

Full Paper

Effect of Clonidine on Renal Sodium Handling in Spontaneously Hypertensive Rats

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Abstract. Up-regulation of kidney α_2 -adrenoceptor expression has been implicated in the development of hypertension in spontaneously hypertensive rats (SHR). This study was carried out to evaluate renal sodium excretion in response to clonidine administration in SHR and control normotensive Wistar-Kyoto (WKY) rats. SHR and WKY rats (12-week-old) were placed in metabolic cages for 4 days: the first 2 days in control conditions and the following 2 days under oral clonidine treatment (100 μ g/kg body weight). Clonidine produced a similar reduction in systolic blood pressure values in SHR and WKY rats, although SHR remained hypertensive. At the end of the study SHR and WKY rats presented similar noradrenaline plasma levels. However, noradrenaline kidney tissue levels were significantly higher in SHR compared to WKY rats. Under control conditions, SHR presented lower urine flow compared to WKY rats. Clonidine produced a significant decrease in urine flow in WKY rats but not in SHR. Furthermore, clonidine also produced a significant reduction in urinary sodium, potassium, and creatinine excretion in WKY rats, but had no effect in SHR. In conclusion, in SHR the reduction in systolic blood pressure and sympathetic activity produced by clonidine was not accompanied by a decrease in urine volume and sodium excretion.

Keywords: clonidine, spontaneously hypertensive rats (SHR), kidney, α_2 -adrenoceptor, sympathetic nervous system (SNS)

Introduction

The kidney plays a major role in the regulation of blood pressure via modulation of sodium and water excretion (1). Evidence from clinical and experimental studies supports the idea that altered renal sodium handling plays an important role in the development and maintenance of hypertension (2) both in humans and in an animal model of this disorder, the spontaneously hypertensive rat (SHR) (3). One proposal for the initiation and maintenance of hypertension centers on a reduced capacity of the kidney to excrete salt and water in proper relation to intake (4). Renal sympathetic nerves and circulating catecholamines are involved in the regulation of sodium and water excretion in the kidney (3, 5). The catecholamine noradrenaline is the major endogenous

neurotransmitter in renal sympathetic nerves and mediates sympathetic regulation of blood pressure. Noradrenaline interacts with both the α - and β -adrenoceptors in the renal proximal tubules (6 – 8). Studies have shown that renal nerves, acting through α -adrenoceptors, enhance proximal tubular sodium reabsorption in the kidney. These studies suggest that noradrenaline acting via α -adrenoceptors may contribute to the development of hypertension (5, 9).

Considerable evidence indicates that the antihypertensive effect of clonidine is exerted in central α_2 -adrenoceptors (10), thereby inhibiting peripheral sympathetic tone and also markedly affecting renal function (11). The effects on urinary output have been ascribed to activation of α_2 -adrenoceptors (12) both on the renal vasculature and along the nephron (13). Studies using radioligand binding and autoradiographic techniques have shown that renal α_2 -adrenoceptors seem to be located postsynaptically (14). Activation of renal α_2 -adrenoceptors mediates an inhibition of renin release

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(15) and a facilitation (16) or inhibition (17) of water and sodium excretion suggesting that renal tubular α_2 -adrenoceptors may be involved in modulating the overall excretion of water and sodium. Autoradiographic findings have also demonstrated that the distribution of α_2 -adrenoceptors is increased in SHR at pre-hypertensive (3-week-old), early development (7-week-old), and established stages (22-week-old) compared with age matched normotensive control Wistar Kyoto (WKY) rats (18). Although renal α_1 -adrenoceptors are important in the initiation and development of SHR hypertension by increasing sodium absorption in the distal tubules, as hypertension progresses and sodium returns to levels similar to normotensive animals, a molecular switch between α_1 -adrenoceptor and α_2 -adrenoceptor signaling occurs in the distal tubules of SHR (19). Hence, it has been suggested that in SHR with established hypertension and age-matched WKY rats, renal α_2 -adrenoceptors play a more prominent role in the balance of sodium transport.

In view of the above, the general aim of this study was to evaluate the potential role of the adrenergic system in the regulation of solute and fluid secretion, since abnormalities in noradrenergic activity and in sodium handling have been implicated in the pathogenesis of hypertension. Therefore, the study was designed to determine whether any differences exist between WKY rats and SHR in the natriuretic and diuretic effect of the α_2 -adrenoceptor agonist clonidine. The clonidine dose selected for this study [(100 $\mu\text{g/kg}$ body weight (b.w.)) is at the lower end of the spectrum of doses (30 $\mu\text{g/kg}$ to 50 mg/kg b.w.) that have previously been used to investigate α_2 -adrenoceptor-mediated functions in rodents (20–22).

Materials and Methods

Animals

Eight-week-old male SHR ($n = 8$ animals) and WKY rats ($n = 8$ animals) were obtained from Charles River (Barcelona, Spain). The health reports of the animals were in accordance with the recommendations by Federation of European Laboratory Animal Science Associations. The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996) and the experiments were performed according to the Portuguese law on animal welfare. The animals were kept under controlled environmental conditions (12:12-h light/dark cycle and room temperature $22^\circ\text{C} \pm 2^\circ\text{C}$). All animals were fed ad libitum throughout the study with standard rat chow (PANLAB, Barcelona, Spain), containing $1.9 \text{ g}\cdot\text{kg}^{-1}$ of sodium. Animals were

selected after a 5-day period of stabilization and adaptation to blood pressure measurements. Systolic blood pressure and heart rate were measured in conscious restrained animals between 7:00 and 10:00 a.m., using a photoelectric tail-cuff pulse detector (LE 5000; Letica, Barcelona, Spain). Five determinations were made each time and the mean was used for further calculation.

