

Altered urinary sodium excretion response after central cholinergic and adrenergic stimulation of adult spontaneously hypertensive rats

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Abstract In this study, we hypothesized that blunting of the natriuresis response to intracerebroventricularly (i.c.v.) microinjected cholinergic and adrenergic agonists is involved in the development of hypertension in spontaneously hypertensive rats (SHR). We evaluated the effect of i.c.v. injection of cholinergic and noradrenergic agonists, at increasing concentrations, and of muscarinic cholinergic and $\alpha 1$ and $\alpha 2$ -adrenoceptor antagonists on blood pressure and urinary sodium handling in SHR, compared with age-matched Wistar Kyoto rats (WR). We confirmed that CCh and NE microinjected into the lateral ventricle (LV) of conscious rats leads to enhanced natriuresis. This response was associated with increased proximal and post-proximal sodium excretion accompanied by an unchanged rate of glomerular filtration. We showed that cholinergic-induced natriuresis in WR and SHR was attenuated by previous i.c.v. administration of atropine and was significantly lower in the hypertensive strain than in WR. In both groups the natriuretic effect of injection of noradrenaline into the LV was abolished by previous local injection of an $\alpha 1$ -adrenoceptor antagonist (prazosin). Conversely, LV $\alpha 2$ -adrenoceptor antagonist (yohimbine) administration potentiated the action of noradrenaline. The LV yohimbine pretreatment normalized urinary sodium

excretion in SHR compared with age-matched WR. In conclusion, these are, as far as we are aware, the first results showing the importance of interaction of central cholinergic and/or noradrenergic receptors in the pathogenesis of spontaneous hypertension. These experiments also provide good evidence of the existence of a central adrenergic mechanism consisting of $\alpha 1$ and $\alpha 2$ -adrenoceptors which works antagonistically on regulation of renal sodium excretion.

Keywords Central nervous system · Renal function · Natriuresis · Intracerebroventricular · Cholinergic system · Adrenergic system · Hypertension · SHR

Abbreviations

0.15 M NaCl	Vehicle
Atr	Selective cholinergic (atropine) antagonist
AV3V	Anterior portion of the third ventricle
AVP	Vasopressin
C_{Li}	Lithium clearance
C_{Na}	Sodium clearance
CCh	Carbachol
CNS	Central nervous system
FE_{Na}	Fractional sodium excretion
FEP_{Na}	Fractional proximal sodium excretion
$FEPP_{Na}$	Fractional post-proximal sodium excretion
GFR	Creatinine clearance
i.c.v.	Intracerebroventricular
LV	Right lateral ventricle
MHS	Hypertensive rats of the Milan strain
NE	Norepinephrine
Pz	$\alpha 1$ -Adrenoceptor (prazosin) antagonist
SHR	Spontaneously hypertensive rats
WR	Wistar Kyoto rats
Yo	$\alpha 2$ -Adrenoceptor (Yohimbine) antagonist

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Introduction

The involvement of the central nervous system (CNS) in the control of blood pressure and water–electrolyte homeostasis has been demonstrated in several studies [1–4]. It has long been known that there is an association between the hypothalamus and water and electrolyte excretion by the kidneys [1, 5, 6]. Cholinergic and adrenergic stimulation of the septal area, lateral hypothalamus, subfornical organ, and the anterior region of the third ventricle (AV3V) induces dose-related natriuresis accompanied by, but to a lesser extent, kaliuresis [1, 7–12]. Studies have also shown that electrolytic lesion of the hypothalamic regions of conscious rats reduces water and salt intake, and the pressor response to angiotensinergic, cholinergic, and noradrenergic activation of the median preoptic nucleus [13], further suggesting involvement of the hypothalamus in hydroelectrolyte homeostasis and cardiovascular control. Studies of the CNS pathways involved in regulation of electrolyte and water excretion have also revealed that these stimulation and inhibitory effects are mediated by cholinergic receptors and by alpha and beta-adrenoceptors respectively, [1, 10, 14–18]. It has also been postulated that the kidneys are of crucial importance in the pathogenesis of essential hypertension, as a consequence of a primary defect in kidney function and/or renal hemodynamics that promotes retention of sodium [19–22]. Although the precise mechanism by which blood pressure increases in spontaneously hypertensive rats (SHR) also remains to be elucidated, renal control of the fluid and electrolyte balance is believed to dominate long-term control of arterial blood pressure. Sodium metabolism disturbances seem to be important in the pathogenic process in the Okamoto–Aoki strain of SHR, because chronic consumption of excess sodium increases whereas sodium restriction usually attenuates hypertension in this species [23, 24]. In addition, cross-transplantation studies indicate that a defect in renal function is important in determining arterial pressure [25, 26]. Previous balance studies [20, 27, 28] examining urinary sodium excretion throughout 3–7-week periods provided evidence of renal dysfunction in young genetically hypertensive rats of the Milan strain (MHS), SHR, and the stroke-prone sub-strain of SHR. These studies revealed that excretion of urinary salt and water was lower than for paired age-matched normotensive Wistar Kyoto rats (WR). Similar results were reported by Herlitz et al. [29] for 7-week-old SHR compared with normotensive Wistar rats, although sparse data were reported for the appropriate genetic control, WR. In view of these results, we hypothesized that presumable blunting of the natriuresis response to centrally injected cholinergic and noradrenergic agonists may affect renal tubule sodium and water

transport, resulting in the inability of the kidneys to handle the hydroelectrolytic balance, and consequently, causing blood pressure enhancement. To test this hypothesis, in this study we evaluated the effect of intracerebroventricular microinjection of cholinergic and noradrenergic agonists at increasing concentrations, and muscarinic cholinergic, $\alpha 1$, and $\alpha 2$ -adrenoceptor antagonists on blood pressure and urinary sodium handling in SHR, and compared the results with those from age-matched normotensive WR.

