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Na^+-ATPase in spontaneous hypertensive rats: Possible AT_1 receptor target in the development of hypertension

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ABSTRACT

Clinical and experimental data show an increase in sodium reabsorption on the proximal tubule (PT) in essential hypertension. It is well known that there is a link between essential hypertension and renal angiotensin II (Ang II). The present study was designed to examine ouabain-insensitive Na^+-ATPase activity and its regulation by Ang II in spontaneously hypertensive rats (SHR). We observed that Na^+-ATPase activity was enhanced in 14-week-old but not in 6-week-old SHR. The addition of Ang II from 10^{-12} to 10^{-6} mol/L decreased the enzyme activity in SHR to a level similar to that obtained in WKY. The Ang II inhibitory effect was completely reversed by a specific antagonist of AT_2 receptor, PD123319 (10^{-8} mol/L) indicating that a system leading to activation of the enzyme in SHR is inhibited by AT_2-mediated Ang II. Treatment of SHR with losartan for 10 weeks (weeks 4–14) prevents the increase in Na^+-ATPase activity observed in 14-week-old SHR. These results indicate a correlation between AT_1 receptor activation in SHR and increased ouabain-insensitive Na^+-ATPase activity. Our results open new possibilities towards our understanding of the pathophysiological mechanisms involved in the increased sodium reabsorption in PT found in essential hypertension.

1. Introduction

The kidneys play a crucial role in the long-term regulation of systemic blood pressure (BP) [1–3].This role is strictly correlated to the ability of the kidneys to fine tune the level of the sodium excretion at different BP levels [4,5]. According to Guyton's hypothesis, whenever arterial blood pressure is elevated an increase in natriuresis pressure occurs and, consequently, in renal sodium excretion [6,7]. This hypothesis predicts that the mechanism that resets this relationship towards higher pressure leads to hypertension [8].

The renal mechanisms of Na^+ transport are the main determinants of urinary Na^+ excretion, constituting the primary regulators of extracellular fluid volume and, therefore, contributing to the maintenance of BP [9]. The observation that patients with essential hypertension as well as spontaneously hypertensive rats (SHR) show increased sodium reabsorption on the proximal tubule (PT) indicates that this segment plays an important role during the genesis of the hypertension [8,10–13]. Furthermore, before the onset of hypertension, SHR already show increased sodium and water reabsorption [11], and the PT has been implicated in this sodium retention [13,14].

The basolateral sodium transport is one of the limiting steps to PT sodium reabsorption [15,16]. Two sodium pumps have been described in this renal segment: the classic (Na^++K^+)ATPase and the ouabain-insensitive, furosemide-sensitive Na^+-ATPase [15,17,18]. It was shown that in SHR the (Na^++K^+)ATPase activity in PT is increased in 5-week-old rats but is not changed in animals more than 12 weeks of age [19,20]. On the other hand, it has been observed that the increased sodium reabsorption in PT cells is maintained even in adult animals and the transporter involved in this process is still to be determined.

SHR has been used as a model of essential hypertension [21]. It is well known that there is a link between essential hypertension and the rennin/angiotensin system (RAS) [22]. It is well recognized that PT function is regulated by both circulating and locally formed angiotensin II (Ang II) [23]. Matsushima et al. [24] observed that the content of Ang II in the renal cortex is increased in 14-week-old SHR. Our group showed that the PT Na^+-ATPase is the target for some peptides such as Ang II [25–27]. Then, the selective modulation of Na^+-ATPase by Ang II may be an important mechanism of extracellular fluid volume control and, consequently, of arterial blood

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pressure control. If this assumption is true, it could be expected that the activity of this enzyme would be changed in hypertension. Therefore, the present study was designed to examine the role of Na^+-ATPase in SHR and its regulation by Ang II.

2. Materials and methods

2.1. Materials

Adenosine triphosphate (magnesium salt; Mg-ATP), ouabain, furosemide, sodium chloride, potassium chloride, magnesium chloride, ethylenediaminetetracetic acid (EDTA), N-2-hydroxyethyl-piperazine N'-2-ethanesulfonic acid (HEPES), (trishydroxymethyl)-aminomethane (Tris), histone, bovine serum albumin (BSA), phenylmethylsulfonyl fluoride (PMSF), PD123319 and guanosine 5-[β-thio]diphosphate (GDPβS) were purchased from Sigma Aldrich Company (St. Louis, MO). Sucrose and choline chloride were from Merck (Darmstadt, Germany). The AT_1 receptor selective antagonist, losartan, was obtained from Medley S.A. (São Paulo, Brazil). [^{32}Pi]Pi was obtained from the Brazilian Institute of Energetic and Nuclear Research, São Paulo, Brazil. [γ-^{32}Pi]ATP was synthesized according to the procedures described by Maia et al. [28]. All other reagents were of the highest purity available.