Metabolic study

Having reached the age of 12 weeks, rats were placed in metabolic cages (TECNIPLAST, Buguggiate-VA, Italy) for 4 days. The 1st day (day 1) was to allow adaptation; the 2nd day (day 2) to establish control conditions, and the following 2 days (day 3 and day 4) under oral clonidine treatment (100 $\mu\text{g/kg}$ b.w.). Urine volume, faecal weight, solid intake, and liquid intake were measured after every 24 h. Urine samples were collected to measure the ionogram in the 2nd day of control (day 2) and the 2nd day of treatment (day 4). At the end of the study, rats were anesthetized with sodium pentobarbital (60 mg/kg b.w., i.p.) and blood was collected from the vena cava and placed in tubes containing heparin for plasma catecholamine and ionogram determination. The right kidney was placed in a vial containing 1 mL of 0.2 mol/L perchloric acid (PCA) for the catecholamine assay.

Assay of catecholamines

The assay of catecholamines dopamine and noradrenaline in plasma and kidney was performed by high performance liquid chromatography with electrochemical detection (HPLC-ED) as previously described (23). In brief, 500- μL aliquots of the PCA in which the tissues had been kept or 1000- μL aliquots of the plasma supernatant were placed in 5-mL conical base glass vials containing 50 mg of alumina, and the pH of the samples was adjusted to 8.6 by addition of Tris buffer. 3,4-Dihydroxybenzylamine hydrobromide was used as the internal standard. The adsorbed catecholamines were then eluted from the alumina with 200 μL of 0.2 mol/L PCA in Costar Spin-X microfilter tubes; 50 μL of the eluate was injected into an HPLC-ED system (Gilson Model 141; Gilson Medical Electronics, Villiers Le Bel, France). The lower limit of detection of catecholamines and L-DOPA ranged from 350 to 1000 fmol.

Urine sodium, potassium, and creatinine

Urinary sodium and potassium were measured by flame photometry and urine and plasma osmolality, by means of an osmometer (24). Urinary and plasma creatinine and plasma urea were measured by a wavelength photometer (24).

Statistical analyses

Results are expressed as the mean \pm standard error of the mean (S.E.M.). The significance of differences between means was evaluated using Student's *t*-test or one-way analysis of variance (ANOVA). Values were considered statistically different when $P < 0.05$ by using Newman-Keuls multiple comparisons test to compare values. A *P*-value less than 0.05 was assumed to denote a significant difference.

Results

Cardiovascular parameters

To evaluate changes in cardiovascular function, systolic blood pressure and heart rate were measured in WKY rats and SHR, 4 weeks before treatment with clonidine. At 8 weeks, systolic blood pressure values were already significantly higher in SHR compared to age-matched WKY rats and steadily increased until the animals reached 12 weeks of age. Therefore, in the control stage of the study, SHR had significantly higher systolic blood pressure values compared to WKY rats (Fig. 1A). Clonidine produced a significant reduction in systolic blood pressure values both in SHR and WKY rats, al-

though values remained significantly elevated in SHR compared to WKY rats (Fig. 1A). Heart rate was higher in SHR in the control phase and after treatment with clonidine, and remained unchanged in both strains after clonidine treatment (Fig. 1B).

Weight, solid intake, and fecal weight

Animal weight was similar between strains and remained unchanged after treatment with clonidine (Table 1). SHR and WKY rats had similar solid intake in the control stage and clonidine produced a significant reduction in solid intake in both strains (Table 1). Control values of fecal weight were similar between SHR and WKY rats (Table 1). After treatment with clonidine, both SHR and WKY rats had a significant reduction in fecal weight (Table 1).

Liquid intake and urine volume

Before treatment with clonidine, SHR and WKY rats had similar liquid intake values (Fig. 2A). Treatment with clonidine progressively and significantly reduced liquid intake in SHR. On the other hand, treatment of WKY rats with clonidine did not change liquid intake values (Fig. 2A). In regard to urine volume, in control

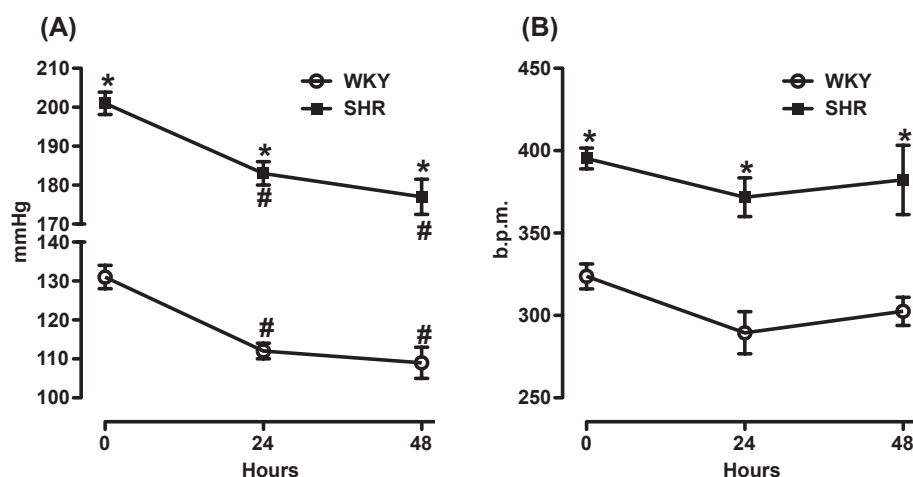


Fig. 1. Systolic blood pressure (A) (mmHg) and heart rate (B) (b.p.m.) in Wistar Kyoto rats (WKY) and spontaneously hypertensive rats (SHR) before (0 h) and after (24 and 48 h) treatment with clonidine. Symbols represent the mean and vertical lines show S.E.M. ($n = 8$). * $P < 0.05$ vs. WKY. # $P < 0.05$ vs. corresponding control values.