Methods

Animals and surgical procedures

The general guidelines established by the Brazilian College of Animal Experimentation (COBEA) were followed throughout the investigation. The renal tests were conducted on age-matched, male offspring of sibling-mated SHR and randomly outbred WR (aged 12 weeks after weaning). Our local colonies originated from breeding stock supplied by CEMIB/Unicamp, Campinas, SP, Brazil. Male WR and SHR (180–250 g) were chronically instrumented with an right lateral ventricle (LV) guide cannula, and kept under controlled temperature (25 °C) and lighting conditions (07:00 h–19:00 h) in individual metabolic cages, with free access to tap water and standard laboratory rodent chow (Purina rat chow: Na^+ content: $135 \pm 3 \mu\text{Eq/g}$; K^+ content: $293 \pm 5 \mu\text{Eq/g}$) for seven days before the experiments. Briefly, the animals were anesthetized by intraperitoneal injection of sodium pentobarbital (50 mg kg^{-1} body weight) and a stainless steel cannula was stereotaxically implanted into the right LV, by use of techniques reported elsewhere and pre-established coordinates: anteroposterior +0.2 mm from bregma; lateral +1.5 mm; and vertical –4.5 mm [30, 31]. Rats were allowed one-week recovery before testing for cannula patency and position. The position of the cannula was visually confirmed by infusion of blue Evans 2 % through the i.c.v. cannula at the end of the experiment. Data from animals with incorrectly placed cannulas were excluded from statistical analysis.

Renal function test

Renal function tests were performed at 12 weeks of age on conscious, unrestrained SHR and WR. To evaluate the effect of intracerebroventricular cholinergic drug carbamylcholine chloride (carbachol) and noradrenergic (norepinephrine) drug microinjections on tubular sodium handling, the rats were randomly assigned to one of three groups (a, b, and c). Fourteen hours before the renal test,

60 $\mu\text{mol LiCl}/100 \text{ g}^{-1}$ body weight was given by gavage. At 8:00 a.m., after an overnight fast with free access to water, each animal received tap water by gavage (5 % of body weight), followed by the same volume 1 h later. Immediately before experiments, the animals' (WR 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 completely filled with test solution. Afterwards, 30 min after the second volume of water, the drugs carbachol (CCh, $n = 12$ for each dose) (group a), norepinephrine (NE, $n = 12$ for each dose) (group b), Sigma Chemical, or a similar volume of 0.15 M NaCl (vehicle, $n = 12$) (group c) was microinjected into the LV in a volume of 3 μl at different concentrations (CCh: 0.7, 1.4, 2.8 and 5.6 nmol; NE: 7.5, 15.0, 30.0 and 60.0 nmol of a different group of animals) with a 10- μl Hamilton microsyringe and urine spontaneously voided over four periods of 30 min was collected into graduated centrifuge tubes. To examine the effect of antagonist after LV administration of 2.8 nmol CCh or 30 nmol NE, rats were randomly assigned to one of four specific experimental groups and a selective cholinergic antagonist (4 nmol atropine, Atr) (group d), $\alpha 1$ -adrenoceptor antagonist (4 nmol prazosin, Pz) (group e), $\alpha 2$ -adrenoceptor antagonist (4 nmol Yohimbine, Yo) (group f), or vehicle (0.15 M NaCl) (group g) was LV microinjected 30-min before the agonist in a volume of 1 μl . At the end of the experiment, blood samples were drawn by cardiac puncture from anesthetized rats and urine and plasma samples were collected for analysis [17, 18, 20, 31].

Blood pressure measurement

For some of the animals in all the groups, systolic blood pressure (SBP) was measured 30 min before and after LV cholinergic (2.8 nmol CCh) and 30 nmol noradrenergic agonist and/or antagonist administration, then twice at 60-min intervals, for conscious SHR and WR, by use of an indirect tail-cuff method with an electrospygmanometer combined with a pneumatic pulse transducer and/or amplifier (IITC Life Science BpMonWin Monitor Version 1.33). This indirect approach enabled repeated measurements with close correlation (correlation coefficient = 0.975), compared with direct intra-arterial recording [4, 20, 30]. Systolic blood pressure was measured, in the morning, from 9:00 to 11:00 h. The mean of three consecutive readings was used as the blood pressure.

Data presentation and statistical analysis

All numerical results are expressed as mean \pm SD for the number of experiments indicated. Plasma and urine sodium,

potassium, and lithium concentrations were measured by flame photometry (Micronal B262, São Paulo, Brazil). 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 [20, 30, 31]. Fractional sodium excretion (FE_{Na}) was calculated as $C_{\text{Na}}/C_{\text{Cr}} \times 100$, 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 $C_{\text{Li}}/C_{\text{Cr}} \times 100$ and $C_{\text{Na}}/C_{\text{Li}} \times 100$, respectively. Data obtained over time were analyzed by use of repeated measures two-way 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 performed by use of a Student's t test. The level of significance was set at $P \leq 0.05$.

Results

Blood pressure and renal function data (expressed as mean \pm SD) for 12-week-old SHR ($n = 12$ in each experimental group) and WR ($n = 12$ in each experimental group) are summarized in Figs. 1, 2, 3, 4, and 5 and Table 1. Basal tail arterial blood pressure among 12-week-old SHR averaged 185.7 ± 9 mmHg for SHR and 126 ± 11 mmHg for WR ($P \leq 0.01$). There were no significant differences between the weekly sodium consumption, serum sodium, and potassium and lithium levels (Table 1) in WR compared with the age-matched SHR group. Initial body weight was slightly, but significantly, lower among SHR. However, SHR grew less rapidly over their lifetime, and a significant difference was observed 12 weeks of life compared with the age-matched WR group. This study revealed a rapid, transient, but significant blood pressure increase after LV microinjection of 2.8 nmol CCh, action blocked by previous administration of 4 nmol atropine. Similarly, norepinephrine LV injection also enhanced, transiently, arterial blood pressure, an effect which, in turn, was attenuated by $\alpha 1$ -adrenoceptor antagonists (4 nmol prazosin) (Fig. 1b) and was unchanged by $\alpha 2$ -receptor antagonist (4 nmol yohimbine) (Fig. 1c).

