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The role of AT1 receptor-mediated reproductive function in renovascular hypertension in male rats

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ABSTRACT

There is an association between hypertension and reproductive dysfunction. Angiotensin II (Ang II) is involved in the pathogenesis of hypertension and the regulation of reproduction. The present study aimed to determine whether the angiotensinergic system mediates the effects of hypertension on reproductive function in male rats subjected to a two-kidney, one-clip (2K1C) model. Sexual behavior parameters, gametogenesis and plasma concentrations of Ang II, testosterone, prolactin and corticosterone were evaluated in male rats 28 days after 2K1C or sham surgery and losartan (Los) treatment (a type 1 angiotensin II (AT1) receptor antagonist) or vehicle (V) treatment. The animals were divided into Sham + V, 2K1C + V, Sham + Los and 2K1C + Los groups. The 2K1C + V group showed a hypertensive response, inhibition of sexual behavior, spermatogenesis dysfunction, and increases in plasma Ang II and prolactin. Conversely, plasma testosterone decreased, and plasma corticosterone remained constant. Losartan treatment normalized blood pressure and prevented the changes in plasma testosterone and prolactin, sexual behavior and spermatogenesis in the 2K1C + Los group. In addition, losartan treatment caused an additional increase in circulating Ang ll in both groups (Sham + Los and 2K1C + Los). Together, these results suggest that Ang ll, acting through the AT1 receptor, modulates behavioral and endocrine parameters of reproductive function during renovascular hypertension. In addition, the effects of circulating Ang II on plasma testosterone and prolactin seem to contribute to the spermatogenic and sexual dysfunctions in hypertensive rats.

Introduction

Normal reproductive function requires the integration of several physiological mechanisms, involving the central and peripheral nervous systems, cardiovascular system, pituitary, and reproductive organs (Speth et al., 1999). Chronic high blood pressure can cause progressive damage to the arterial vessels, brain, heart, kidney, and sex organs. Hypertension is a risk factor for sexual dysfunction in males (Manolis and Doumas, 2008). However, the mechanisms underlying reproductive dysfunction associated with hypertension remain poorly understood.

Angiotensin II (Ang II) is a peptide that plays an important role in the pathogenesis of hypertension (Fitzsimons, 1980; Reid et al., 1978) and is also involved in the regulation of reproduction function (Ganong, 1995). Central Ang ll has an inhibitory role in the modulation of sexual behavior in male rats (Clark, 1988). Our laboratory reported that microinjection of Ang II into the medial amygdaloid nucleus decreases sexual behavior in male rats (Breigeiron et al., 2002). Peripherally, Ang II affects testicular function, modifying seminiferous tubule contraction (Rossi et al., 2002), and could be involved in the inhibition of spermatogenesis. Male rats subjected to the two-kidney, one-clip model (2K1C) exhibit reproductive hormone and sexual behavior changes and impaired spermatogenesis. The treatment of hypertensive rats with nifedipine, a Ca^{++} channel blocker, prevents the increases in blood pressure and plasma prolactin, as well as the decrease in sexual behavior. However, the impairment of spermatogenesis and reductions of plasma testosterone and FSH are not prevented by nifedipine (Breigeiron et al., 2007). Therefore, the mechanisms underlying reproductive dysfunction associated with renovascular hypertension remain to be clarified.

The effects of Ang II on cardiovascular and reproductive functions are mainly mediated through Ang II type 1 (AT1) receptor (Chan et al., 1999; Keaton and Clark, 1998). Therefore, specific AT1 antagonists are potentially useful for treating hypertension (Chiu et al., 1990; Tsunoda et al., 1993) and for improving sexual dysfunction in hypertensive patients (Della Chiesa et al., 2003). Ang II can modulate reproductive function by affecting the expression of hormonal regulators of reproduction or stress, such as gonadotropins, prolactin, and corticosterone (Donadio et al., 2004; Dornelles and Franci, 1998; Ganong, 1995; Rivier and Vale, 1983). However, effects of the interactions among Ang II and these hormones on reproduction need to be better investigated.

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In this study, we used an experimental model of elevated Ang II (2K1C) and the AT1 receptor antagonist losartan to analyze the role of the angiotensinergic system as a mediator of the effects of hypertension on reproductive function in male rats. Considering that Ang II is involved in hypertension and in the regulation of reproductive function, we hypothesize that Ang II, as mediated by AT1 receptor, can play an inhibitory role in sexual behavior and spermatogenesis.

Materials and methods

All procedures used in this study followed the guidelines for the care and use of Laboratory Animals from the National Institutes of Health and were approved by the Ethics Committee in Research of the Federal University of Rio Grande do Sul (Protocol # 13434).