Table 1. Body weight (g), solid intake ($\text{g} \cdot \text{kg b.w.}^{-1} \cdot 24 \text{ h}^{-1}$), and faecal weight ($\text{g} \cdot \text{kg b.w.}^{-1} \cdot 24 \text{ h}^{-1}$) in Wistar Kyoto rats (WKY) and spontaneously hypertensive rats (SHR) before (0 h) and after (24 and 48 h) clonidine treatment

	Body weight		Solid intake		Faecal weight	
	WKY	SHR	WKY	SHR	WKY	SHR
0 h (control)	307 \pm 5	286 \pm 10	65 \pm 2	70 \pm 3	23 \pm 2	26 \pm 2
24 h (clonidine)	303 \pm 5	291 \pm 10	51 \pm 2 [#]	57 \pm 3 [#]	14 \pm 2 [#]	16 \pm 1 [#]
48 h (clonidine)	308 \pm 5	288 \pm 10	49 \pm 3 [#]	53 \pm 1 [#]	15 \pm 3 [#]	17 \pm 2 [#]

Values are given as the mean \pm S.E.M. ($n = 8$). # $P < 0.05$ vs. corresponding control values.

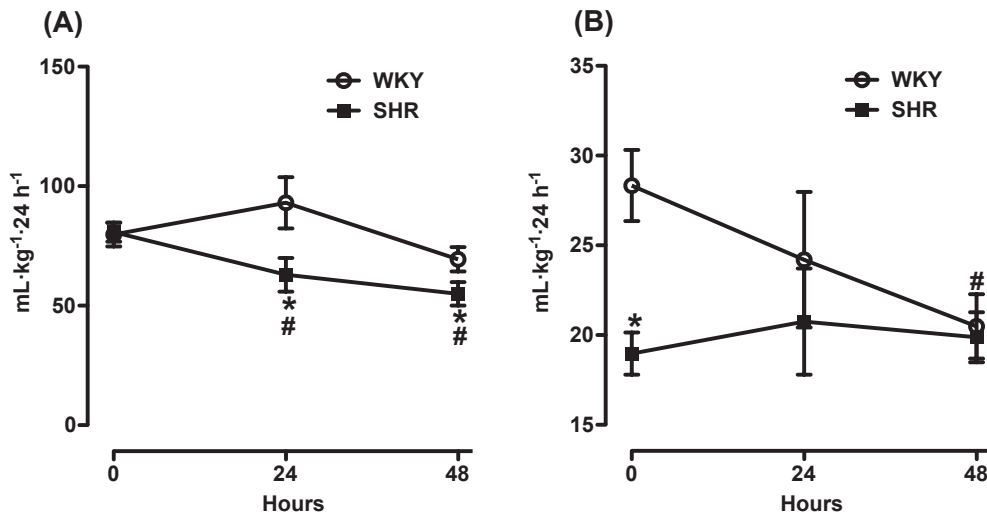


Fig. 2. Liquid intake (A) and urine volume (B) ($\text{mL} \cdot \text{kg} \text{ b.w.}^{-1} \cdot 24 \text{ h}^{-1}$) for Wistar Kyoto rats (WKY) and spontaneously hypertensive rats (SHR) before and after treatment with clonidine. Symbols represent the mean and vertical lines show S.E.M. ($n = 8$). * $P < 0.05$ vs. WKY. # $P < 0.05$ vs. corresponding control values.

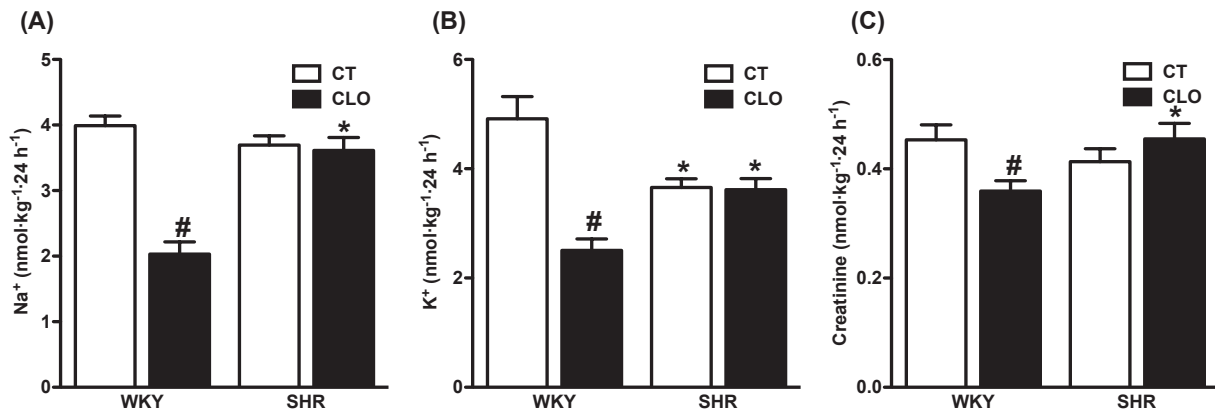


Fig. 3. Urinary sodium (A), potassium (B), and creatinine (C) ($\text{nmol} \cdot \text{kg} \text{ b.w.}^{-1} \cdot 24 \text{ h}^{-1}$) for Wistar Kyoto rats (WKY) and spontaneously hypertensive rats (SHR) before (CT) and after (CLO) 48-h treatment with clonidine. Columns represent means and vertical lines represent S.E.M. ($n = 8$). * $P < 0.05$ vs. WKY. # $P < 0.05$ vs. corresponding control values.

conditions, SHR had a lower urine volume when compared to WKY rats (Fig. 2B). Although treatment with clonidine failed to produce an effect on urine volume values in SHR, treatment with clonidine for 48 h significantly reduced urine volume in WKY rats to values similar to those of SHR (Fig. 2B).