Renal function data—dose-response curve for CCh-induced sodium excretion response

The data for renal function among 12-week-old normotensive and hypertensive (WR and SHR) groups are summarized in Figs. 1, 2, 3, 4, and 5. Urinary flow (data not included) and glomerular filtration rates, estimated by

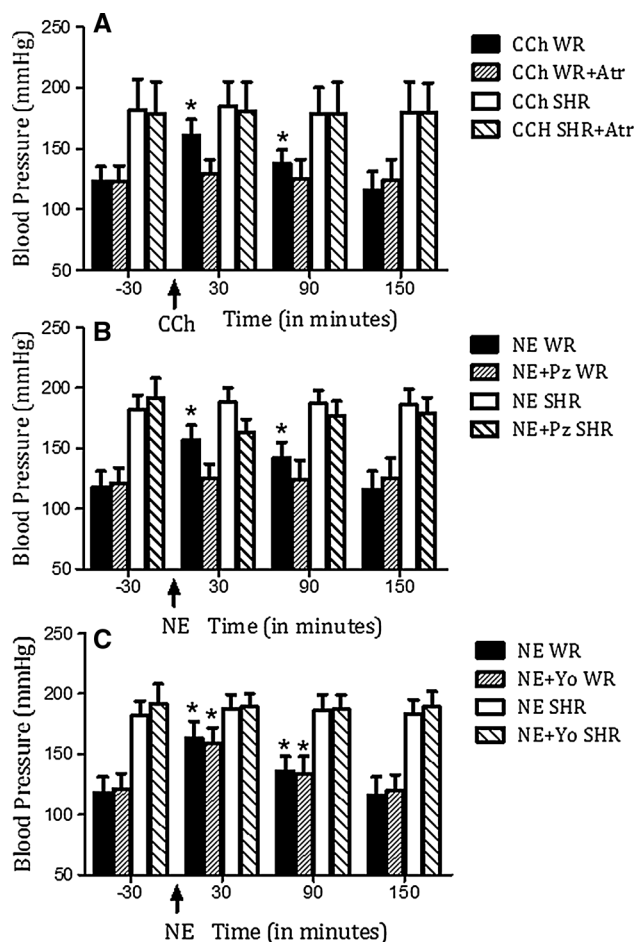


Fig. 1 Effect of lateral ventricle (LV) microinjection of agonists 2.8 nmol carbachol, 30 nmol norepinephrine, and their antagonists, 4 nmol atropine (a), 4 nmol prazosin (b) and 4 nmol yohimbine (c), on blood pressure among Wistar Kyoto rats (WR) compared with spontaneously hypertensive rats (SHR). Results are reported as mean \pm SD. * $P \leq 0.05$ vs. -30 min (ANOVA two-way and Bonferroni's contrast test)

measurement of creatinine clearance, did not differ significantly among the groups during the renal tubule sodium handling studies. Intracerebroventricular microinjections of 0.7, 1.4, 2.8, and 5.6 nmol of carbachol (CCh) in 3 μ l induced a dose-dependent increase in urinary sodium, lithium, and potassium excretion by 12-week-old WR and SHR rats (Fig. 2) compared with controls (0.15 M NaCl). This increased urinary ions excretion was accompanied by predicted antidiuresis similar to that observed after CCh LV administration at the 40th minute for both groups, WR and SHR. After 60 min, i.e., 30 min after CCh microinjection, natriuresis and kaliuresis increased, reaching maximum values after 40 and 60 min. After dose–response experiments (Fig. 2), a dose of 2.8 nmol CCh was selected as optimum for the rest of the study. The effect of 2.8 nmol CCh on increasing renal fractional sodium excretion was significantly higher for WR than for SHR ($P \leq 0.001$)

