

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

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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 C_{Cr} , 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 (AT_{1R}), and the angiotensin type 2 receptor (AT_{2R}) [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 AT_{1R} , which respond rapidly to an AngII stimulus [4]. Most studies conclude that centrally administered AngII induces thirst by activating AT_{1R} 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)

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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 cages, with free access to tap water and standard laboratory rodent chow (Purina rat chow: Na⁺ content: 135 ± 3 μEq/g; K⁺ content: 293 ± 5 μEq/g) for 7 days before the experiments. Briefly, the animals were anesthetized with sodium pentobarbital (50 mg/kg body wt, i.p.) and, after loss of corneal and pedal reflexes, were positioned on a Stoelting stereotaxic apparatus. A 23-gauge guide stainless steel cannula with an indwelling 30-gauge obturator was stereotaxically implanted into the lateral cerebral ventricle (LCV) using previously reported techniques and pre-established coordinates: anteroposterior, 0.2 mm from bregma, lateral 1.5 mm and vertical –2.8 mm [12]. Rats were allowed a 1-week

recovery before testing for cannula patency and position. Cannulas were considered patent and correctly positioned on the basis of appropriate infusion of Evans blue through the i.c.v. at the end of the experiments. Tail arterial pressure was estimated 1 day before the renal test, in conscious and conditioned rats by the tail-cuff method, using an electrospigmomanometer (Narco Bio-System, Austin, TX, USA). This indirect approach enables repeated measurements with close correlation with direct intra-arterial recordings (correlation coefficient = 0.975) [13].

Protocols for AngII-thirst induction evaluation

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

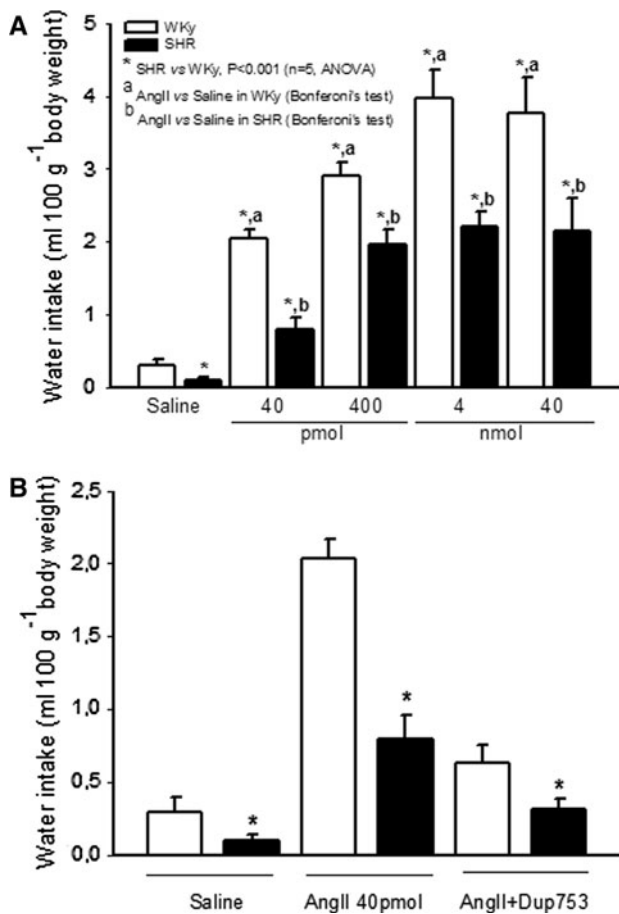


Fig. 1 Effect of i.c.v. dose–response injection of AngII on drinking behavior in WKy and SHR rats. Rats were i.c.v. cannulated and received 3.0 μ l saline, 3.0 μ l of a solution containing AngII or 1 μ l DUP753 before 3 μ l AngII (amounts stated in the figure). In all groups, the volume of spontaneous water consumption was measured over the next 30 min. In all experiments, $n = 5$; $*P \leq 0.05$ versus saline-treated (ANOVA)