Urinary ionogram

In the control stage, sodium urinary values were similar between WKY rats and SHR (Fig. 3A), but potassium urinary values were significantly reduced in SHR (Fig. 3B). In WKY rats, clonidine produced a significant reduction in sodium and potassium urinary levels (Fig. 3: A and B). In contrast, clonidine did not change urinary sodium or potassium in SHR (Fig. 3: A and B), both being significantly increased when compared to WKY rats treated with clonidine. Treatment with clonidine also

produced a significant reduction in creatinine values in the urine of WKY rats, but did not produce an effect in creatinine values in urine of SHR (Fig. 3C).

Plasma and kidney dopamine and noradrenaline

Data on dopamine and noradrenaline kidney tissue content and plasma levels were obtained at the end of the study after animals had been treated with clonidine. After treatment with clonidine, SHR and WKY rats had similar noradrenaline and dopamine plasma levels (Fig. 4: A and B). However kidney tissue content of noradrenaline and dopamine in SHR were significantly higher compared to WKY rats (Fig. 4: C and D). Nevertheless, the dopamine/noradrenaline ratio in the kidney was significantly higher in SHR compared to WKY rats after clonidine treatment (WKY: 0.033 ± 0.001 ; SHR: $0.050 \pm 0.007^*$, * $P < 0.05$).

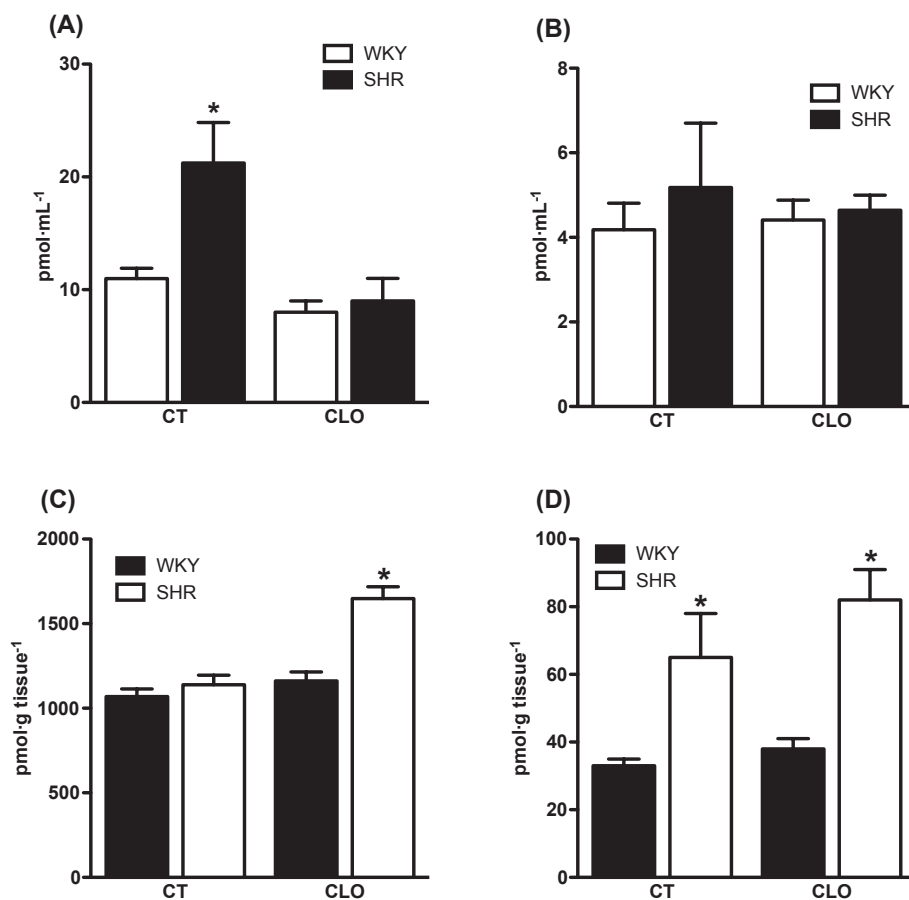


Fig. 4. Effect of clonidine on noradrenaline (A) and dopamine (B) plasma levels (pmol/mL) and noradrenaline (C) and dopamine (D) kidney tissue content (pmol·g tissue⁻¹) in Wistar Kyoto rats (WKY) and spontaneously hypertensive rats (SHR). Columns represent means and vertical lines represent S.E.M. (n = 8). * $P < 0.05$ vs. WKY.

Discussion

In the present study, we have shown that in basal conditions water and potassium but not sodium excretion rates were lower in hypertensive SHR compared to WKY rats. Administration of clonidine for two consecutive days produced a significant decrease in blood pressure values in both strains, but whereas water and sodium excretion decreased in the WKY rats, these parameters remained unchanged in SHR.

The effect of clonidine on the renal system in SHR had been the subject of a previous study (25). However, the influence of hypertension was not investigated, as the study did not include normotensive controls. Furthermore, this and another study from the same group that used Sprague-Dawley rats as the normotensive model (12) only addressed the acute effects of clonidine. Recent studies suggest that different rat strains are associated to differences in renal handling of electrolytes (26, 27). In this view, in the present study we evaluated the sub-acute effect of clonidine and have used the WKY rat as the control strain not only to better understand the effect of clonidine on renal sodium handling in SHR, but also to

provide a background to evaluate the possible influence of hypertension on this mechanism. Given the differences in sympathetic activity and α_2 -adrenoceptor function between WKY rats and SHR, data obtained from this study would allow further insight into the physiological mechanisms of blood pressure regulation through the kidney relating to the sympathetic nervous system.