Animals and general procedure

Three-month-old Wistar rats were obtained from the colony maintained in the Federal University of Rio Grande do Sul Laboratory Animal Facility. Animals were kept on a 12 h light/dark cycle (lights off at 6:00 PM) and in a stable environmental temperature (21 °C) with ad libitum access to rat chow (Nutrilab, Colombo, Brazil) and water. On the night before surgical procedures, male rats were selected for sexual behavior analysis, and only those with at least 6 intromission behaviors in a 10 min test were used. Male sexual behavior was recorded 28 days after sham or 2K1C surgery, at night before decapitation. The systolic blood pressure was recorded the morning following the sexual behavior test, 4–5 h before sacrifice. Immediately after decapitation, the blood was collected for hormone assays, and the testes were excised, weighed, and frozen for later spermatogenesis analysis.

Three-month-old female Wistar rats were used to record male sexual behavior. The females were gonadectomized and induced to sexual receptiveness by a sequential subcutaneous injection of 20 μg of estradiol (Benzo-ginoestril, Sanofi, São Paulo, Brazil) 48 h before testing and 20 μg of estradiol plus 500 μg of progesterone (4-Pregnene-3,20-dione, Sigma, St. Louis, MO, USA) 6 h before testing. All females were first tested with nonexperimental, sexually active males, and only females that were receptive (lordotic) were used.

Experimental groups

Male rats were divided into 4 groups: sham surgery with vehicle (water) (Sham + V; n = 11), 2K1C surgery with vehicle (water) (2K1C + V; n = 11), sham surgery with losartan treatment (Sham + Los; n = 11), and 2K1C surgery with losartan treatment (2K1C + Los; n = 11).

Surgical procedures

The 2K1C model was employed in male rats weighing 290 to 330 g (n = 22). These animals were anesthetized with i.p. administration of a ketamine–xylazine cocktail (100 mg/kg of ketamine and 50 mg/kg of xylazine; Syntec, São Paulo, Brazil) for the surgical procedures. The left renal artery was isolated, and a small segment was dissected free of the renal vein to place a silver clip (internal diameter 0.23 mm) around the main renal artery. As controls, sham-operated rats (n = 22) underwent the same surgical procedure without receiving the clip.

Losartan treatment

The dose of losartan was determined according to the findings of a previous study (Nobre et al., 2006). Losartan (DUP 753, Du Pont, DE, USA) was dissolved in the drinking water of the 2K1C and sham rats for 21 days before decapitation. Seven days after surgery, when animals were recovered from surgery, systolic blood pressure was recorded in 2K1C and sham rats. All normotensive sham rats received losartan treatment, but in the 2K1C group, only hypertensive animals received this treatment. The losartan dose (10 mg/kg/day) was adjusted according to each rat's water intake and body weight previously established in our laboratory. Solutions with losartan were changed every 24 h to measure the volume (in mL) that was drunk per day by each rat.

Arterial pressure recording

Systolic blood pressure (SBP) was indirectly measured (tail-cuff method) with a pressure transducer connected to a Kent Scientific system (RTBP 1001 Rat Tail Blood Pressure System; Litchfield, USA). Recordings were quantified using WinDaq Data Acquisition Software (DATAQ Instruments Inc., Akron, Ohio, USA). Prior to recording, the animals were habituated to the cardiovascular laboratory for 1 h. In the morning 4–5 h before sacrifice, the animal was kept in an acrylic tube with the tail free for placement of the sphygmomanometer and blood pressure transducer for arterial pressure recording. Arterial pressure was recorded for 5 min, and we obtained 5–6 SBP measurements from each animal as references for analysis. Only 2K1C rats with mean SBP of more than 150 mm Hg were included in this study.

Reproductive function

Male sexual behavior

Adult males had their sexual behaviors recorded 1–2 h after the beginning of the dark phase. Each male rat was habituated to the observation cage for 15 min before behavioral recording. After this period, a sexually receptive female was introduced into the observation cage, and the behavior was videotaped. The following parameters of the male's behavior were recorded: latency to the first intromission: time (seconds) from the introduction of the female in the cage up to the first intromission; intromission frequency: the number of intromissions; and the ejaculation latency: the time (seconds) from the first intromission of a series up to the first ejaculation (Agmo, 1997). The animals were videotaped until ejaculation or a maximum of 30 min if no ejaculation was observed, and then the test was finished. The intromission latency and intromission frequency were analyzed in the first 10 min of the 30 min recording period, because in most non-hypertensive rats the recording session corresponded to 10 min. When the frequency of intromissions was zero, the intromission latency was considered to be 600 s. In rats that did not show intromission, the ejaculation latency was considered to be 1800 s. The number of animals showing intromissions and ejaculation was recorded (Breigeiron et al., 2002, 2007).