(SHR and WKy). Fourteen hours before the renal test, 60 μ 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 μ l with a 10- μ 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-flashed 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 β -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- β -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 \pm 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 (C_{Li}) was used to assess proximal tubule output [12, 16, 17]. Fractional sodium excretion (FE_{Na}) was calculated as $C_{Na}/C_{Cr} \times 100$, where C_{Na} is sodium clearance and C_{Cr} is creatinine clearance. Fractional proximal (FEP_{Na}) and post-proximal ($FEPP_{Na}$) sodium excretion were calculated as $C_{Li}/C_{Cr} \times 100$ and $C_{Na}/C_{Li} \times 100$, respectively. Data obtained over time were analyzed using appropriate ANOVA. Post-hoc comparisons between selected means were performed with Bonferroni's contrast test when initial ANOVA indicated statistical differences between experimental groups. Comparisons involving only two means within or between groups were carried out using a Student's *t* test. The results of blots are presented as direct comparisons of bands in autoradiographs and quantified by densitometry using Scion Image software. The Tukey–Kramer test (ANOVA) was used for statistical analysis. The level of significance was set at $P \leq 0.05$.

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 ($P \leq 0.001$). 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 ($n = 5$, $P \leq 0.05$ 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 \pm 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 ($P \leq 0.01$). 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 FE_{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 FE_K 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 urinary 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.c.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 FE_{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 ($P \leq 0.05$).

Fig. 2 Effect of i.c.v. injection of 40 pmol AngII upon renal function behavior of rats. Rats were i.c.v. cannulated and received 3.0 μ l saline or 3.0 μ l of a solution containing AngII. Creatinine clearance (C_{Cr}), fractional sodium excretion (FE_{Na}), proximal (FEP_{Na}) and post-proximal (FEP_{Na}) fractional sodium excretion, fractional potassium excretion (FE_K), and absolute urinary sodium excretion ($UNaV$) in 12-week-old SHR and WKy rats. The data are reported as the mean \pm SD. * $P \leq 0.05$ versus WKy (ANOVA and Bonferroni's contrast test, $n = 10$). See "Results" for details of statistical analysis