Clonidine was given orally and this means that both central and peripheral α_2 -adrenoceptors will be targeted (Fig. 5). Thus, the kidney will be subjected to two influences: a) withdrawal of sympathetic tone, causing an increase in renal blood flow and decreased proximal tubule reabsorption due to direct action of the renal sympathetic nerves at the kidney (28); b) the α_2 -adrenoceptors along the distal nephron will be activated. So, how can one account for the decrease in fluid reabsorption in this situation? Large falls in blood pressure which in itself would have had a major impact on the renal fluid reabsorption, that is, a pressure natriuresis effect (29). Thus as pressure decreased in response to clonidine, even without a change in sympathetic tone, fluid excretion should have decreased. It was clear that this happened in WKY rats, but not in the SHR. One possible explanation

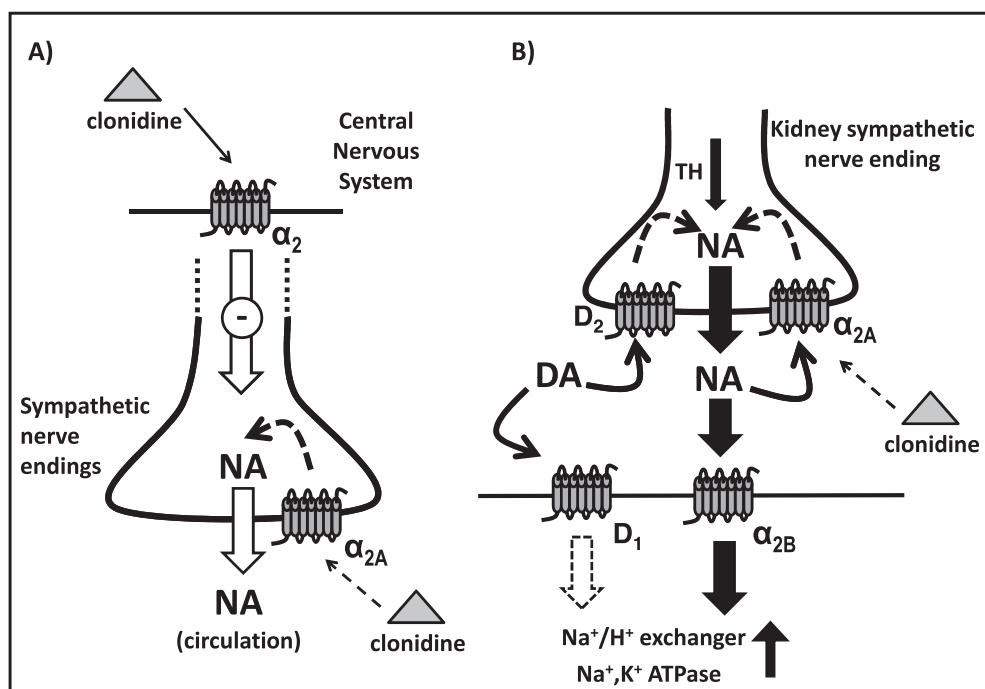


Fig. 5. Effect of clonidine on spontaneously hypertensive rats (SHR). A) Clonidine reduces sympathetic activation by acting on central α_{2A} -adrenoceptors. B) The effect of clonidine on the kidney noradrenergic system is blunted due to a down-regulation of the inhibitory effect of pre-synaptic α_{2A} -adrenoceptors and due to increased noradrenaline synthesis; increased noradrenaline drive activates post-synaptic α_{2B} -adrenoceptors, leading to altered renal sodium handling. Dopamine receptors are also unresponsive to increased dopamine. DA, dopamine; NA, noradrenaline; TH, tyrosine hydroxylase; D_1 , D_2 , dopamine receptors 1 and 2. Dashed arrows represent a desensitized pathway. Open arrows represent an inhibitory mechanism. Bold arrows represent increased activity.

for this is the blunted pressure natriuresis shown to be present in the SHR (30).

Treatment with clonidine for two days produced a significant decrease in solid intake in WKY rats and SHR, a side effect that is often observed with sedative drugs. This effect of clonidine has been well studied and is explained by its action on α_2 -adrenoceptors in the brain (31). Activation of α_2 -adrenoceptor in the intestine not only reduces intestinal motility, but also increases absorption of sodium chloride and fluid simultaneously due to a reduction in ion secretion, overall contributing to the anti-diarrhea effect of clonidine (32). Again, this effect was also clearly observed since rats treated with clonidine had lower fecal weight and what is more, this effect was similar between the normotensive and the hypertensive animals. Hydrosaline retention is another side effect that has been described for clonidine (32). The findings of the present study show that in WKY rats, clonidine increased sodium and water reabsorption in the kidney, as evidenced by the decrease in urine volume and sodium urinary excretion. However, in SHR no changes in these parameters were observed, and in fact, SHR already had a lower urinary volume under control conditions. Furthermore, it should be noted that in SHR, despite the reduction in liquid intake produced by clonidine, probably due to the sedative effect of clonidine, there was no effect in urine flow, an indication that the physiological response to a decrease in liquid intake (a decrease in urine volume) is impaired in the hypertensive animals.