Spermatogenesis

All rats had their right testis analyzed for measurement of the spermatid quotient (SQ) (number of spermatids $\times 10^6$) and epididymal transit (ET) (transit time/days for the maturation of the sperm in the epididymis). The testes were weighed, homogenized and sonicated in 8 mL of a T-X solution (NaCl .9% and Triton X-100 .05%). Aliquots of 100 mL of the homogenate were diluted in 900 mL of the T-X solution. Homogenization-resistant testicular spermatids in the testes and sperm in the caput/corpus epididymides and cauda epididymides were enumerated in Neubauer chambers. SQ was calculated by dividing the total number of homogenization-resistant spermatids in testis by the testicular parenchyma weight. Daily sperm production per testis was determined by dividing the total number of homogenization-resistant spermatids per testis by 6.1 days (the number of days of a seminiferous cycle in which these spermatids are present) (Robb et al., 1978). Transit times through the caput/corpus of epididymis and cauda of epididymis were calculated by dividing the number of sperm within each of these regions by the daily sperm production (Breigeiron et al., 2007).

Plasma hormones

Enzyme-linked immunosorbent assay (ELISA)

Plasma concentration of Ang II (ng/mL) was measured by ligand ELISA kits. We collected the animal's trunk blood in Eppendorf tubes containing a cocktail of protease inhibitors phenylmethanesulfonyl fluoride and pepstatin (Sigma, USA). Blood samples were centrifuged at 3000 rpm for 20 min at 4 °C. Each plasma sample was subsequently transferred into a test tube and stored at −80 °C. Then, plasma Ang II was measured using a commercial Angiotensin II kit — EIA (Peninsula Laboratories Inc., San Carlos, USA) according manufacturer's protocol. The lower detection limit was 0.02–0.04 ng/mL.

Radioimmunoassay (RIA)

Plasma concentrations of prolactin, testosterone and corticosterone (ng/mL) were measured by RIA. Trunk blood was collected and centrifuged at 3000 rpm for 20 min at 4 °C. Each plasma sample was transferred into a test tube and stored at −80 °C for hormone assays. Plasma prolactin was measured using specific kits provided by the National Institute of Diabetes and Digestive and Kidney Diseases (Baltimore, MD, USA). The lower detection limit was 0.09 ng/mL. Plasma samples for testosterone concentration underwent an extraction process with distilled ethylic ether. The testosterone concentration was performed using a tritiated hormone (1,2,6,7-^{3}H testosterone) acquired from New England Nuclear (USA) and a testosterone standard and specific antibody acquired from Sigma (USA). The separation of the free and bound fractions was performed by incubation with dextran-coal. The detectable minimum doses were 0.03 ng/mL. Plasma samples for corticosterone concentration underwent an extraction process with distilled ethylic ether. Plasma concentration of corticosterone was measured using a corticosterone standard and specific antibody acquired from Sigma (USA) and tritiated corticosterone from Amersham (USA). The detectable minimum doses were 0.2 ng/mL. For each hormone, all samples were run in duplicate in one assay.

Statistical analysis

Data are expressed as mean ± SEM, except for the latency data and frequency data (sexual behavior) for which the median (interquartile interval) was used. Sexual behavior data were analyzed by the nonparametric Kruskal–Wallis test for comparison between the main effects (renal artery clipping and losartan treatment) followed by the Dunn's multiple range test for the comparison of the groups. The percentage of animals that ejaculated was analyzed using the chi-square test and data are expressed as percentage of rats in each group. All other data were analyzed using two-way ANOVA for the comparison of means and to determine whether there were effects of renal artery clipping and losartan treatment or interaction between treatments, followed by Bonferroni's post hoc test pairwise comparison of groups. In all cases, the level of significance was set at $P<0.05$.

Results

Systolic blood pressure

Fig. 1 shows a significant increase in mean SBP in the 2K1C + V group. Two-way ANOVA indicated that the clipping effect ($F(1.43)=30.52$), losartan effect ($F(1.43)=51.81$), and interaction ($F(1.43)=35.01$) were significant ($P<0.0001$). The 2K1C + V group showed a significant increase in mean SBP compared to the other groups (Bonferroni test: $P<0.001$) and losartan treatment prevented the increase in mean SBP.