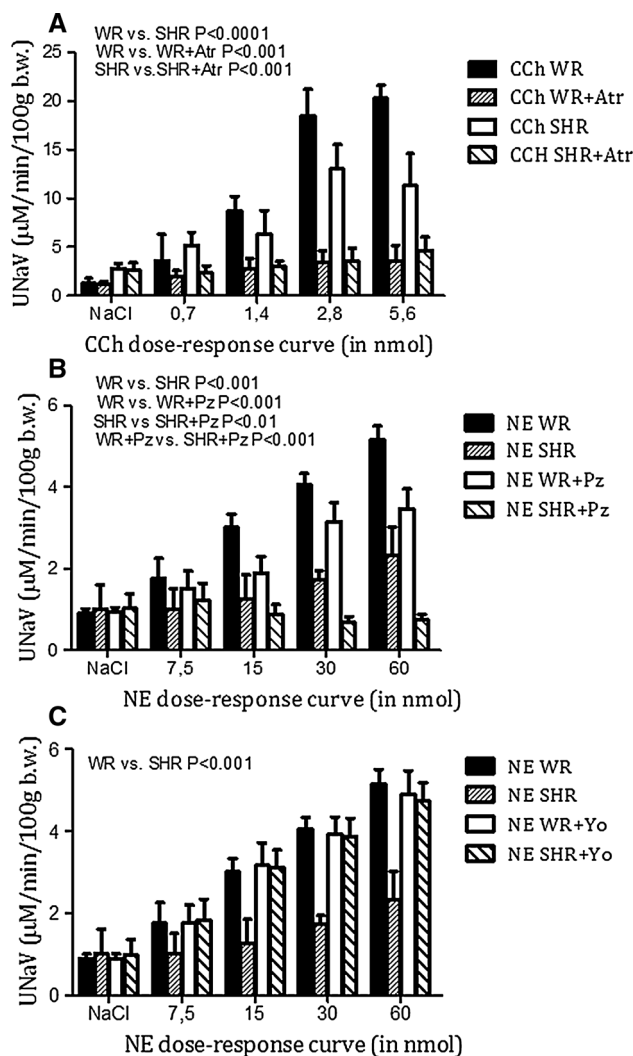
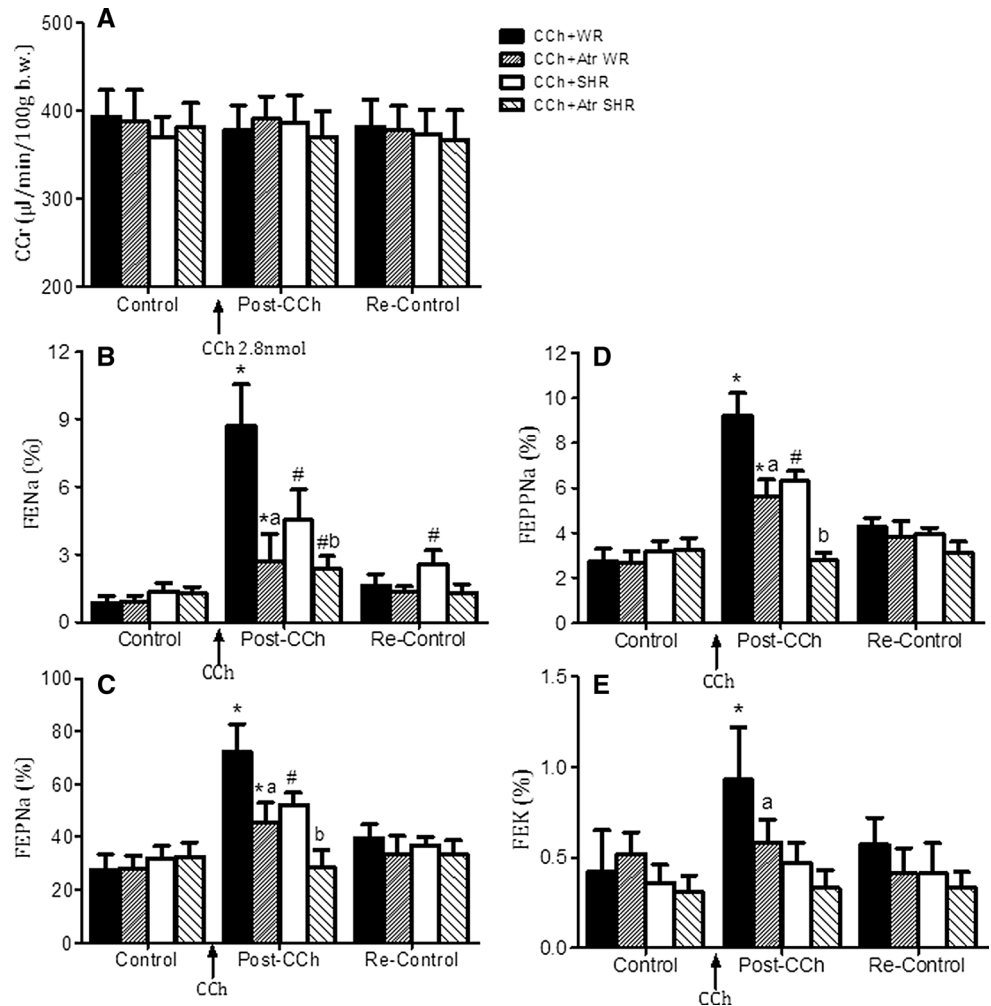


Fig. 2 Dose–response curve for LV microinjection of agonists 2.8 nmol carbachol, 30 nmol norepinephrine, and their antagonists, 4 nmol atropine (a), 4 nmol prazosin (b), and 4 nmol yohimbine (c), on urinary sodium excretion ($UNaV$) by Wistar Kyoto rats (WR) compared with the response of spontaneously hypertensive rats (SHR). Results are reported as mean \pm SD. *vs. WR Control; ^avs. CCh WR; [#]vs. SHR Control; ^bvs. CCh SHR; $P \leq 0.05$ (ANOVA two-way and Bonferroni's contrast test)

(Fig. 3b). This consistently increased FE_{Na} for WR was accompanied by significant enhancement of proximal (from 26.7 ± 8.6 to 77.5 ± 12.8 %, $P = 0.001$) and post-proximal (from 2.86 ± 0.49 to 9.36 ± 2.96 %, $P = 0.001$) sodium excretion and, for SHR, by a smaller but significant increase in proximal (from 29.6 ± 6.8 to 52.6 ± 8.1 %) and post-proximal (from 3.09 ± 0.37 to 6.14 ± 0.62 %) sodium excretion ($P = 0.002$ and $P = 0.001$, respectively) (Fig. 3c, d). The increase occurred in association with unchanged C_{Cr} (Fig. 3a). Likewise, the C_{Cr} and higher natriuresis and kaliuresis responses observed for WR and SHR were significant and similarly attenuated by previous LV treatment of animals with 4 nmol atropine (Figs. 2, 3).

Fig. 3 Effect of previous lateral ventricle (LV) microinjection of 0.15 M NaCl (*Control*), 2.8 nmol carbachol, or 2.8 nmol carbachol plus 4 nmol atropine on creatinine clearance (*CCr*, **a**), fractional sodium excretion (*FENa*, **b**), proximal (*FEPNa*, **c**) and post-proximal (*FEPPNa*, **d**) fractional sodium excretion, and fractional potassium excretion (*FEK*, **e**), 60 min (*Post-CCh*, $n = 12$) and 120 min (*Re-Control*) after LV drug administration to Wistar Kyoto rats (*WR*) compared with the response of spontaneously hypertensive rats (*SHR*). Results are reported as mean \pm SD. *vs. WR Control; ^avs. CCh WR; #vs. SHR Control; ^bvs. CCh SHR; $P \leq 0.05$ (ANOVA two-way and Bonferroni's contrast test)

