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Research Article

Enhanced angiotensin II induced sodium appetite in renovascular hypertensive rats

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ABSTRACT

Renovascular hypertensive 2-kidney, 1-clip (2K1C) rats have an increased activity of the renin-angiotensin system and an initial transitory increase in daily water and NaCl intake. However, the dipsogenic and natriorexigenic responses to angiotensin II (ANG II) have not been tested yet in 2K1C rats. Therefore, in the present study, we evaluated water and 0.3 M NaCl intake induced by water deprivation (WD)-partial rehydration (PR) or intracerebroventricular (icv) ANG II in 2K1C rats. In addition, the cardiovascular changes to these treatments were also evaluated. Male Holtzman rats received a silver clip around the left renal artery to induce 2K1C renovascular hypertension. In the 5th week, a group of animals received a guide cannula in the lateral ventricle for icv injections. Daily water intake increased from the 3rd week after surgery and remained elevated until the 6th week (last recording week), whereas daily 0.3 M NaCl intake transiently increased from the 2nd to the 5th week after surgery. On the 6th week, in spite of comparable daily 0.3 M NaCl intake between 2K1C and sham rats, WD-PR and icv ANG II induced an increased 0.3 M NaCl intake in 2K1C rats. Water intake induced by WD-PR, not by icv ANG II, also increased in 2K1C rats. The increase in arterial pressure to WD-PR or icv ANG II was similar in sham and 2K1C rats. Therefore, these results suggest that 2K1C rats are more responsive to the natriorexigenic effects of ANG II, whereas other responses to ANG II are not modified.

1. Introduction

The renin-angiotensin system (RAS) has a major role in cardiovascular regulation and fluid-electrolyte balance [12,32]. Angiotensin II (ANG II), the main peptide produced by activation of the RAS, acts in brain areas lacking blood brain barriers and rich in ANG II receptors affecting several physiological functions, including arterial pressure regulation, vasopressin secretion and the induction of thirst and salt appetite [18,25,36,45]. For instance, it is well described that intracerebroventricular (icv) injection of ANG II induces water and NaCl intake [7,8,12,36] and that ANG II acting in the brain is also involved on water deprivation-induced water and NaCl intake [2,21,39]. In normotensive rats, water intake and the expression of angiotensinergic AT_1 receptors in forebrain areas related to fluid electrolyte balance, such as the median preoptic nucleus and subfornical organ (SFO) are increased in rats submitted to multiple injections of ANG II [27]. Repeated injections or continuous infusion of icv ANG II may also sensitize sodium intake in normovolemic/normohydrated rats [4]. Taken together, these studies suggest that ANG II-induced thirst or sodium appetite can be sensitized.

Increases in RAS is an important mechanism involved in the development/maintenance of the 2-kidney 1-clip (2K1C) renovascular model of hypertension [10,22,31]. ANG II injected icv produces a more pronounced effect in mean arterial pressure (MAP) in anesthetized, sinoaortic denervated and cervical vagotomized 2K1C rats [47] compared to sham normotensive rats. Similarly, icv ANG II produced an increased sympathoexcitation and pressor response in spontaneously hypertensive rats (SHR) compared to normotensive rats, although ANG II-induced water intake and vasopressin release were not different from normotensive rats [13]. Rats previously exposed to a low dose (subpressor) of ANG II have a greater increase in MAP in a subsequent ANG II treatment, suggesting that ANG II-induced pressor responses can also be sensitized [46].

Although 2K1C rats display an increased daily sodium intake and

Abbreviations: 2K1C, 2-kidney 1-clip; ACE, angiotensin converting enzyme; ANG II, angiotensin II; HR, heart rate; icv, intracerebroventricular; LK/RK, left kidney/right kidney weight ratio; LV, lateral ventricle; MAP, mean arterial pressure; P_{osm}, plasma osmolality; PRA, plasma renin activity; RAS, renin-angiotensin system; SFO, subfornical organ; SHR, spontaneous hypertensive rats; S_{Na}, serum sodium concentration; TP, total serum protein; WD-PR, water deprivation–partial rehydration; WR, water replete

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RAS overactive [29,40], it is not known if these animals are more sensitive to natriorexigenic protocols, such as central ANG II and water deprivation. Another response to be confirmed is if the pressor response to central ANG II in unanesthetized 2K1C rats is similar to that described for anesthetized, sinoaortic denervated and cervical vagotomized 2K1C rats [47]. Therefore, in the present study, we investigated the ingestion of 0.3 M NaCl and water in 2K1C hypertensive rats submitted to water deprivation or to icv injection of ANG II. In addition, considering that cardiovascular changes can either increase or decrease the ingestive behaviors [11,42], the effect of icv ANG II or water deprivation on the arterial pressure in 2K1C rats was also investigated.

2. Material and methods

2.1. Animals

Male Holtzman rats (initial weight of 150–180 g) were used. Animals were housed in individual stainless steel cages with free access to normal sodium diet (BioBase Rat Chow, Águas Frias, Brazil), water and 0.3 M NaCl. Room temperature was maintained at 23 ± 2 °C, humidity at 55 ± 10% and on a 12:12 light-dark cycle. Experimental protocols were approved by the Ethical Committee in Animal Use (CEUA) of the School of Dentistry – UNESP (Proc. CEUA # 06/2015).