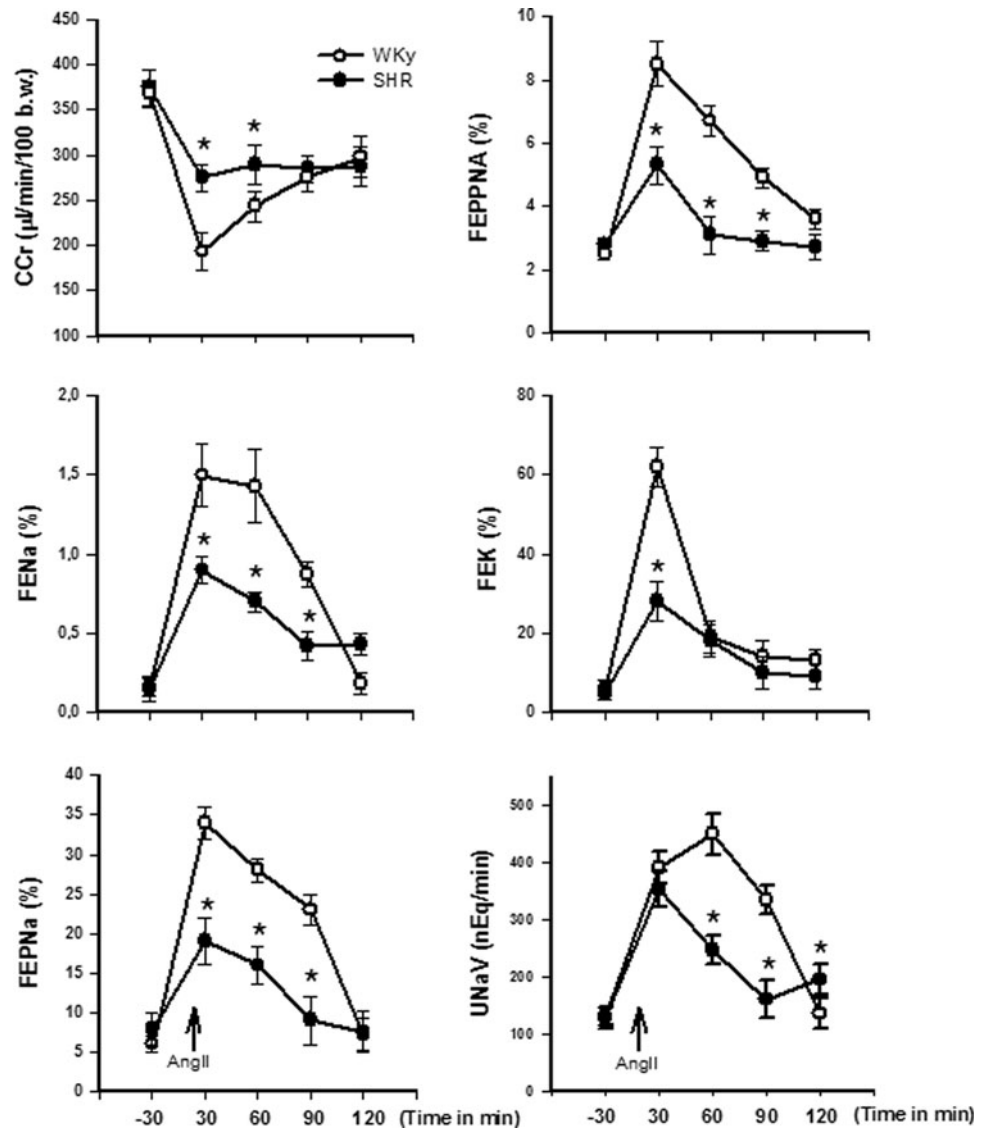


Table 1 Body weight as related to age, sodium intake, serum sodium, potassium, and lithium levels in spontaneously hypertensive (SHR) and strain normotensive (WKy) rats fed a standard diet

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

Data are reported as mean \pm SD

* $P \leq 0.05$ versus WKy (Student's t test)

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 μ l) 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

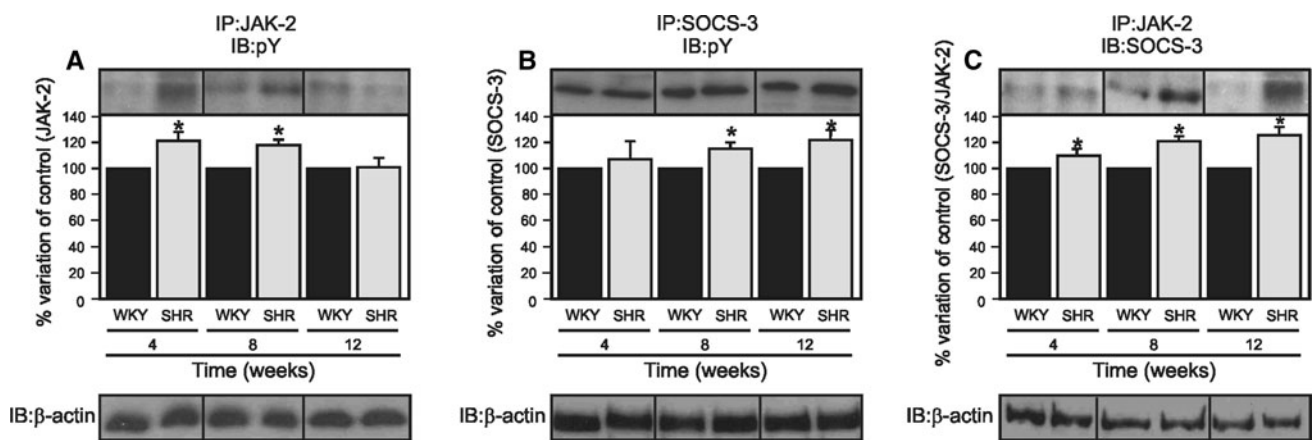


Fig. 3 Age-related (at 4, 8, and 12-week-old) SOCS-3 expression and activation of JAK-2 and SOCS-3/JAK-2 association signal transduction in the hypothalami of SHR and WKY rats. After 4, 8, and 12 weeks, hypothalami were obtained and total protein was extracted, according to the protocols described in “Materials and methods”. Immunocomplexes were separated by SDS-PAGE, transferred to

nitrocellulose membranes and blotted (IB) with anti-SOCS-3 or anti-phosphotyrosine (pY) antibodies. In all experiments, $n = 5$; $*P < 0.05$ SHR versus WKY rats (ANOVA). At the bottom, we show representative densitometric expression of β -actin as the internal standard for protein loading

reducing basal or AngII-stimulated SOCS-3 association to JAK-2 by 80% ($P \leq 0.05$) 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⁻¹ 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).