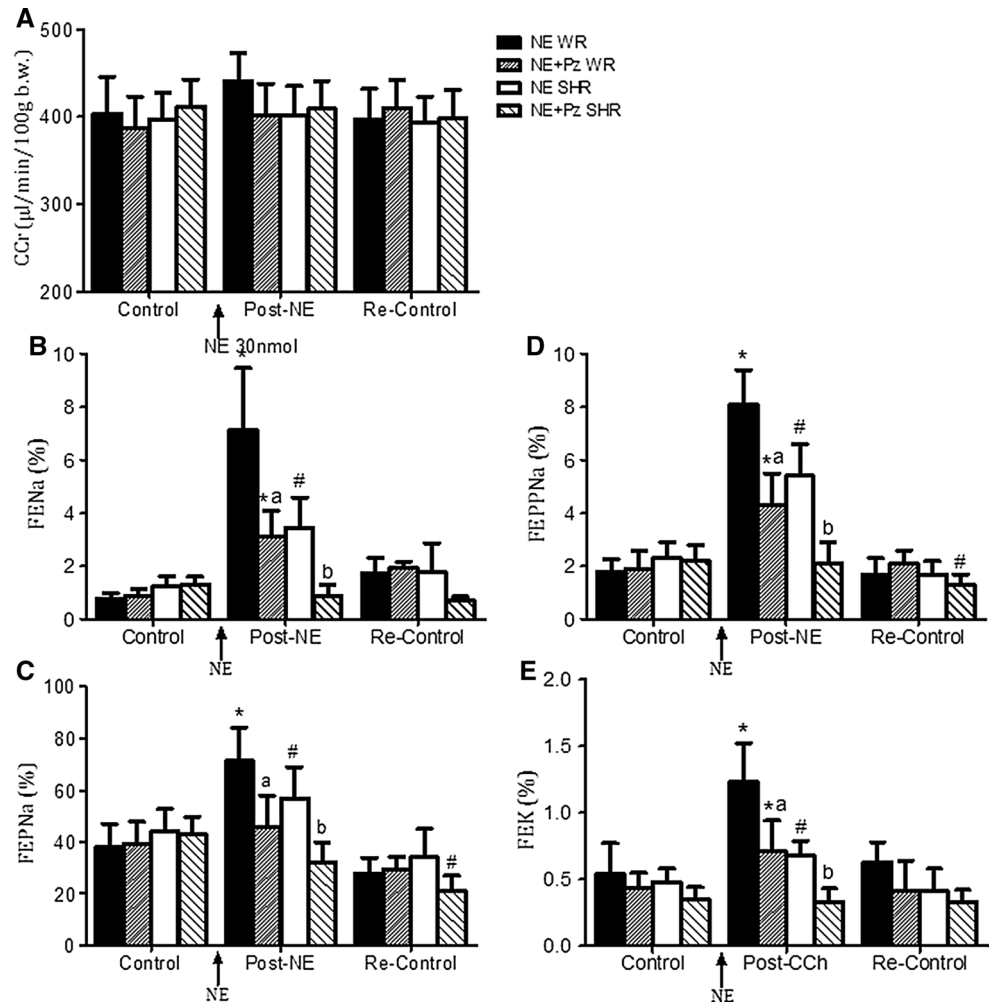


Dose-response curve for NE-induced sodium excretion response

Intracerebroventricular microinjections of 7.5, 15.0, 30, and 60 nmol NE in 3 μ l volume promoted dose-dependent increases in urinary sodium and potassium excretion over 120 min among 12-week-old WR; this effect was significantly attenuated in WR and SHR (Fig. 2). No changes in plasma sodium, lithium, or potassium levels were also observed in any of the experimental groups. After dose-response experiments (Fig. 2), a dose of 30 nmol NE was selected as optimum for the rest of the study. The effect of 30 nmol NE on increasing renal fractional sodium excretion was significantly higher for WR than for SHR ($P \leq 0.001$) (Figs. 4b, 5b). This consistent increase of FE_{Na} among WR was accompanied by significant enhancement of proximal (from basal 38.3 ± 9.5 to 73.3 ± 21.2 %, $P = 0.001$) and post-proximal (from basal 1.86 ± 0.37 to 7.13 ± 2.74 %, $P = 0.001$) sodium excretion; for SHR a smaller but significant increase in proximal (from 42.9 ± 8.3 to 54.7 ± 7.6 %) and post-proximal

(from 2.36 ± 0.38 to 5.12 ± 0.62 %) sodium excretion was observed ($P = 0.002$ and $P = 0.001$, respectively) (Figs. 4c, d, 5c, d). The increase occurred in association with unchanged C_{Cr} (Figs. 4, 5a). Likewise, the C_{Cr} , higher natriuresis, and kaliuresis responses observed for WR and SHR were significantly and similarly attenuated by previous LV treatment of the animals with 4 nmol prazosin (Figs. 2, 3, 4, and 5). The effects of blockage of LV adrenoceptors on urinary sodium excretion was also studied for both types of rat bearing implanted cannulas. This study revealed the participation of LV $\alpha 1$ and $\alpha 2$ -adrenergic receptors in regulation of renal sodium and potassium excretion. The natriuretic and kaliuretic effects of 30 nmol noradrenaline injection on LV were abolished by previous local injection of an $\alpha 1$ -adrenergic antagonist (4 nmol prazosin) ($P = 0.001$) for both types of rat (Figs. 2, 4, and 5). These findings also support the observation that LV pre-injection of 4 nmol yohimbine, an $\alpha 2$ -adrenergic antagonist, synergically potentiated the action of noradrenaline in SHR (Fig. 5). Note that prazosin (Fig. 4b) significantly inhibited fractional sodium excretion

Fig. 4 Effect of lateral ventricle (LV) microinjection of 0.15 M NaCl (*Control*), and 30 nmol norepineprine (*NE*) or 30 nmol norepineprine + 4 nmol prazosin on creatinine clearance (*CCr*, **a**), fractional sodium excretion (*FENa*, **b**), proximal (*FEPNa*, **c**) and post-proximal (*FEPPNa*, **d**) fractional sodium excretion and fractional potassium excretion (*FEK*, **e**) 60-min (*Post-CCh*, $n = 12$) and 120-min (*Re-Control*) after LV drug administration to Wistar Kyoto rats (*WR*) compared with the response of spontaneously hypertensive rats (*SHR*). Results are reported as mean \pm SD. *vs. WR Control; ^avs. NE WR; [#]vs. SHR Control; ^bvs. NE SHR; $P \leq 0.05$ (ANOVA two-way and Bonferroni's contrast test)



whereas yohimbine (Fig. 5b) enhanced fractional sodium excretion. Surprisingly, the LV yohimbine pretreatment normalized urinary sodium excretion by SHR compared with age-matched WR. ($P \leq 0.001$).

