensin II in spontaneously hypertensive rats. Brain Res 1028:9–18
22. Beresford MJ, Fitzsimons JT (1992) Intracerebroventricular angiotensin II-induced thirst and sodium appetite in rat are blocked by the AT1 receptor antagonist, losartan (DUP753) but not by the AT2 antagonist, CGP42112B. Exp Physiol 77:761–764
23. Hall JE, Brands MW (2000). The renin-angiotensin-aldosterone systems. In: Seldin DW, Giebisch G (eds) The kidney: physiology and pathophysiology, chap 40. Lippincott Williams & Wilkins, New York
24. Kannan H, Nakamura T, Jin XJ, Hayashida Y, Yamashita H (1991) Effects of centrally administered angiotensin on sympathetic nerve activity and blood flow to the kidney in conscious rats. J Auton Nerv Syst 34:201–210
25. Hall JE, Guyton AC, Brands MW (1996) Pressure-volume regulation in hypertension. Kidney Int 55:S35–S41
26. Veerasingham SJ, Raizada MK (2003) Brain renin-angiotensin system dysfunction in hypertension: recent advances and perspectives. Br J Pharmacol 139(2):191–202
27. Zhu F, Liao YH, Li LD, Cheng M, Wei F, Wei YM, Wang M (2006) Target organ protection from a novel angiotensin II receptor (AT1) vaccine ATR12181 in spontaneously hypertensive rats. Cell Mol Immunol 3:107–114
28. Cross TG, Scheel-Toellner D, Henriquez NV, Deacon E, Salmon M, Lord JM (2000) Serine/threonine protein kinases and apoptosis. Exp Cell Res 256:34–41
29. Naka T, Narazaki M, Hirata M, Matsumoto T, Minamoto S, Aono A, Nishimoto N, Kajita T, Taga T, Yoshizaki K, Akira S, Kishimoto T (1997) Structure and function of a new STAT-induced STAT inhibitor. Nature 387:924–929
30. Yoshimura A, Ohkubo T, Kiguchi T, Jenkins NA, Gilbert DJ, Copeland NG, Hara T, Miyajima A (1995) A novel cytokine-inducible gene CIS encodes an SH2-containing protein that binds to tyrosine-phosphorylated interleukin 3 and erythropoietin receptors. EMBO J 14:2816–2826
31. Simonnet G, Rodriguez F, Fumoux F, Czemichow P, Vincent JD (1979) Vasopressin release and drinking induced by intracranial injection of angiotensin II in monkey. Am J Physiol 237:R20–R25