2.2. Animals

All the animal procedures were conducted in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Ethics Committee of Federal University of Rio de Janeiro (number IBCCF004). Adult male Wistar Kyoto (WKY) and Spontaneously Hypertensive Rats (SHR) aged 6 or 14 weeks were purchased from Centro de Desenvolvimento de Modelos Experimentais, Escola Paulista de Medicina, São Paulo, Brazil. The animals were reared four per cage with free access to food and water in a room maintained at 25 ± 1 °C, 55 ± 5% humidity with a 12-h light–dark cycle. In agreement with data from other authors [29–31], we observed that 6-week-old SHR did not develop hypertension, while at 14 weeks, hypertension is already established.

2.3. Treatment

The SHR were randomly divided into four groups according to the treatment: Group 1, WKY rats, used as normotensive controls, received vehicle (water) for 10 weeks (WKY-V); Group 2, SHR treated with losartan for 6 weeks and then vehicle (water) for 4 weeks (SHR-L up to 10 weeks old); Group 3, SHR treated with losartan for 10 weeks (SHR-L up to 14 weeks old); and Group 4, SHR treated only with vehicle (water, SHR-V). The treatments were administered by gavage once daily and body weight was measured daily for adjustment of the losartan dose to 30 mg/kg per day. Arterial blood pressure was measured weekly by the tail-cuff compression method (Non-invasive Blood Pressure Instrument LE 5001, Panlab, Barcelona, Spain) for the duration of the treatment. After the treatment the animals were anesthetized with 100 mg/kg ketamine and 20 mg/kg xylazine and then the kidneys were removed to prepare the microsomal fraction of the kidney cortex as described below.

2.4. Non-invasive BP measurement

Twenty male rats were used for the non-invasive blood pressure measurements using a Letica LE 5001 instrument (Barcelona, Spain). The tail-cuff method was carried out as previously reported [32]. For accurate blood pressure measurements, the animals were kept in a warm environment (30–32 °C) for at least 30 min until arterial tail pulsations were detected. Three consecutive, consistent readings of the systolic blood pressure (SBP), diastolic blood pressure (DBP), mean blood pressure (MBP) and heart rate (HR) were taken in each individual, and the values were averaged.

2.5. Preparation of the microsomal fraction

The microsomal fraction was obtained using a previous method with a few modifications [33]. Briefly, the kidneys were removed and maintained in cold solution containing (mmol/L): sucrose 250, HEPES–Tris (pH 7.6) 10, EDTA 2 and PMSF 1. Thin slices of the cortex (cortex corticis) were removed with a Stadie Riggs microtome using a scalpel. After dissection, the slices were homogenized in the same cold solution using a Teflon and glass homogenizer. The homogenate was centrifuged at 3000 rpm for 10 min at 4 °C in a Hitachi-Himac SCR-20B centrifuge using a RPR 20.2 rotor. The supernatant was centrifuged at 6000 rpm for 20 min at 4 °C using the same centrifuge and rotor. The new supernatant was ultracentrifuged at 60,000 rpm for 1 h at 4 °C in a Beckman XL100 centrifuge using a type 70 Ti rotor. The final pellet containing a microsomal fraction was re-suspended and homogenized in 250 mmol/L sucrose to a final concentration of 2–3 mg of protein/mL. This preparation was stored at −4 °C and protein concentrations were determined by the Folin phenol method [34] using bovine serum albumin (BSA) as standard.