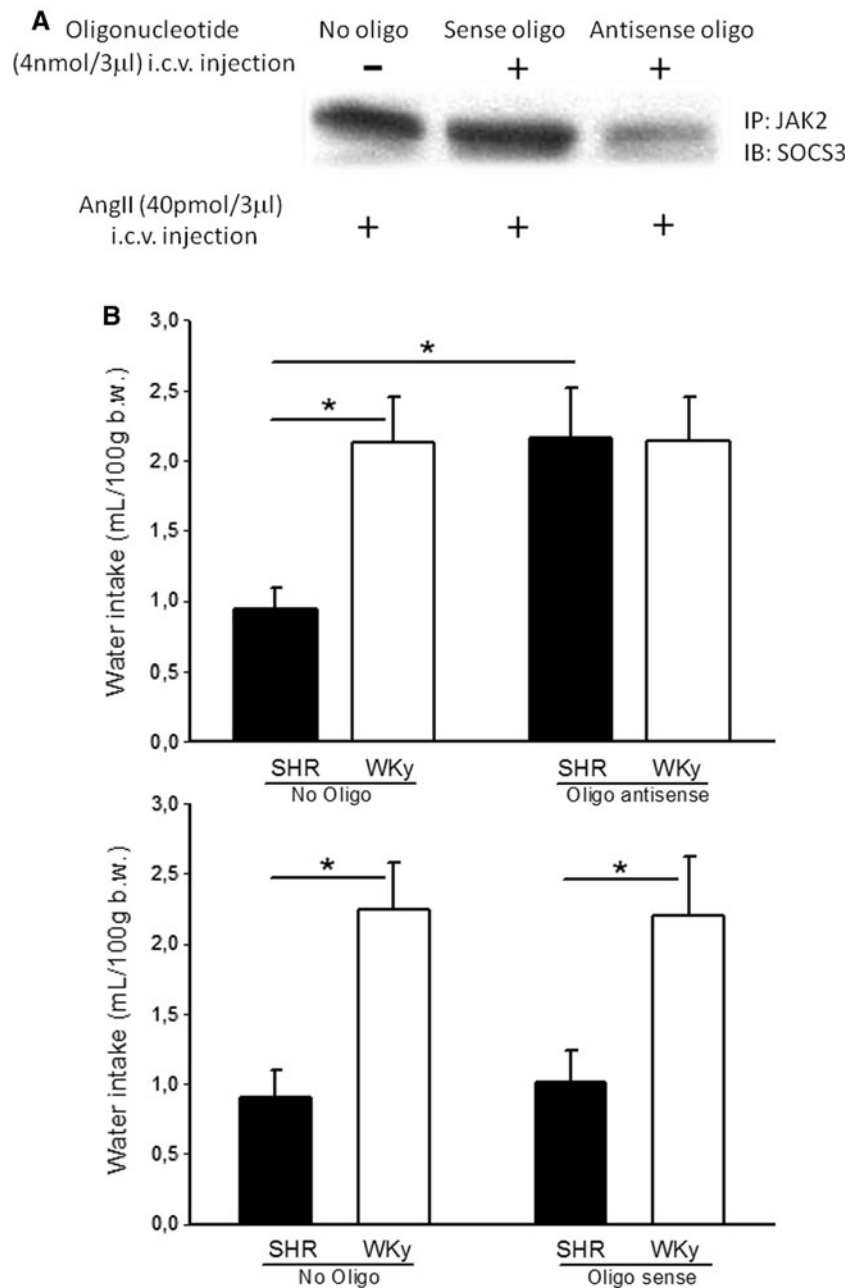
Discussion

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

Fig. 4 Effect of SOCS-3 partial blockade upon AngII-elicited SOCS-3 drinking behavior of SHR. Two groups of rats (**a**, **b**) were i.c.v. cannulated and received either SOCS-3 antisense or SOCS-3 sense phosphorothioate-modified oligonucleotides (4.0 nmol in 3.0 μ l solution). Thirty minutes after SOCS-3 antisense or sense, a dose of AngII (40 pmol) was injected and water intake was measured over the next 30 min. In all experiments $n = 5$; $*P \leq 0.05$ versus No oligo (no oligonucleotides) (ANOVA and Bonferroni's contrast test)



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 C_{Cr} 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.

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