Normal sympathetic nervous system regulation and function occurs via α_2 -adrenoceptors (33), and almost all presynaptic inhibitory autoreceptor function in the central sympathetic nervous system is exerted by the α_{2A} subtype (31). Increased noradrenaline synthesis and release and decreased α_{2A} -adrenoceptor inhibitory function, reflected by elevated noradrenaline plasma levels, are prominent features of SHR hypertension (34–36). We have recently shown that administration of clonidine to SHR in the pre-hypertensive phase not only reduced sympathetic activation, as evidenced by the reduction in noradrenaline and adrenaline plasma levels, but also completely prevented the rise in blood pressure in the early stages of the development of SHR hypertension (37). We have also previously shown that 12-week-old SHR have significantly higher noradrenaline plasma levels compared to age-matched WKY rats (23). In this study we show that, two days after clonidine administration, noradrenaline plasma levels were similar between normotensive WKY rats and SHR, but kidney noradrenaline tissue levels were significantly higher in SHR compared to WKY rats.

Clonidine may act at 2 anatomic sites to lower blood pressure. In several brain stem nuclei, activation of α_2 -adrenoceptors leads to a reduction in sympathetic tone. In addition, clonidine may activate presynaptic inhibitory α_2 -adrenoceptors on postganglionic sympathetic fibers to lower sympathetic noradrenaline release. One possible explanation for the increased noradrenaline levels in the

kidney is a down-regulation of pre-synaptic α_{2A} -adrenoceptors. Therefore, although clonidine normalizes sympathetic activity as evidenced by the similar noradrenaline levels when compared to WKY rats, this effect is not present in the kidney due to a reduced function of pre-synaptic α_{2A} -adrenoceptors. In support of this notion, a reduced expression and defective modulation of solute excretion in SHR rats due to alteration in the α_{2A} -adrenoceptor-subtype gene and function has been reported and shown to play a causal role in the pathogenesis of SHR hypertension (38). Another possibility could be due to increased noradrenaline synthesis by increased tyrosine hydroxylase activity, the rate-limiting step in catecholamine synthesis. In fact, increased tyrosine hydroxylase activity and increased noradrenaline levels have been demonstrated in the kidney of SHR (39). The inability to turn off noradrenaline activity may lead to excess sodium retention and prolonged peripheral vasoconstriction, ultimately leading to manifest hypertension (Fig. 5).

Abnormalities in the renal dopaminergic system relating to sodium handling have been implicated in the pathogenesis of hypertension. At the cellular level, dopamine inhibits sodium reabsorption (attenuation of Na^+, K^+ -ATPase and Na^+/H^+ antiport activity) and inhibits noradrenaline release from postganglionic sympathetic nerves. In the proximal tubule, Na^+, K^+ -ATPase activity is determined by the balance between the stimulatory effect of α -adrenoceptors and the inhibitory effect of dopamine receptors. As opposed to dopamine, noradrenaline stimulates renal Na^+, K^+ -ATPase activity, thereby decreasing sodium excretion. In a situation when sodium excretion needs to be enhanced, the dopamine/noradrenaline ratio should be increased, as the two hormones have opposite actions on sodium reabsorption (excretion/retention) and vascular resistance (dilation/constriction). Our study shows that after clonidine treatment, not only dopamine tissue levels were elevated in SHR compared to WKY rats but also the dopamine/noradrenaline ratio was significantly elevated in SHR compared to WKY rats. There are reports on a defective D_1 -receptor adenylyl cyclase coupling in proximal tubules of SHR and a decreased renal response to D_1 -receptor stimulation in SHR (40). The higher dopamine levels in SHR may either be due to increased synthesis to compensate for the defect in the D_1 -receptor or be a means to compensate for the increased blood pressure in SHR. Another possibility could be an adaptive change to the increase sympathetic tone as manifested by the increase in noradrenaline synthesis and release. The inability of the kidney to respond to the increased dopaminergic system activity further underscores the idea that an increased activity of the noradrenergic system may be responsible for the imbalance

in sodium, ultimately leading to manifest hypertension (Fig. 5).

Functional studies have shown that renal vascular responsiveness mediated by the α_2 -adrenoceptor is greater in SHR than in WKY rats (41, 42) and that noradrenaline stimulates the tubular reabsorption of sodium more pronouncedly in SHR (43). This enhanced responsiveness in SHR kidney may be partially explained by α_{2B} -adrenoceptor-mediated acceleration of Na^+/H^+ exchange. The latter may be explained by an increased activity of the Na^+/H^+ exchanger (NHE) in the kidney of the hypertensive rat strains, namely the SHR (44, 45) that is unresponsive to inhibitory action (46). This increased expression of NHE in the kidney of SHR compared to WKY rats (47) has been related to the development of hypertension in these animals (48). What is more, α -adrenoceptor agonists stimulate Na^+/H^+ exchange activity (49), and higher renal α_{2B} -adrenoceptor expression coupled with the increased noradrenergic tone during the development of hypertension in SHR (19) may explain the higher activity of NHE (Fig. 5).

In conclusion, the data presented show that clonidine treatment 1) normalized noradrenaline plasma levels in SHR, a measure of sympathetic drive, but not kidney noradrenaline or dopamine levels which remained elevated; 2) decreased sodium excretion only in WKY rats; and 3) albeit reducing blood pressure in both strains, this effect was less pronounced in SHR that remained hypertensive. Our data further underscore the idea that altered renal sodium balance in SHR may represent a consequence of increased noradrenaline synthesis and release due to increased tyrosine hydroxylase activity and decreased α_{2A} -adrenoceptor inhibitory function, respectively, that result in enhanced activation of kidney α_{2B} -adrenoceptors (Fig. 5).