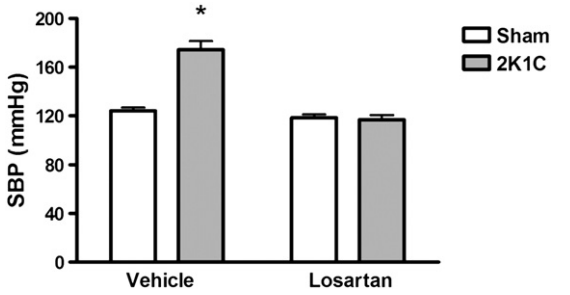

Fig. 1. Systolic blood pressure (SBP; mm Hg) recordings 28 days after sham or 2K1C surgery under vehicle (V) or losartan (Los) treatment. The rats were divided into 4 groups: Sham + V (n = 11), 2K1C + V (n = 11), Sham + Los (n = 11), and 2K1C + Los (n = 11). Values are means ± SEM. There were significant effects of clipping, losartan treatment and the interaction between clipping and losartan treatment (ANOVA: $P<0.0001$). * Significant difference between 2K1C + V and others groups (Bonferroni test: $P<0.001$).

Plasma hormones

Fig. 2 shows plasma Ang II, testosterone, prolactin and corticosterone concentrations. Two-way ANOVA indicated that there was a significant increase in plasma Ang II by clipping effect: ($F(1.43)=7.08$; $P<0.05$) and losartan effect ($F(1.43)=6.02$; $P<0.05$), but there was no interaction. Thus, clipping increased plasma Ang II in 2K1C groups and losartan treatment promoted an additional increase in Ang II levels. By two-way ANOVA, there was a significant effect of clipping ($F(1.38)=4.39$; $P<0.05$) and losartan treatment ($F(1.38)=10.07$; $P<0.05$) on plasma concentration of testosterone, indicating that the 2K1C + V group presented lower plasma testosterone and losartan treatment prevented this change. Plasma prolactin increased in the 2K1C + V group. Two-way ANOVA indicated that there was a significant effect of clipping ($F(1.43)=6.54$; $P<0.05$), but there was no effect of losartan treatment and no interaction between the two treatments. There was a significant difference between the vehicle groups, with the 2K1C + V group showing a significantly higher prolactin compared to the Sham + V group (Bonferroi test: $P<0.05$). Plasma corticosterone showed no significant changes in any group.

Reproductive function

Male sexual behavior

Fig. 3 shows the effects of renovascular hypertension on the sexual behavior of male rats 28 days after 2K1C or sham surgery. Kruskal–Wallis indicated a significant difference in the medians for the intromission frequency ($P=0.0001$), intromission latency ($P=0.0006$) and ejaculation latency ($P=0.0007$). Hypertensive rats (2K1C + V) presented a reduced intromission frequency compared to the other groups (Dunn's multiple comparison test: $P<0.05$). Conversely, the intromission latency increased in the 2K1C + V group when compared to the Sham + V animals (Dunn's multiple comparison test: $P<0.001$). Ejaculation latency was increased in hypertensive rats compared to the other groups (Dunn's multiple comparison test: $P<0.05$). In the 2K1C + V group, only 64% of the animals ejaculated. Losartan treatment prevented all changes in sexual behavior.

Spermatogenesis

Fig. 4 shows the spermatid quotient and epididymal transit in each group. Two-way ANOVA indicated there was a significant effect of clipping ($F(1.39)=17.56$; $P<0.0002$) for spermatid quotient analysis, but no effect of losartan and no interaction. The spermatid quotient in the 2K1C + V group was reduced compared to others (Bonferroni test: $P<0.05$), while groups treated with losartan did not show any