Discussion

Studies have shown that the CNS regulatory mechanisms affect the kidney, maintaining sodium homeostasis under conditions in which arterial blood pressure remains stable [1, 3, 13–19]. The importance of the renal sympathetic nerves to natriuretic control in SHR is indicated by studies showing that chronic renal denervation abolishes the reduced sodium excretion responses among these rats [19, 20, 27, 28]. Many investigators, studying mammalian species, have demonstrated that administration of cholinergic and noradrenergic agonists into different cerebral areas elicits a large increase in renal sodium excretion accompanied by changes in arterial pressure [5, 6, 17, 18, 32–34]. In this study we evaluated the concentration-

dependent effect of intracerebroventricular cholinergic and noradrenergic agonists, and administration of antagonists, on blood pressure and urinary sodium handling, estimated on the basis of lithium clearance by adult SHR compared with appropriate age-matched WR controls. Of particular interest, confirming results from different stimulation techniques [5, 6, 14, 16], this study revealed a rapid, transient, but significant blood pressure increase after LV microinjection of CCh. The study also confirmed that i.c.v. cholinergic-induced natriuresis among WR and SHR was significantly abolished by previous LV administration of atropine. However, the natriuretic effect of i.c.v. CCh administration was significantly lower among hypertensive rats than among the normotensive strain and was associated with transient and similar antidiuresis for both WR and age-matched SHR.

Similarly, noradrenergic stimulation of LV also transiently enhances blood pressure; this effect was, in turn, attenuated by $\alpha 1$ -adrenoceptor antagonist and unchanged by $\alpha 2$ -receptor antagonist i.c.v. microinjections. This study confirms the participation of LV $\alpha 1$ and $\alpha 2$ -adrenoceptors

Fig. 5 Effect of lateral ventricle (LV) microinjection of 0.15 M NaCl (*Control*), and 30 nmol norepinephrine (*NE*) or 30 nmol norepinephrine + 4 nmol yohimbine on creatinine clearance (*CCr*, **a**), fractional sodium excretion (*FENa*, **b**), proximal (*FEPNa*, **c**) and post-proximal (*FEPPNa*, **d**) fractional sodium excretion and fractional potassium excretion (*FEK*, **e**) 60-min (*Post-CCh*, $n = 12$) and 120-min (*Re-Control*) after LV drug administration to Wistar Kyoto rats (*WR*) compared with the response of spontaneously hypertensive rats (*SHR*). Results are reported as mean \pm SD. *vs. WR Control; ^avs. NE WR; [#]vs. SHR Control; ^bvs. NE SHR; $P \leq 0.05$ (ANOVA two-way and Bonferroni's contrast test)

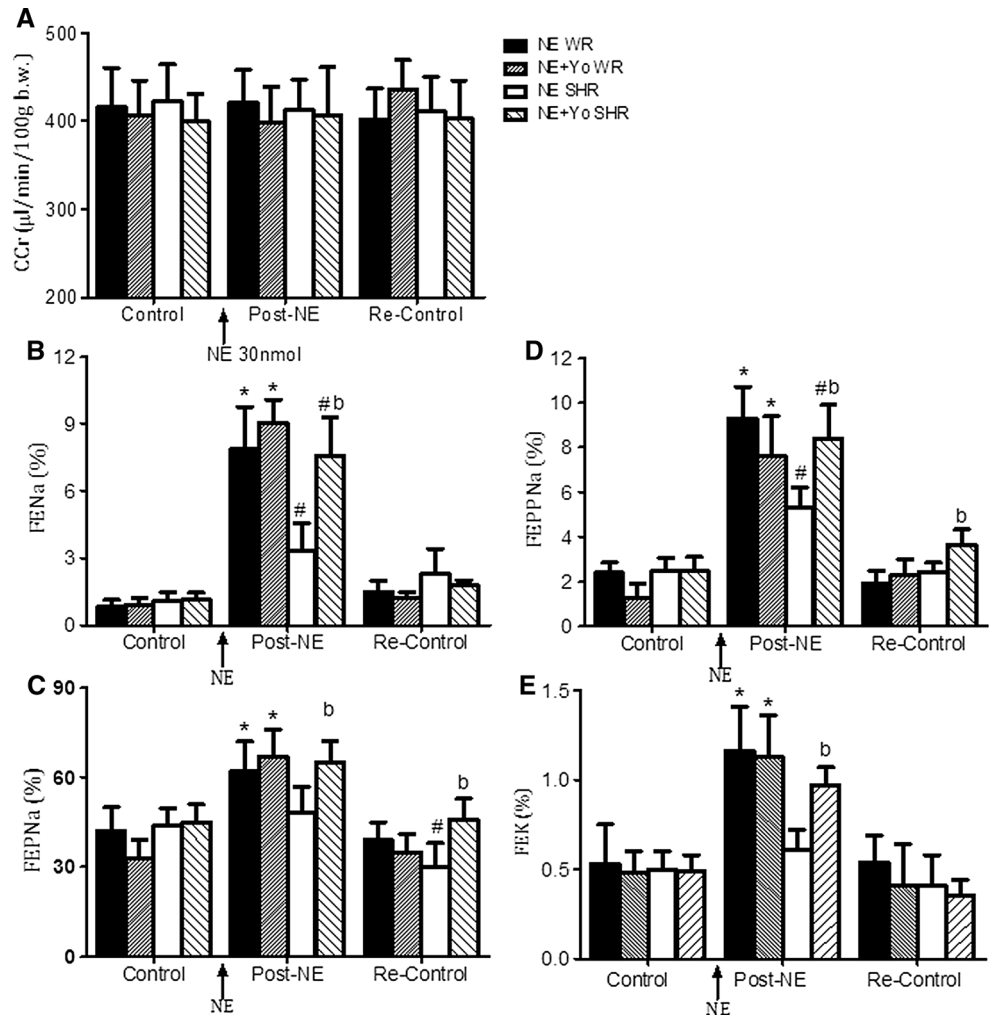


Table 1 Body weight, sodium intake, serum sodium, potassium and lithium levels for spontaneously hypertensive (SHR) compared with normotensive (WR) rats fed a standard diet