2.6. Measurements of ATPase activity

The ATPase activity was measured according to the method described by Grubmeyer and Penefsky [35]. The composition of the assay medium for the measurement of ATPase activity (0.1 ml) was: 2 mmol/L $MgCl_2$, 20 mmol/L HEPES–Tris (pH 7.0), 4 mmol/L ATP (as ATP-Mg), and 120 mmol/L NaCl. [γ-^{32}P]ATP was added to a specific activity of 3000 cpm/nmol. The Na^+-ATPase activity was determined from the difference between [^{32}P]Pi released in the absence and in the presence of 2 mM furosemide (an Na^+-ATPase specific inhibitor), both in the presence of 1 mM ouabain (an $(Na^+ + K^+)$ATPase specific inhibitor) and in the absence of KCl. For measurement of $(Na^+ + K^+)$ ATPase activity, 30 mmol/L KCl was added and the activity was determined from the difference between [^{32}P]Pi released in the absence and in the presence of 1 mM ouabain. In the absence of NaCl or KCl, choline chloride was added to maintain a constant ionic strength. The reaction was started by the addition of the microsomal fraction to the assay medium to a final protein concentration of 0.3–0.5 mg/mL at 37 °C and stopped after 10 min by the addition of 0.1 mol/L HCl-activated charcoal. The [^{32}P]Pi released was measured in an aliquot of the supernatant obtained after centrifugation of the samples for 5 min at 2000 rpm in a clinical centrifuge. The radioactivity was quantified by liquid scintillation counting (Packard Tri-Carb 2100 TR). Spontaneous hydrolysis of [γ-^{32}P]ATP was measured simultaneously in tubes to which protein was added after the acid.

2.7. Kinetic parameters

The kinetic parameters were derived from the experimental data by using the statistical curve-fitting package SigmaPlot® (San Jose, CA, USA). This program calculates the kinetic parameters using a non-linear regression method. The interpolation is automatically obtained by derivation of the Michaelis–Menten equation. The non-linear regression coefficient obtained was always higher than 0.99 under all conditions.

2.8. Statistical analysis

The means were compared by one-way analysis of variance (ANOVA) taking into account the treatment of the experimental

groups. The magnitude of the differences was evaluated using the multiple comparative Bonferroni test. Then values correspond to the results obtained from different microsomal fraction preparations of different animals.

3. Results

3.1. Sodium pump activities in SHR and WKY

In the first experimental group we measured ouabain-insensitive Na^+-ATPase and ($Na^+ + K^+$)ATPase activities in 6- or 14-week-old SHR and in age-matched WKY used as controls. The Na^+-ATPase activity was not different in 6-week-old SHR (Fig. 1A) but it was significantly enhanced in 14-week-old SHR compared with the controls (Fig. 1B). On the other hand, ($Na^+ + K^+$)ATPase activity was increased in 6-week-old SHR (Fig. 1A) but was not different in 14-week-old SHR (Fig. 1B). It is important to note that the ATPase activity in the absence of Na^+ was not different in both 6 and 14-week-old SHR (data not shown). Our results show that ouabain-insensitive Na^+-ATPase and ($Na^+ + K^+$)ATPase activities are modulated at different ages of SHR during the development of hypertension.

Fig. 2 shows the Na^+ dependence of the hydrolytic activity of ouabain-insensitive Na^+-ATPase in the presence of 1 mmol/L ouabain and in the absence of K^+, in 6- and 14-week-old WKY and SHR. Under such conditions, ($Na^+ + K^+$)ATPase is completely inhibited. The increase in Na^+ concentration enhanced the ATPase activity in a dose-dependent manner in both SHR and WKY. The Na^+ concentration that promoted half-maximal stimulation ($K_{0.5}$) of Na^+-ATPase and the maximal rate (V_{max}) in 6-week-old WKY was 2.5 ± 1.4 mmol/L and 44.7 ± 2.3 nmol Pi mg^{-1} min^{-1} (Fig. 2A). Although the V_{max} was not different ($V_{max} = 37.8 \pm 2.3$ nmol Pi mg^{-1} min^{-1}) in age-matched SHR, the $K_{0.5}$ for Na^+ decreased 18 times to 0.15 ± 0.06 mmol/L. On the other hand, in 14-week-old SHR, differences in both parameters, V_{max} and $K_{0.5}$, was observed (Fig. 2B). The $K_{0.5}$ for Na^+ decreased from 10.7 ± 2.8 mmol/L (WKY) to 0.38 ± 0.12 mmol/L and V_{max} increased from 24.7 ± 2.2 (WKY) to 51.1 ± 2.7 nmol Pi mg^{-1} min^{-1}. The $K_{0.5}$ for Na^+ found in 14-week-old WKY agrees with the value obtained by other authors in isolated basolateral membrane (BLM) of proximal tubule [36,37]. The Na^+-ATPase activity in 6-week-old WKY is higher than the activity in 14-week-old WKY, but in SHR the activity is maintained in 14-week-old animals.