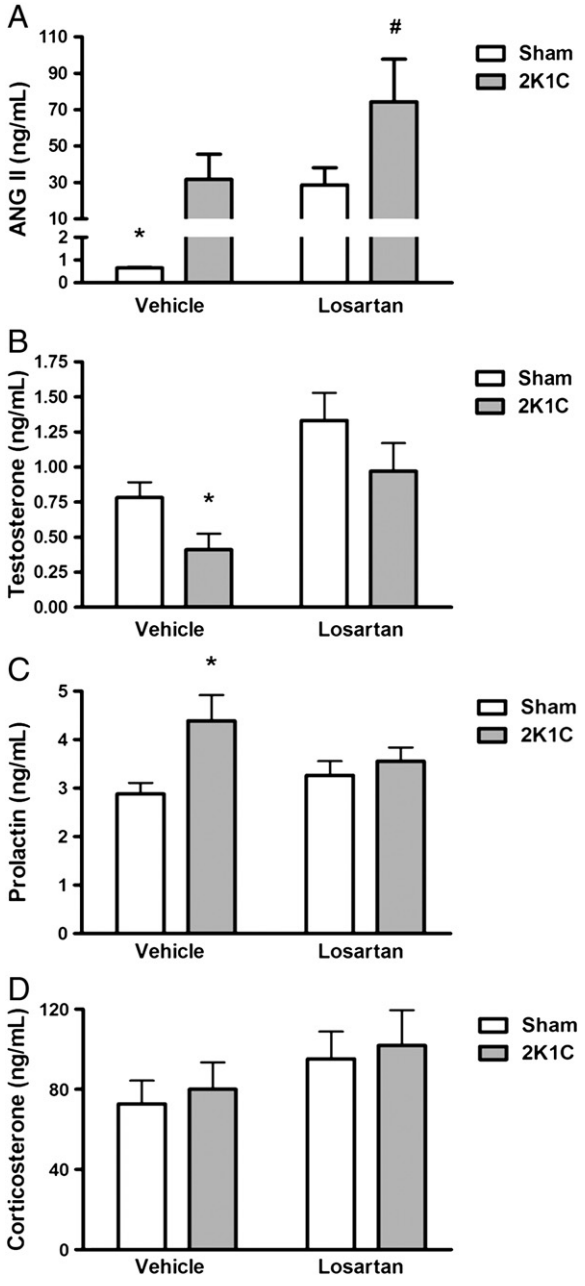

Fig. 2. Plasma concentrations of angiotensin II (Ang II), testosterone, prolactin, and corticosterone in male rats 28 days after sham or 2K1C surgery under vehicle or losartan treatment. The rats were divided into 4 groups: Sham + V (n = 11), 2K1C + V (n = 11), Sham + Los (n = 11), and 2K1C + Los (n = 11). Values are means ± SEM. A) Ang II: there were significant effects of clipping and losartan treatment but no interaction between clipping and losartan treatment (ANOVA: $P<0.05$). * Significant difference between Sham + V and others groups. # Significant difference between 2K1C + Los and others groups (Bonferroni test: $P<0.05$); B) testosterone: there was a significant effect of clipping and losartan treatment but no interaction between clipping and losartan treatment (ANOVA: $P<0.05$). * Significant difference between 2K1C + V and others group (Bonferroni test: $P<0.05$); C) prolactin: there was a significant effect of clipping (ANOVA: $P<0.05$) but no effect of losartan treatment and no interaction between clipping and losartan treatment. * Significant difference between 2K1C + V and Sham + V (Bonferroni test: $P<0.05$); D) corticosterone: there were no significant effects.