Group	Na ⁺ (mM)	K ⁺ (mM)	Li ⁺ (µM)	Body weight (g) 12-week-old	Sodium intake (mmol/week/100 g b.w.)
WKy ($n = 12$)	138 \pm 2.6	4.3 \pm 0.6	85 \pm 19	232 \pm 15	12.7 \pm 2.3
SHR ($n = 12$)	142 \pm 3.1	4.1 \pm 0.4	79 \pm 15	180 \pm 13*	11.8 \pm 1.9

Results are reported as mean \pm SD. * $P \leq 0.05$ vs. WR (Student's *t* test)

in regulation of renal sodium and potassium excretion. This conclusion is based on results showing that the natriuretic and kaliuretic effects of injection of noradrenaline into the LV are abolished by previous local injection of an α 1-adrenergic antagonist (prazosin), in both groups. These findings also support the observation that LV pre-injection of yohimbine, an α 2-adrenoceptor antagonist, synergistically potentiated the action of noradrenaline. Note that prazosin significantly inhibited fractional sodium excretion whereas yohimbine enhanced fractional sodium excretion. Surprisingly, LV yohimbine pretreatment normalized urinary

sodium excretion in SHR compared with age-matched WR. This study confirmed that CCh and NE, when centrally microinjected into conscious rats, leads to a very predictable and reproducible absolute and fractional natriuretic response accompanied by a smaller amount of kaliuresis. This response was associated with an increase in proximal and post-proximal sodium excretion without changes in glomerular filtration rate. The similar plasma lithium, sodium, and potassium levels of the experimental groups, with the absence of variation in glomerular filtration rate, indicate that the values calculated for filtered

amounts of those ions were the same after all treatments. Thus, the observed increase in renal sodium and potassium excretion is probably because of the inability of renal tubules to handle these electrolytes, with disruption of glomerulotubular balance.

Despite repeated demonstration of the natriuretic effect of central CCh and NE administration to a variety of species, to the best of our knowledge there has been no previous description of these effects among SHR and the precise mechanism of this phenomenon remains unknown. Our results suggest that the effect of LV cholinergic and noradrenergic homeostatic on hydroelectrolytic balance and blood pressure control is impaired in SHR. This mechanism be important in the development and maintenance of hypertension in SHR. Several possibilities should be considered. First, the central nervous system may directly affect renal sodium excretion via neural routes. Second, hemodynamic factors may be responsible for alteration of electrolyte excretion. Third, the natriuresis may result from fluctuations in the level of neural-borne factors which may affect sodium and/or water transporters in renal tubules and, consequently, sodium handling. Fourth, the SHR findings suggest the definite lack of a relationship between the activity of LV neurotransmitters and/or expression of receptors and peripheral renal response in that strain.

The time course, magnitude, and specificity of the natriuretic response to central microinjection of carbachol into rats have been well characterized [5, 6, 10, 11, 17, 18]. Mechanisms usually proposed to explain the cause or mediation of natriuresis include inhibition of renal nerve activity [14–16]; increases in renal perfusion pressure, glomerular filtration rate, or renal plasma flow [33, 35]; and release of vasopressin or other hormones that inhibit tubule absorption of sodium [5, 36]. However, previous studies suggest that the natriuresis seen after central cholinergic stimulation in conscious rats is not mediated by inhibition of renal nerve activity or by changes in plasma concentrations of renin and aldosterone [7–14, 33], and is not associated with significant alterations in glomerular filtration rate [16–18].

It is, however, known that central administration of vasopressin (AVP) causes significant natriuresis [37–39]. In addition, acetylcholine stimulates concentration-dependent release of AVP from the hypothalamic nucleus. It also increases AVP release *in vivo* when administered by way of the carotid artery or into the cerebral ventricles [40–42] and it increases the electrical activity of supraoptic neurons when iontophoresed on to them *in vivo* [40, 43] or when added to the superfusion medium *in vitro* [44]. Thus, in this study, the natriuretic effect induced by CCh after LV administration may be caused, at least in part, by enhanced secretion of AVP from the posterior pituitary. Studies

showing elevation of the AVP content of the posterior pituitary and reduced hypothalamic AVP content [45, 46] in adult SHR compared with WR suggest abnormalities in the synthesis and/or release of AVP by SHR. In our study, the attenuation of cholinergic responsiveness among 16-week-old SHR compared with age-matched WR suggests that either that the system is inhibited by the higher blood pressure present at this age or the chronic exposure to this high blood pressure has permanently altered the responsiveness of the hypothalamus–pituitary system to signals controlling AVP release. Thus, over responsiveness might result in significantly greater circulating AVP concentrations, which could potentially contribute to the development of hypertension. Conversely, for adult SHR reduced hypothalamic contents of AVP may explain the attenuated response to LV administration of CCh to these rats.

When central noradrenergic neurons in both SHR and WR were destroyed by intracerebroventricular injection of 6-hydroxydopamine, carbachol-induced vasopressor responses were markedly augmented, and resulted in responses similar to those of SHR. These findings indicate that central noradrenergic vasodepressive neurons are deficient and that the augmented vasopressor responses to carbachol resulted from deranged central noradrenergic mechanisms in SHR. A study by Yamada et al. [47] also revealed a specific increase in muscarinic receptors and a decrease in cholinergic activity in the hypothalamus of SHR [47]. Oparil et al. [48] have demonstrated that increased blood pressure in SHR occurs, at least in part, as a result of reduced norepinephrine release or receptors in nerve ends in the anterior hypothalamic area (AHA), thus reducing activation of sympathoinhibitory neurons. Two mechanisms have been shown to contribute to this effect:

- 1 Reduced noradrenergic input into the AHA via baroreflex pathways; and
- 2 Local inhibition of NE release in the AHA by the inhibitory neuromodulator atrial natriuretic peptide (ANP) [48, 49].