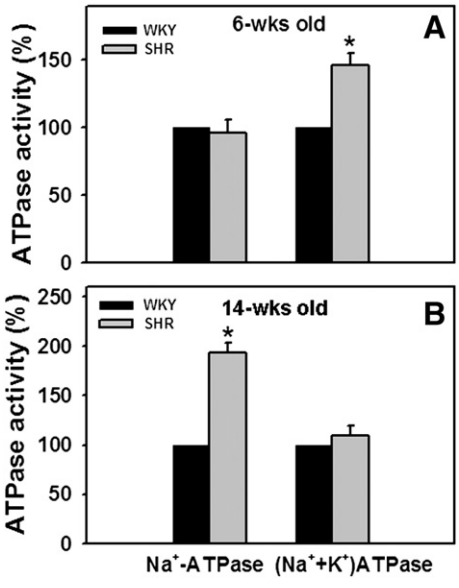
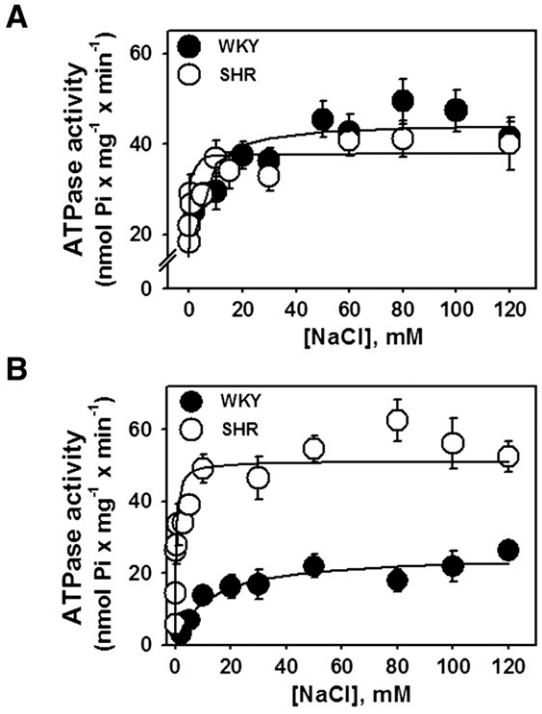


Fig. 1. Differences in PT ATPase activity between SHR and WKY rats. ATPase activity was measured as described in the Materials and methods section. (A) ($Na^+ + K^+$) ATPase and Na^+-ATPase activity in 6-week-old WKY (black bars) and SHR (gray bars) ($n = 10$). (B) ($Na^+ + K^+$) ATPase and Na^+-ATPase activity in 14-week-old WKY (black bars) and SHR (gray bars) ($n = 10$). Results are expressed as mean ± SE. *Statistically significant when compared to respective controls ($p < 0.05$).



C

Parameters	6 wks-old		14 wks-old	
	WKY	SHR	WKY	SHR
$K_{0.5}$ (mM)	2,5 ± 1.4	0.15 ± 0.06*	10.7 ± 2.8	0.38 ± 0.12*
$V_{máx.}$ (nmolPi.mg^{-1}.min^{-1})	47.7 ± 2.3	37.8 ± 2.3	24.7 ± 2.2	51.1 ± 2.7*

Fig. 2. Differences in kinetic parameters of Na^+-ATPase in the PT between SHR and WKY rats. Na^+ concentration dependence of ATPase activity in PT of 6-week-old (A) or 14-week-old (B) SHR (open symbols) and WKY rats (closed symbols) in the presence of 1 mmol/L ouabain and in the absence of KCl. ATPase activity was measured as described in the Materials and methods section and the indicated Na^+ concentrations (as NaCl). (C) The kinetic parameters were calculated using the following equation: $v = V_{max}[S]/K_{0.5} + [S]$ for the 6- and 14-week-old WKY and SHR rats. The data (mean ± SE) correspond to the difference between parallel assays performed in the absence or in the presence of each Na^+ concentration. All experiments were done in duplicate ($n = 8$).