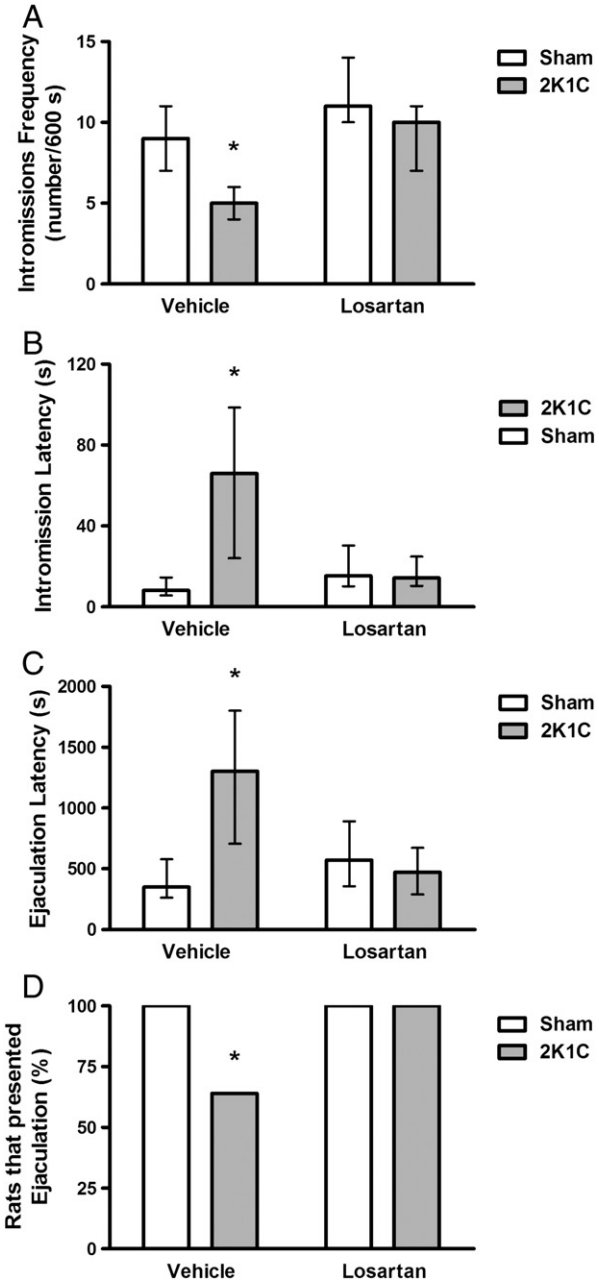

Fig. 3. Sexual behavior of male rats 28 days after sham or 2K1C surgery under vehicle or losartan treatment. The rats were divided into 4 groups: Sham + V (n = 11), 2K1C + V (n = 11), Sham + Los (n = 11), and 2K1C + Los (n = 11). Values are medians + interquartile intervals for frequency and latency of data. The intromission recording session was 600 s, and the ejaculation recording session was 1800 s. Kruskal–Wallis test indicated a significant difference in intomission frequency ($P=0.0001$), intromission latency ($P=0.0006$) and ejaculation latency ($P=0.0007$). A) Intromission frequency was significantly reduced in 2K1C + V compared to the other groups. * Significant difference (Dunn's multiple comparison test: $P<0.05$); B) intromission latency was significantly increased in 2K1C + V compared to Sham + V. * Significant difference (Dunn's multiple comparison test: $P<0.001$); C) ejaculation latency was significantly increased in 2K1C + V compared to the other groups. * Significant difference (Dunn's multiple comparison test: $P<0.05$); D) the percentage of 2K1C + V animals that ejaculated was significantly diminished compared to the other groups * Significant difference (chi-square test: $P=0.036$).

differences. By two-way ANOVA there were significant effects of clipping ($F(1.39)=16.35$; $P<0.0001$), losartan treatment ($F(1.39)=23.31$; $P<0.0003$) and the interaction between clipping and losartan treatment ($F(1.39)=22.45$; $P<0.0001$) in epididymal transit analysis. The epididymal transit increased in 2K1C + V group compared to the others (Bonferroni test: $P<0.001$).

Discussion

This study shows that renovascular hypertension reduces sexual behavior in male rats and impairs spermatogenesis by actions of Ang II at the AT1 receptor. The results confirm that the 2K1C model induces hypertension, due to higher plasma Ang II levels, and indicate an increase in plasma prolactin in contrast to the decrease in plasma

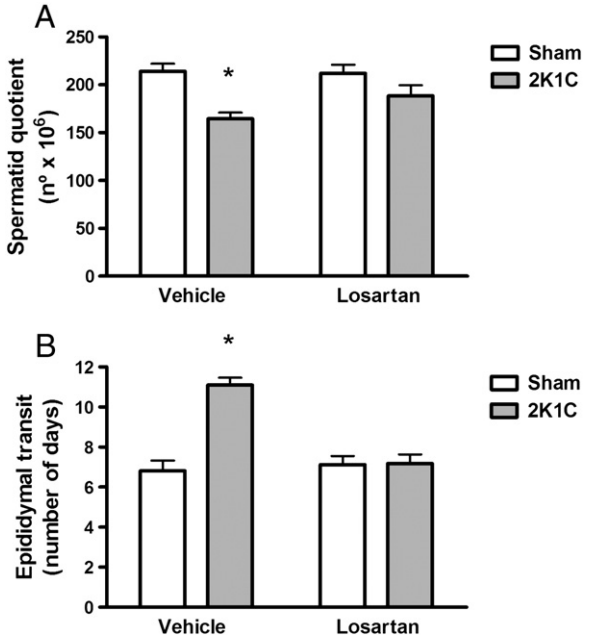

Fig. 4. Spermatid quotient and epididymal transit of male rats 28 days after sham or 2K1C surgery under vehicle or losartan treatment. The rats were divided into 4 groups: Sham + V (n = 10), 2K1C + V (n = 10), Sham + Los (n = 10), and 2K1C + Los (n = 10). Values are means ± SEM. A) There was a significant effect of clipping (ANOVA: P = 0.0002), but no effect of treatment and no interaction in the spermatid quotient analysis. * Significant difference in 2K1C + V compared to the other groups (Bonferroni test: P < 0.05); B) there were significant effects of clipping (ANOVA: P < 0.0001), losartan treatment (ANOVA: P < 0.0003) and the interaction between clipping and losartan treatment on epididymal transit (ANOVA: P < 0.0001). * Significant difference in 2K1C + V compared to the other groups (Bonferroni test: P < 0.001).

testosterone in hypertensive rats, with no change in plasma corticosterone being observed. The effects of 2K1C on blood pressure, sexual behavior, spermatogenesis, plasma testosterone and prolactin were prevented by the systemic treatment with AT1 antagonist. These results suggest that Ang II, acting through AT1 receptor, modulates the reproductive dysfunction associated with renovascular hypertension and that treatment with AT1 antagonists improves sexual behavior and spermatogenesis in hypertensive male rats.