Acknowledgments

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References

- 1 Guyton AC. Blood pressure control--special role of the kidneys and body fluids. *Science*. 1991;252:1813–1816.
- 2 Strazzullo P, Galletti F, Barba G. Altered renal handling of sodium in human hypertension: short review of the evidence. *Hypertension*. 2003;41:1000–1005.
- 3 Kopp UC, Cicha MZ, Smith LA. Impaired interaction between efferent and afferent renal nerve activity in SHR involves increased activation of α_2 -adrenoceptors. *Hypertension*. 2011;57:640–647.
- 4 Guyton AC, Coleman TG, Cowley AV Jr, Scheel KW, Manning

- RD Jr, Norman RA Jr. Arterial pressure regulation. Overriding dominance of the kidneys in long-term regulation and in hypertension. *Am J Med.* 1972;52:584–594.
- 5 Kopp UC, Cicha MZ, Smith LA, Ruohonen S, Scheinin M, Fritz N, et al. Dietary sodium modulates the interaction between efferent and afferent renal nerve activity by altering activation of alpha2-adrenoceptors on renal sensory nerves. *Am J Physiol Regul Integr Comp Physiol.* 2011;300:R298–R310.
- 6 DiBona GF. Neural control of renal function: role of renal alpha adrenoceptors. *J Cardiovasc Pharmacol.* 1985;7 Suppl 8:S18–S23.
- 7 Jin X, Satoh-Otonashi Y, Zamami Y, Takatori S, Hashikawa-Hobara N, Kitamura Y, et al. New molecular mechanisms for cardiovascular disease: contribution of endothelium-derived hyperpolarizing factor in the regulation of vasoconstriction in peripheral resistance arteries. *J Pharmacol Sci.* 2011;116:332–336.
- 8 Wilborn TW, Sun D, Schafer JA. Expression of multiple alpha-adrenoceptor isoforms in rat CCD. *Am J Physiol.* 1998;275: F111–F118.
- 9 DiBona GF, Sawin LL. Role of renal alpha 2-adrenergic receptors in spontaneously hypertensive rats. *Hypertension.* 1987;9: 41–48.
- 10 Onesti G, Schwartz AB, Kim KE, Paz-Martinez V, Swartz C. Antihypertensive effect of clonidine. *Circ Res.* 1971;28 Suppl 2: 53–69.
- 11 Schmitt H. The pharmacology of clonidine and related products. In: Gross F, editor. *Handbook of experimental pharmacology.* Vol. 39: Antihypertensive agents. New York: Springer-Verlag; 1977. p. 299–378.
- 12 Hohage H, Schlatter E, Greven J. Effects of moxonidine and clonidine on renal function and blood pressure in anesthetized rats. *Clin Nephrol.* 1997;47:316–324.
- 13 Barr JG, Kauker ML. Renal tubular site and mechanism of clonidine-induced diuresis in rats: clearance and micropuncture studies. *J Pharmacol Exp Ther.* 1979;209:389–395.
- 14 Schmitz JM, Graham RM, Sagalowsky A, Pettinger WA. Renal alpha-1 and alpha-2 adrenergic receptors: biochemical and pharmacological correlations. *J Pharmacol Exp Ther.* 1981;219: 400–406.
- 15 Pettinger WA, Keeton TK, Campbell WB, Harper DC. Evidence for a renal alpha-adrenergic receptor inhibiting renin release. *Circ Res.* 1976;38:338–346.
- 16 Miller M. Clonidine-induced diuresis in the rat: evidence for a renal site of action. *J Pharmacol Exp Ther.* 1980;214:608–613.
- 17 Gesek FA, Schoolwerth AC. Hormonal interactions with the proximal Na(+)-H⁺ exchanger. *Am J Physiol.* 1990;258: F514–F521.
- 18 Takatori K, Ogura T, Katayama E, Ota Z, Mizukawa K, Ogawa N. Autoradiographic localization and computerized quantification of alpha 1- and alpha 2-adrenoceptors in spontaneously hypertensive rat kidney: [3H]bunazosin and [3H]yohimbine binding studies. *J Int Med Res.* 1991;19:153–160.
- 19 Gesek FA. Alpha1- and alpha2-adrenoceptor control of sodium transport reverses in developing hypertension. *Hypertension.* 1999;33:524–529.
- 20 Tan CM, Wilson MH, MacMillan LB, Kobilka BK, Limbird LE. Heterozygous alpha 2A-adrenergic receptor mice unveil unique therapeutic benefits of partial agonists. *Proc Natl Acad Sci U S A.* 2002;99:12471–12476.
- 21 Tank J, Jordan J, Diedrich A, Obst M, Plehm R, Luft FC, et al. Clonidine improves spontaneous baroreflex sensitivity in conscious mice through parasympathetic activation. *Hypertension.* 2004;43:1042–1047.
- 22 Zhu QM, Lesnick JD, Jasper JR, MacLennan SJ, Dillon MP, Eglen RM, et al. Cardiovascular effects of rilmenidine, moxonidine and clonidine in conscious wild-type and D79N alpha2A-adrenoceptor transgenic mice. *Br J Pharmacol.* 1999;126: 1522–1530.
- 23 Moura E, Pinho Costa PM, Moura D, Guimaraes S, Vieira-Coelho MA. Decreased tyrosine hydroxylase activity in the adrenals of spontaneously hypertensive rats. *Life Sci.* 2005; 76:2953–2964.
- 24 Pestana M, Vieira-Coelho MA, Pinto-do OP, Fernandes MH, Soares-da-Silva P. Assessment of renal dopaminergic system activity during cyclosporine A administration in the rat. *Br J Pharmacol.* 1995;115:1349–1358.
- 25 Hohage H, Hess K, Jahl C, Greven J, Schlatter E. Renal and blood pressure effects of moxonidine and clonidine in spontaneously hypertensive rats. *Clin Nephrol.* 1997;48:346–352.
- 26 Bagrov AY, Agalakova NI, Kashkin VA, Fedorova OV. Endogenous cardiotonic steroids and differential patterns of sodium pump inhibition in NaCl-loaded salt-sensitive and normotensive rats. *Am J Hypertens.* 2009;22:559–563.
- 27 Silva E, Serrao MP, Soares-da-Silva P. Age-dependent effect of ouabain on renal Na⁺,K⁺-ATPase. *Life Sci.* 2011;88:719–724.
- 28 DiBona GF, Kopp UC. Neural control of renal function. *Physiol Rev.* 1997;77:75–197.
- 29 Granger JP, Alexander BT, Llinas M. Mechanisms of pressure natriuresis. *Curr Hypertens Rep.* 2002;4:152–159.
- 30 Roman RJ, Cowley AW Jr. Abnormal pressure-diuresis-natriuresis response in spontaneously hypertensive rats. *Am J Physiol.* 1985;248:F199–F205.
- 31 Knaus AE, Muthig V, Schickinger S, Moura E, Beetz N, Gilsbach R, et al. Alpha2-adrenoceptor subtypes—unexpected functions for receptors and ligands derived from gene-targeted mouse models. *Neurochem Int.* 2007;51:277–281.
- 32 Fedorak RN, Field M, Chang EB. Treatment of diabetic diarrhea with clonidine. *Ann Intern Med.* 1985;102:197–199.
- 33 Martinez AC, Pagan RM, Prieto D, Recio P, Garcia-Sacristan A, Hernandez M, et al. Modulation of noradrenergic neurotransmission in isolated rat radial artery. *J Pharmacol Sci.* 2009;111: 299–311.
- 34 Moura E, Pinto CE, Calo A, Serrao MP, Afonso J, Vieira-Coelho MA. alpha(2)-Adrenoceptor-mediated inhibition of catecholamine release from the adrenal medulla of spontaneously hypertensive rats is preserved in the early stages of hypertension. *Basic Clin Pharmacol Toxicol.* 2011;109:253–260.
- 35 Moura E, Pinto CE, Serrao MP, Afonso J, Vieira-Coelho MA. Adrenal alpha(2)-adrenergic receptors in the aging normotensive and spontaneously hypertensive rat. *Neurobiol Aging.* 2012; 33:969–978.
- 36 Zugck C, Lossnitzer D, Backs J, Kristen A, Kinscherf R, Haass M. Increased cardiac norepinephrine release in spontaneously hypertensive rats: role of presynaptic alpha-2A adrenoceptors. *J Hypertens.* 2003;21:1363–1369.
- 37 Moura E, Afonso J, Serrao MP, Vieira-Coelho MA. Effect of clonidine on tyrosine hydroxylase activity in the adrenal medulla and brain of spontaneously hypertensive rats. *Basic Clin Pharmacol Toxicol.* 2009;104:113–121.
- 38 Intengan HD, Smyth DD. Renal alpha 2a/d-adrenoceptor subtype