3.2. Ang II interacts with different receptors in SHR and WKY

In a previous paper we observed that Ang II modulates ouabain-insensitive Na^+-ATPase activity in isolated basolateral membrane of the pig PT: the AT_1 receptor increases the enzyme activity [25], and the AT_2 receptor inhibits it [38]. In the next experimental group we decided to investigate if the effect of exogenous addition of Ang II on the ouabain-insensitive Na^+-ATPase activity in 6- (Fig. 3A) and 14-week-old (Fig. 3B) SHR could be changed. To test the effect of Ang II, the microsomal fraction was previously incubated with Ang II, at the concentrations indicated, for 30 min, and then the enzyme activity assays were carried out. This procedure was carried out every time we tested for the effect of Ang II. The increase in Ang II concentration from 10^{-12} to 10^{-6} mol/L increased the enzyme activity in 6- and 14-week-old WKY in a similar way to that observed by our group in isolated BLM of PT cells. The same behavior of the effect of Ang II was

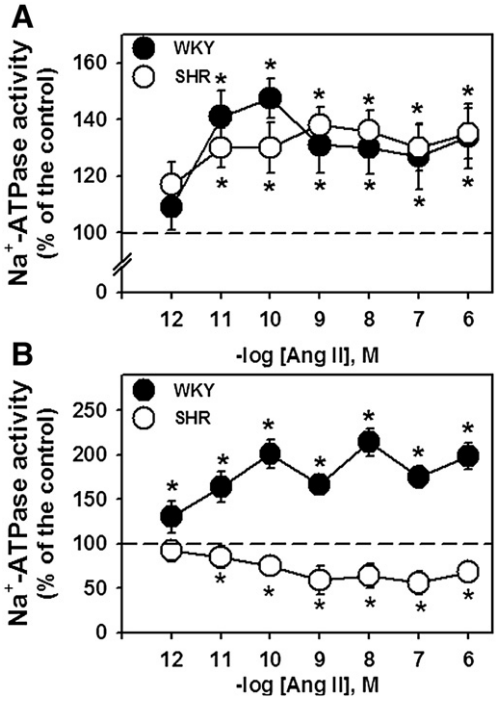


Fig. 3. Modulation of Na^+-ATPase activity by Ang II in 6- and 14-week-old SHR and WKY rats. Na^+-ATPase activity was measured as described in the Materials and methods section. The Ang II concentration ranged from 10^{-12} mol/L to 10^{-6} mol/L. (A) The Ang II effect on 6-week-old WKY (closed symbols) and SHR (open symbols) rats ($n=7$). (B) Ang II effect on 14-week-old WKY (closed symbols) and SHR (open symbols) rats ($n=5$). Results are expressed as percentage of the control (mean ± SE). *Statistically significant when compared to respective controls in the absence of Ang II ($p<0.05$). Dashed line represents the control value of the SHR and WKY taken as 100%.

observed in 6-week-old SHR (Fig. 3A). On the other hand, in 14-week-old SHR, an inhibitory effect of Ang II on the enzyme activity was observed, which is contrary to that observed in age-matched WKY (Fig. 3B). The maximal effect under all conditions was obtained at a concentration of 10^{-10} mol/L. Exogenous Ang II, in 14-week-old SHR, decreased the ouabain-insensitive Na^+-ATPase activity to a level similar that obtained in WKY in the absence of Ang II. These results show that there is an anomaly effect of Ang II on ouabain-insensitive Na^+-ATPase activity that is a consequence of something that happens between birth and 14 weeks in SHR.

In order to identify which receptor could be involved in the effect of exogenous Ang II on the ouabain-insensitive Na^+-ATPase activity in 14-week-old SHR and WKY, we used specific antagonists for AT_1 and AT_2 receptors, losartan and 7PD123319, respectively (Fig. 4A). The stimulatory effect of 10^{-9} mol/L Ang II on Na^+-ATPase activity in WKY was completely reversed by 10^{-6} mol/L losartan but it was not modified by 10^{-8} mol/L PD123319. On the other hand, in SHR, the inhibitory effect of exogenous Ang II was completely reversed by 10^{-8} mol/L PD123319 but it was not modified by 10^{-6} mol/L losartan. The addition of 10^{-8} mol/L PD123319 or 10^{-6} mol/L losartan alone did not change the enzyme activity in both WKY and SHR (data not shown). These results indicate that the modulatory effect of exogenous Ang II on Na^+-ATPase activity is mediated by different receptors, AT_1 and AT_2, in 14-week-old WKY and SHR, respectively.