In the 2K1C model, the hypertension is induced by unilateral renal artery stenosis. The reduced renal perfusion pressure stimulates increased renin synthesis, which increases Ang II production. Circulating Ang II increases total peripheral resistance and raises blood pressure (Pickering, 1989). Additionally, Ang II is present centrally in neural sites that act on blood pressure control, such as the nucleus of the solitary tract (Plunkett and Saavedra, 1985), subfornical organ (Hendel and Collister, 2005), rostral ventrolateral medulla and paraventricular nucleus of the hypothalamus (Campos and Bergamaschi, 2006; Campos et al., 2011). Peripheral Ang II can traverse the circumventricular organs and signal to central areas, contributing to the hypertensive response (Campos, 2009; Maliszewska-Scislo et al., 2008). The AT1 antagonist treatment confirmed that renal artery clipping requires this receptor to produce hypertension, as AT1 antagonist prevented hypertension in the 2K1C + Los group. Furthermore, losartan administration induced compensatory increases in circulating Ang ll in both groups (Sham + Los and 2K1C + Los). These data are in accord with clinical studies in normotensive and hypertensive patients, indicating the increased availability of circulating Ang II, which cannot bind to blocked AT1 receptor (Christen et al., 1991; Mazzolai et al., 1999). In addition, the AT1 receptor antagonism disrupts the negative feedback of Ang II on renin-secreting granular cells, leading to increases in reactive plasma renin activity and the concentration of circulating Ang II.

Our results show that renovascular hypertension in male rats inhibits sexual behavior and spermatogenesis, increases plasma prolactin and reduces plasma testosterone in agreement with previous data from our laboratory using a Ca^{++} channel blocker (Breigeiron et al., 2007). However, in that previous study, treatment with nifedipine prevented only the reduction of sexual behavior and the increase of plasma prolactin but did not alter the reduction of plasma testosterone or spermatogenesis of 2K1C rats. The present study shows that AT1 receptor modulates both behavioral and endocrine parameters of reproductive function during renovascular hypertension. Comparing the effects of nifedipine and losartan treatment on reproduction of hypertensive male rats, we propose that the spermatogenesis dysfunction was caused by the increase of Ang II, induced by renal artery clipping, associated with the reduction of plasma testosterone. This effect might be attributable to activation of AT1 receptors, since losartan treatment prevented changes in spermatogenesis and testosterone reduction. Probably, impairment on sexual behavior and prolactin increase are mainly associated with the effects of hypertension, since both drugs reduced blood pressure and prevented the decrease of sexual behavior and increased prolactin in 2K1C rats.

In 2K1C rats, it is possible that increased Ang II acts on Leydig cells to inhibit the stimulation of testosterone production by LH. The stimulation of Ang II receptors in Leydig cells inhibits the ability of LH to stimulate testosterone production through inhibition of the cAMP pathway (Khanum and Dufau, 1988). This effect seems to be mediated by AT1 receptor because losartan treatment in 2K1C rats prevented the plasma testosterone reduction. Testosterone is essential for the initiation and maintenance of spermatogenesis because somatic Sertoli cells, Leydig cells and peritubular cells have active androgen receptors (Welsh et al., 2009). The reduction of plasma testosterone can damage the nutrition and development of spermatic cells. In fact, hypertensive rats presented a reduced spermatid quotient, which can lead to a deficit in daily sperm production. The increased epididymal transit suggests impairment in maturation and storage capacity of epididymal sperm. Additionally, the presence of functional Ang II receptors in rat testis and epididymis supports its involvement in gonad function (Grove and Speth, 1989; Khanum and Dufau, 1988). Seminiferous tubule contraction is important for spermatogenesis regulation and testicular sperm output. In vitro studies have shown that Ang II, via the AT1 receptor, increases intracellular calcium, cell growth and contraction in rat peritubular myoid cells (Rossi et al., 2002). Losartan treatment prevented the reduction in the spermatid quotient and the increase in epididymal transit caused by 2K1C, confirming the participation of Ang II and AT1 receptor in the regulation of spermatogenesis. We suggest that the combined effects of increased Ang II and reduced testosterone in our 2K1C model contribute to impaired spermatogenesis.

In addition, prolactin seems to be involved in spermatogenesis regulation. Quantitative RT-PCR and in situ hybridization have detected the expression of prolactin in Leydig cells and in spermatogonia, thereby suggesting that prolactin participates in mouse spermiogenesis and spermatogenesis (Ishida et al., 2010). Therefore, hyperprolactinemia alters the mechanism of chromatin condensation during rat spermiogenesis, causing a decrease in spermatozoid quality and inducing infertility (Gill-Sharma, 2009). Prolactin can control testicular function in other ways: acting directly on the pituitary, to inhibit LH and FSH synthesis (Bartke et al., 1977), or on the testes, to regulate testosterone synthesis induced by LH (Zipf et al., 1978). Therefore, we propose that increased plasma prolactin can contribute to delaying spermatid maturation in hypertensive rats. However, prolactin does not appear to be crucial in spermatogenesis, as our previous study showed that nifedipine administration normalizes plasma prolactin but does not alter spermatogenesis impairment or plasma testosterone reduction. In addition, it is important to consider the participation of vascular damage in the decrease of spermatogenesis in hypertensive rats. Studies in spontaneously hypertensive rats suggest that the decline

in Sertoli cell functions is a result of hypertensive vascular changes involving the spermatid number reduction (Itoh et al., 1995).