- function: Wistar as compared to spontaneously hypertensive rats. *Br J Pharmacol.* 1997;121:861–866.
- 39 Burgi K, Cavalleri MT, Alves AS, Britto LR, Antunes VR, Michelini LC. Tyrosine hydroxylase immunoreactivity as indicator of sympathetic activity: simultaneous evaluation in different tissues of hypertensive rats. *Am J Physiol Regul Integr Comp Physiol.* 2011;300:R264–R271.
- 40 Jose PA, Eisner GM, Drago J, Carey RM, Felder RA. Dopamine receptor signaling defects in spontaneous hypertension. *Am J Hypertens.* 1996;9:400–405.
- 41 Kobrin I, Pegram BL, Frohlich ED. Acute pressure increase and intrarenal hemodynamics in conscious WKY and SHR rats. *Am J Physiol.* 1985;249:H1114–H1118.
- 42 Uchino K, Frohlich ED, Nishikimi T, Isshiki T, Kardon MB. Spontaneously hypertensive rats demonstrate increased renal vascular alpha 1-adrenergic receptor responsiveness. *Am J Physiol.* 1991;260:R889–R893.
- 43 Steele TH, Underwood JL. Blunted norepinephrine natriuresis in the isolated spontaneously hypertensive rat kidney. *Am J Physiol.* 1978;235:F425–F429.
- 44 LaPointe MS, Sodhi C, Sahai A, Batlle D. Na⁺/H⁺ exchange activity and NHE-3 expression in renal tubules from the spontaneously hypertensive rat. *Kidney Int.* 2002;62:157–165.
- 45 Morduchowicz GA, Sheikh-Hamad D, Jo OD, Nord EP, Lee DB, Yanagawa N. Increased Na⁺/H⁺ antiport activity in the renal brush border membrane of SHR. *Kidney Int.* 1989;36:576–581.
- 46 Pedrosa R, Gomes P, Zeng C, Hopfer U, Jose PA, Soares-da-Silva P. Dopamine D3 receptor-mediated inhibition of Na⁺/H⁺ exchanger activity in normotensive and spontaneously hypertensive rat proximal tubular epithelial cells. *Br J Pharmacol.* 2004;142:1343–1353.
- 47 Pettinger WA, Sanchez A, Saavedra J, Haywood JR, Gandler T, Rodes T. Altered renal alpha 2-adrenergic receptor regulation in genetically hypertensive rats. *Hypertension.* 1982;4:188–192.
- 48 Stanko CK, Smyth DD. Proximal tubular alpha 2-adrenoceptor density in the spontaneously hypertensive rat. *Am J Hypertens.* 1991;4:64–67.
- 49 Siczkowski M, Davies JE, Ng LL. Sodium-hydrogen antiporter protein in normotensive Wistar-Kyoto rats and spontaneously hypertensive rats. *J Hypertens.* 1994;12:775–781.