The Ang II receptors, AT_1 and AT_2, have seven transmembrane domains and belong to the family of G protein-coupled receptors (GPCR) [39,40]. Fig. 4A shows that the effects of Ang II on the enzyme activity in 14-week-old WKY and SHR were completely abolished by 10^{-8} mol/L GDPβS, a specific inhibitor of trimeric G protein. This scenario indicates that in hypertensive animals, activation of some system occurs leading to the increase in enzyme activity which is inhibited by addition of Ang II activating AT_2 receptor.

3.3. AT_1 receptor is responsible for activation of the ouabain-insensitive Na^+-ATPase in adult SHR

It is known that the level of AT_1 receptor is increased in 4-week-old SHR but it is not changed in adult animals (14–16 weeks old) [41]. Therefore, it is possible to imagine that changes in this period could be responsible for changes in sodium transport in adult animals. To address this question, we treated 4-week-old SHR or age-matched WKY daily with 30 mg/kg losartan by gavage as described in the Materials and methods section (Fig. 5). After the treatment, the microsomal fraction of the kidney cortex was prepared separately from each animal in different groups and then the Na^+-ATPase activity assay was carried out.

Treatment of SHR with losartan for 10 weeks (weeks 4–14) or treated with losartan for 6 weeks (weeks 4–10) and vehicle for a further 4 wks (weeks 10–14) avoided the development of hypertension (Fig. 5A) and the increase in ouabain-insensitive Na^+-ATPase activity (Fig. 5B) observed in 14-week-old SHR. Under these conditions, ouabain-insensitive Na^+-ATPase activity was decreased by 50% compared to age-matched WKY-V. Treatment of SHR with vehicle for the same period of time did not change the enzyme activity and mean arterial blood pressure (MAP) when compared to untreated SHR. $(Na^+ + K^+)$ATPase activity in 14-week-old animals was not changed under any of the conditions tested (data not shown). These results indicate a clear correlation between AT_1 receptor activity and ouabain-insensitive Na^+-ATPase activation of kidney cortex.

In the last experimental group, we tested whether treatment of the SHR with losartan avoids the changes in kinetic parameters of the Na^+-ATPase (Fig. 6). SHR were treated with losartan (SHR-L) or treated with vehicle (water, SHR-V) for 10 weeks. WKY treated with

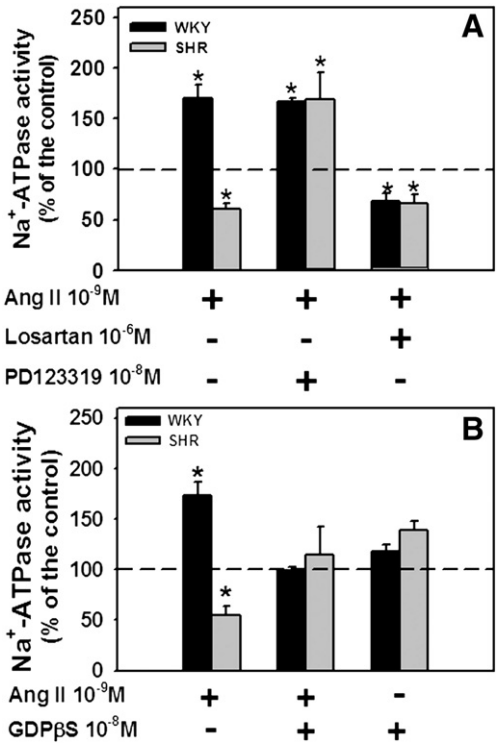


Fig. 4. Identification of the angiotensin receptor involved in the Ang II effect on adult SHR Na^+-ATPase. ATPase activity was measured as described in the Materials and methods section. (A) Effect of the AT_1 antagonist, losartan, and AT_2 antagonist, PD123319, on Ang II modulation of Na^+-ATPase activity in 14-week-old WKY (black bars) and SHR (gray bars) rats ($n=9$). (B) Effect of the G protein inhibitor, GDPβS, on the Ang II effect ($n=5$). Results are expressed as % of the control (mean ± SE). *Statistically significant when compared to the respective controls in the absence of Ang II or GDPβS ($p<0.05$). Dashed line represents the control value of SHR and WKY taken as 100%.