The plasma prolactin increase in the 2K1C + V group was probably due to the predominantly stimulatory effect of Ang II on the pituitary, involving the AT1 receptor, because the 2K1C + Los group showed no significant change in plasma prolactin. There is evidence that Ang II acts directly on the lactotropes of the anterior pituitary, stimulating prolactin release, an effect mediated by the AT1 receptor (Aguilera et al., 1982; Steele and Myers, 1990). The combined increases in plasma Ang II and prolactin could explain the great impairment of sexual behavior in 2K1C animals because clinical and experimental data indicate that prolactin is involved in the regulation of reproduction function (Drago, 1984; Drago and Lissandrello, 2000; Gill-Sharma, 2009). Prolactin receptor expression in the medial preoptic area (MPOA) and arcuate nucleus confirm the hypothesis that prolactin participates in reproductive function (Kokay et al., 2011). Chronic hyperprolactinemia is related to sexual dysfunction in humans and rats (Corona et al., 2007; Cruz-Casallas et al., 1999), whereas the prolonged stimulation of central dopamine transmission can lead to hyposensitivity or downregulation of dopamine receptor systems (Mohankumar et al., 1997), causing sexual dysfunction.

We observed that hypertensive rats presented a sexual behavior inhibition, as represented by a reduction of the intromission frequency and greater intromission and ejaculation latencies. In fact, hypertensive animals showed a decrease in the ejaculation behavior. Intromission and ejaculation abilities are indicators of erectile function in rats (Chan et al., 1999; Keaton and Clark, 1998). Losartan treatment prevented all changes in sexual behavior. These data indicate an association between plasma Ang ll increases and the impairment of erectile function in hypertensive rats, as mediated through AT1 receptor. The renin–angiotensin system affects reproductive function by acting at central and peripheral levels. Actions of Ang II in the central nervous system can be exerted by locally produced Ang II, as well as by peripheral Ang II, through the circumventricular organs (Mangiapane and Simpson, 1980; Vieira et al., 2010). The presence of Ang II and its receptors in brain areas that exert control over reproductive function, such as MPOA and the medial amygdaloid nucleus (MeA) (Von Bohlen und Halbach and Albrecht, 1998a, 1998b), support the participation of Ang II in sexual behavior. Along these lines, Ang II inhibits sexual behavior when injected into the brain (Clark, 1988), which is an effect that involves AT1 and AT2 receptors (Breigeiron et al., 2002). It is important to consider that the pathogenesis of sexual dysfunction associated with hypertension may be multifactorial. Impaired penile arterial function has been observed in impotent patients with hypertension (Kochar et al., 1999; Müller et al., 1991). Ang II is physiologically involved in the initiation of penile detumescence in men (Becker et al., 2001), and therapies using AT1 receptor antagonists have been associated with the ability to maintain adequate erections (Della Chiesa et al., 2003). In contrast, some classes of antihypertensive drugs such as diuretics or β-blockers can interfere with sexual function, causing erectile dysfunction, decrease libido and impaired ejaculation in patients (Ferrario and Levy, 2002).

Our data show no changes in plasma corticosterone in hypertensive rats, despite increases of plasma Ang ll. This result is in agreement with findings in which systemic treatment of rats with exogenous angiotensin does not change the basal level of corticosterone (Müller et al., 2007). The results of our study suggest the activation of compensatory mechanisms in response to the higher plasma Ang II, thereby preventing increases in plasma corticosterone in 2K1C animals. We hypothesized that Ang II would stimulate the paraventricular nucleus of the hypothalamus to synthesize corticotropin-releasing factor, but an increase in corticosterone would be prevented by feedback inhibition in the hypothalamo-pituitary-adrenal (HPA) axis, which has receptors for Ang II (Von Bohlen und Halbach and Albrecht, 1998a, 1998b). In this case, areas such as the hippocampus would inhibit the HPA axis and consequently prevent the increase in corticosterone. This knowledge is consistent with recent study that shows, the action of the renin–angiotensin system on the hippocampus of hypertensive rats mediating cognitive responses (Pelisch et al., 2011).

We conclude that renovascular hypertension inhibits reproductive function in male rats, and this effect is mediated in part by Ang II acting through AT1 receptor. Moreover, the effects of the increased circulating Ang II on plasma prolactin and testosterone seem to contribute to the sexual and spermatogenic dysfunctions in hypertensive rats. The major finding in this study was the evidence that the AT1 receptor promotes a plasma testosterone reduction and impairs spermatogenesis in renovascular hypertensive rats.

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