A previous study has revealed that plasma irANP and irBNP were higher in adult SHR than in control WR, whereas ANP and BNP values of young SHR did not differ from those of control WR [49]. In the WR, excitation of NTS neurons by baroreflex afferents leads to activation of sympathoinhibitory neurons in the NTS and AHA, strong inhibition of sympathetic nervous system outflow, and a decrease in arterial pressure. However, in the SHR brain ANP acts at the levels of the NTS and AHA to perturb this baroreflex regulatory pathway. Thus, we may suppose that ANP acts at several sites in brain to facilitate the development and maintenance of sympathetically mediated hypertension in the SHR model by reducing the number or activation of central sympathoinhibitory neurons.

The novel findings of this work show that the reduced natriuretic effect of central NE stimuli in SHR was associated with unchanged creatinine clearance and reduced ion delivery from the proximal tubule incompletely compensated by more distal nephron segments. This effect demonstrates diminished NE graded-fashion responses with a rightward shift of the dose–response curve, providing evidence of down-regulation of target organ responsiveness to SHR LV stimuli. The mechanisms underlying this phenomenon are still not well understood but may be related to renal sodium handling. We have previously demonstrated that the urinary sodium excretion response to central insulin, angiotensin II, and hypertonic saline was strikingly and similarly attenuated in adult SHR compared with age-matched WR controls [30, 31, 50]. Because hemodynamic and natriuretic responses seem to depend on the magnitude of increased blood pressure, the inability of hypertensive kidneys to achieve effective natriuresis after centrally administered NE may reflect the ineffectiveness of inhibiting sympathetic activity responses and/or modifying the higher basal arterial pressor levels in SHR. There is evidence of the importance of renal sympathetic nerve activity in the pathogenesis of experimental models of hypertension [3, 19, 20]. In this case, the significant reduced natriuretic response among young SHR compared with WR may reflect differences in efferent renal nerve activity, which is reported to be higher for SHR as young as 5 weeks [20, 27, 28].

It is well known that stimulation of α_2 -adrenoceptors in the brainstem, a cardiovascular region, induces a reduction in blood pressure. Carrettiero et al. [49] reported fewer α_2 -adrenoceptors in SHR than in WR in all NTS subnuclei and for different ages. This reveals the importance of α_2 -receptor distribution within the NTS with regard to neural control of blood pressure and the development of hypertension. Other studies have also revealed that after central α_2 -adrenoreceptor stimulation in conscious rats, urinary sodium excretion is selectively mediated by downstream $G\alpha_2$, but not $G\alpha_1$, $G\alpha_3$, $G\alpha_o$, or $G\alpha_s$, subunit GTP-binding regulatory protein signal transduction pathways [51–53]. These authors revealed, in particular, that the brain $G\alpha_2$ -protein-mediated sympathoinhibitory renal nerve-dependent pathway and is of critical importance in the central neural mechanisms activated to maintain fluid and electrolyte homeostasis. The underlying mechanisms by which brain $G\alpha_2$ -subunit protein-gated pathways induce α_2 -adrenoreceptor-evoked sodium control in vivo are unknown. In this study, given the intimate association between fluid and electrolyte homeostasis and the long-term control of arterial pressure, we may speculate that downregulation of brain $G\alpha_2$ protein expression in SHR may lead to elevated sympathetic drive, renal sodium retention, and the development of renal nerve-dependent hypertension.

Our experiments furnished good evidence of the existence of a central adrenergic mechanism consisting of α_1 and α_2 receptors which work antagonistically on regulation of renal sodium excretion. We may suppose that stimulation of central nervous system α_2 -adrenergic receptors prevents basal increased renal sympathetic overexcitability in conscious SHR. This conclusion is based on two main findings. First, the effect of LV administration of norepinephrine on natriuresis is significantly attenuated in SHR. Second, pretreatment with α_2 -adrenergic receptor antagonists reversed the effects of the LV NE injection, which demonstrates that central α_2 -adrenergic receptors are involved in the reduced natriuresis observed for the hypertensive strain [54–56]. Because LV administration of yohimbine alone increases the natriuretic response in SHR but not in WR, we may hypothesize that the basal activity of these central α_2 -adrenergic receptors may be very high. However, we may not discount the possibility that SHR neural synapses have more α_2 -adrenergic receptors than those of WR. In fact, the natriuresis resulting from injection of norepinephrine into the LV of SHR was potentiated by yohimbine and blocked by prazosin. Because adrenergic agonist or antagonists did not alter glomerular filtration rate, changes in renal hemodynamics do not explain the natriuresis. The natriuresis resulting from yohimbine could also be because of:

- 1 inhibition of renal sympathetic nerve activity by a central α_2 -adrenoceptor mechanism; and/or
- 2 α_2 -adrenergic receptor-induced inhibition of vasopressin release from the central nervous system [17, 18, 28, 49].

It is more likely that natriuresis is a result of reduced renal sympathetic nerve activity and a consequent decrease in renal tubular reabsorption of sodium. Norepinephrine administration into the ventromedial hypothalamus, lateral hypothalamus, septal area, and third ventricle of conscious rats increases urinary sodium excretion; the natriuresis is prevented by central α -adrenergic receptor blockade and potentiated by α_2 -adrenergic receptor blockade [17, 18, 54–56]. These studies, with our results, suggest an inhibitory effect of central α_2 -adrenergic receptors on urinary sodium excretion and an excitatory effect of central α_1 -adrenoceptors. In conclusion, our results suggest striking participation of central cholinergic and/or adrenergic receptors in the renal pathogenesis of genetic hypertension in SHR. Although the precise mechanism of the different natriuretic response of WR and SHR is still uncertain, these results led us to speculate that inappropriate neural cholinergic and noradrenergic pathways may have crucial effects on renal tubule sodium and water transport, resulting in the inability of kidneys to control hydroelectrolytic balance, and, consequently, an increase in blood pressure.

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Conflict of interest We affirm there is no conflict of interest.

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