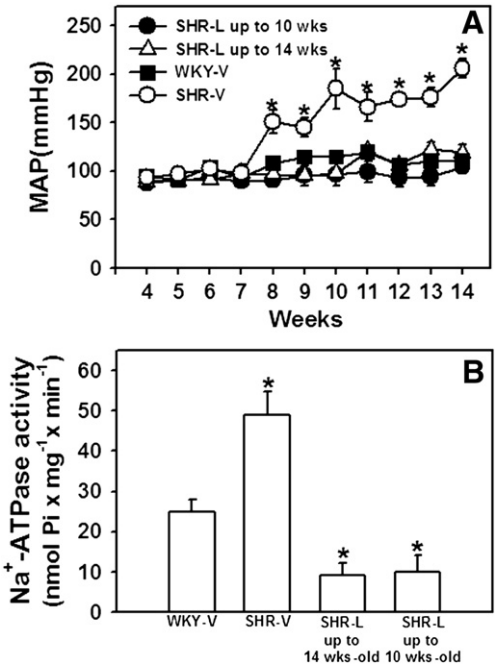


Fig. 5. The AT_1 receptor is involved in the activation of Na^+-ATPase in adult SHR. Animals were treated as described in the Materials and methods section. WKY-V, WKY rats received vehicle (water) for 10 weeks; SHR-V, SHR treated only with vehicle (water); SHR-L up to 10 weeks old, SHR treated with losartan for 6 weeks and then vehicle (water) for 4 weeks; and SHR-L up to 14 weeks old, SHR treated with losartan for 10 weeks ($n=6$/group). (A) mean arterial blood pressure (MAP) measured weekly in all groups; (B) renal cortex Na^+-ATPase activity in the same conditions described in panel A. Results are expressed as % of the control (mean ± SE). *Statistically significant when compared to the respective controls ($p<0.05$).

vehicle for 10 weeks (WKY-V) were used as controls. Losartan treatment avoided the decrease in $K_{0.5}$ for Na^+ observed in SHR. Furthermore, under these conditions losartan decreased V_{max} even when compared to WKY-V (in Fig. 6B).

4. Discussion

Our study shows for the first time that ouabain-insensitive Na^+-ATPase activity is activated in adult spontaneously hypertensive rats. Contrary to $(Na^+ + K^+)$ATPase, ouabain-insensitive Na^+-ATPase activity is increased in adult SHR when hypertension is already established. The activation of the ouabain-insensitive Na^+-ATPase in SHR is correlated to AT_1 receptor activity. Thus, we hypothesize that the increase in expression of AT_1 receptor expression in young animals (4 weeks old), observed in other studies, promotes modifications in the adult phase that lead to changes in ouabain-insensitive Na^+-ATPase activity and, consequently, in proximal tubule sodium reabsorption.

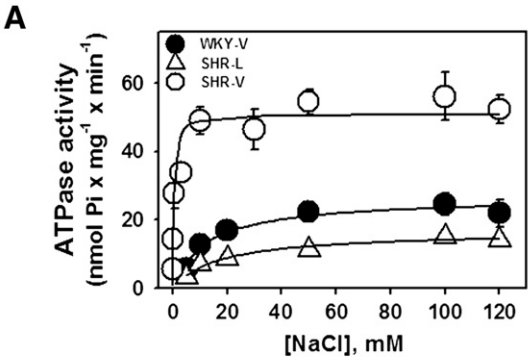
The link between Na^+-stimulated ATPase activity and proximal tubule Na^+ transport has been demonstrated by several authors [42–45]. Furthermore, it has been proposed that ouabain-insensitive Na^+-ATPase is involved in fine tuning, whereas $(Na^+ + K^+)$ATPase is responsible for most of the Na^+ reabsorption in the PT. In this way, any change in ouabain-insensitive Na^+-ATPase leads to significant changes in sodium reabsorption in proximal tubule cells as those observed in SHR. The possible effect of furosemide on other ATPases or on $Na^+/K^+/2Cl^-$ cotransporter can be ruled out as discussed in a previous study [46].

The increase in ouabain-insensitive Na^+-ATPase activity observed in adult SHR is a consequence of the changes in both V_{max} and apparent affinity for Na^+. Based on the intracellular Na^+ concentration, it is plausible to postulate that the Na^+-ATPase is working at almost maximal rate in both 6- and 14-week-old SHR. This result fits very well with the abnormal increase in proximal tubule sodium reabsorption observed in SHR [11–14] or primary hypertensive patients [8,10] indicating the involvement of this enzyme, at least in part, in this process. The role of this enzyme in hypertensive animal models was also shown in a recent paper by Beltowski and coworkes [47]. The authors showed that abnormal regulation of PT ouabain-insensitive Na^+-ATPase activity is involved in hypertension associated with the metabolic syndrome.

It was shown that AT_1 receptor interacts with $(Na^+ + K^+)$ATPase through arrestins, which could lead to changes in the enzyme activity [48]. However, this hypothesis is unlikely in the modulation of ouabain-insensitive Na^+-ATPase activity by the AT_1-mediated Ang II effect because we showed that the Ang II effect is completely reversed by specific inhibitors of the PI-PLC/PKC pathway and by GDPβS [25–27,38,40].

Here we have shown that the increase in Na^+-ATPase activity of kidney cortex is a consequence of AT_1 receptor activity because losartan treatment avoids the increase in enzyme activity. It has been shown that the contribution of the PT in essential hypertension is independent of age and salt-induced variations in renal hemodynamics [49]. The changes in sodium reabsorption in PT could be due to acquired renal tubular defects and/or molecular alterations. Thus, it is possible to postulate that the increase in AT_1 receptor expression in young SHR leads to some modifications in adult SHR responsible for the increase in PT Na^+-ATPase activity.

The final effect of Ang II on PT Na^+-ATPase activity is a consequence of the action of this peptide in two receptors: AT_1 and AT_2 [25,38]. Usually, the level of the AT_2 receptor is lower than the AT_1 receptor and the final effect of Ang II observed is that mediated by the AT_1 receptor. We showed in a previous paper that when AT_1 receptor is blocked by losartan Ang II inhibits the Na^+-ATPase of pig proximal tubule in a PD123319-sensitive way [25,38]. This result agrees with the proposed role of the AT_2 receptor in counteracting the AT_1 receptor to achieve hydroelectrolytic balance in normotensive



B

Parameters	WKY-V	SHR-V	SHR-L
$K_{0.5}$ (mM)	12.2 ± 2.8	0.5 ± 0.2*	16.9 ± 3.4#
V_{max} (nmol Pi.mg-1.min-1)	26.4 ± 1.6	51.0 ± 2.5*	16.4 ± 0.9*,#

Fig. 6. Kinetic parameters of Na^+-ATPase after losartan treatment. (A) Na^+ concentration dependence of ATPase activity in 14-week-old WKY-V (closed circles, WKY treated only with water), SHR-V (open circles, SHR treated only with water) and SHR-L (open triangles, SHR treated with losartan for 10 weeks). All experiments were carried out in the presence of 1 mmol/L ouabain and in the absence of KCl. ATPase activity was measured as described in the Materials and methods section and at the indicated Na^+ concentrations (as NaCl). (B) The kinetic parameters were calculated using the following equation: $v = V_{max}[S]/K_{0.5}+[S]$. The data (mean ± SE) correspond to the difference between parallel assays performed in the absence or in the presence of each Na^+ concentration. All experiments were done in duplicate ($n=8$). *Statistically significant when compared to WKY-V and #statistically significant when compared to SHR-V ($p<0.05$).

animals. However, if the AT_1 receptor is already maximally stimulated, the addition of exogenous Ang II could work as a preferential agonist of AT_2 receptor, even when the expression of this receptor is decreased. This hypothesis could explain the observation that exogenous Ang II inhibited the ouabain-insensitive Na^+-ATPase activity in SHR through AT_2 receptor. A possible increase in expression of AT_2 receptor in SHR can be ruled out because the observation that the expression of AT_2 receptor in mesenteric artery is decreased in SHR [50].

The precise functional role of the renal AT_2 receptor is so far poorly defined, especially in the kidney. Evidence accumulated in the last few years suggests that the major function of AT_2 receptors is related to functional antagonism of the vasoconstrictor action of AT_1 receptors. Our results agree with recent observations that the AT_2 receptor represents a counter-regulatory mechanism of kidney protection in a 5/6 uninephrectomy model [51,52]. Further investigations are necessary to elucidate the role of AT_2 receptor in the genesis of the primary hypertension.

Clinical and experimental data confirm the critical role of the kidneys in essential hypertension. This mechanism seems to be associated with the establishment of essential hypertension and it could be caused, at least in part, by the high level of Ang II in the renal cortex. We observed a correlation between Na^+-ATPase activity and AT_1 receptor activation with the establishment of essential hypertension. Furthermore, our findings indicate that Na^+-ATPase is a key target during the development of hypertension. Our results open up new possibilities in our understanding of the pathophysiological mechanisms involved in the increased sodium reabsorption in PT found in essential hypertension.

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