2.2. Induction of 2K1C renovascular hypertension

Rats were anesthetized with intraperitoneal injection of ketamine [União Química Farmacêutica Nacional, Embu-Guaçu SP, Brazil, 80 mg/kg body weight (wt.)] combined with xylazine (União Química Farmacêutica Nacional, Embu-Guaçu SP, Brazil, 7 mg/kg body wt.) and the left renal artery was partially occluded by a silver clip with an opening of 0.2 mm. Another group of animals was submitted to the same surgical procedure without artery occlusion (sham surgery) and was referred as sham rats. At the end of surgery, animals received an intramuscular injection of antibiotic (benzylpenicillin – 80,000 IUs plus streptomycin – 33 mg; Pentabiótico Veterinário – Pequeno Porte, Fort Dodge Saúde Animal Ltda., Campinas, Brazil) and a sc injection of analgesic/anti-inflammatory (ketoprofen 1% – 0.03 ml/rat; Ketoflex, Mundo Animal, São Paulo, Brazil).

2.3. Brain surgery

Rats were anesthetized as described above, placed in a stereotaxic apparatus (Kopf, Tujunga, CA, USA) and the skull was leveled between bregma and lambda. A stainless steel 23-gauge cannula (12 × 0.6 mm) was implanted in direction to the lateral ventricle (LV) using the following coordinates: 0.1 mm rostral to bregma, 1.4 mm lateral to bregma and 3.3 mm below surface of the skull. The cannula was fixed to the cranium using dental acrylic resin and jeweler screws. Post-surgical treatment was administered as described above and the rats were allowed to recover for one week before starting the experiments.

2.4. Arterial pressure and heart rate recordings

The day before the arterial pressure recordings, animals were anesthetized as described above and a polyethylene tubing (PE-10 connected to a PE-50, Clay Adams, Parsippany, USA) was inserted into the abdominal aorta through the femoral artery, tunneled subcutaneously and exposed on the back of the rat to allow access in conscious freely moving rats. To record pulsatile arterial pressure, MAP and heart rate (HR), the arterial catheter was connected to a Statham Gould (P23 Db) pressure transducer (Statham Gould, Cleveland, USA) coupled to a preamplifier (model ETH-200 Bridge Bio Amplifier, CBSciences Inc., Dover, USA) that was connected to a Powerlab computer data acquisition system (model Powerlab 16SP, ADInstruments, Castle Hill, Australia).

2.5. Drugs

ANG II (acetate salt, human, synthetic) purchased from Sigma Chemical Co (St. Louis, MO, USA) was used at the dose of 25 ng/1 µl, dissolved in sterile saline (0.15 M NaCl). Injections were made using 10 µl-Hamilton syringe connected by PE-10 polyethylene tubing to a needle that was introduced into the brain through the guide cannula. Needles for injection into the LV were 2 mm longer than the guide cannula and volume injection was 1.0 µl.

2.6. Water and 0.3 M NaCl intake

Daily water and 0.3 M NaCl intake of each animal was recorded using 100 ml capacity-polypropylene bottles with 1 ml-divisions. For salt appetite and thirst tests, the polypropylene bottles were removed from the cage and burettes with 0.1 ml divisions that were fitted with metal drinking spouts were provided.

2.7. Urine and blood collection and analysis

Urine samples were collected in 0.1-ml graduated polypropylene tubes and urinary volume was measured. Urinary sodium concentration was analyzed using an electrolyte analyzer (Nova Biomedical, Waltham, USA). Sodium urinary excretion was calculated as the product of urine volume times the urinary concentration of sodium. Sodium balance was calculated as the difference between the amount ingested and the amount excreted.

Trunk blood was collected after decapitation for determination of serum sodium concentration, total serum protein, plasma osmolality, and plasma renin activity (PRA). Samples were collected in pre-refrigerated tubes at 4 °C containing a separating gel or sodium EDTA (2 mg/ml of blood) to obtain serum and plasma, respectively. All samples were centrifuged for 20 min at 3000 revolutions/min. Serum sodium concentration was measured by ion-specific electrode (Nova Biomedical, Waltham, USA). Total serum protein concentration was measured in a refractometer (Atago, Tokyo, Japan). Plasma osmolality was determined by freezing point depression (Advanced Instruments Inc., Norwood, USA). PRA was determined by radioimmunoassay utilizing rabbit antibody for angiotensin I from Maia Biodata Kit (BioChem Immunosystems, Montreal, Canada).

2.8. Experimental protocols

All experiments were done 6–7 weeks after 2K1C or sham surgeries, a phase that hypertension reached the plateau [1,3]. The intakes of 0.3 M NaCl and water were taken daily starting immediately after renal surgery and averaged each week. The urine excretion was taken at the end of each week. For this, once a week the animals were transferred to a metabolic cage for urine collection to determine urinary excretion as described above. For water and 0.3 M NaCl intake tests (described below), each group of rats was submitted to 2 different intake tests with an interval of at least 3 days between them. In each test, the group of rats was divided into two and half of the group received one treatment and the other half received another treatment described below. The sequence of treatments in different tests was randomized. Animals did not have access to food during the water and 0.3 M NaCl intake tests.

2.8.1. Tests in water deprived rats

Since water deprivation induces thirst and salt appetite [44], it is difficult to discriminate between these two responses when animals have access to both water and NaCl at the same time. We used the water deprivation (WD)-partial rehydration (PR) protocol (WD-PR) [39], where water-deprived rats are allowed to drink only water until satiety (PR) and then animals have access to a hypertonic NaCl solution for a salt appetite test. Thus, a group of 2K1C and sham rats were deprived of water and 0.3 M NaCl for 24 h with only regular food available (water

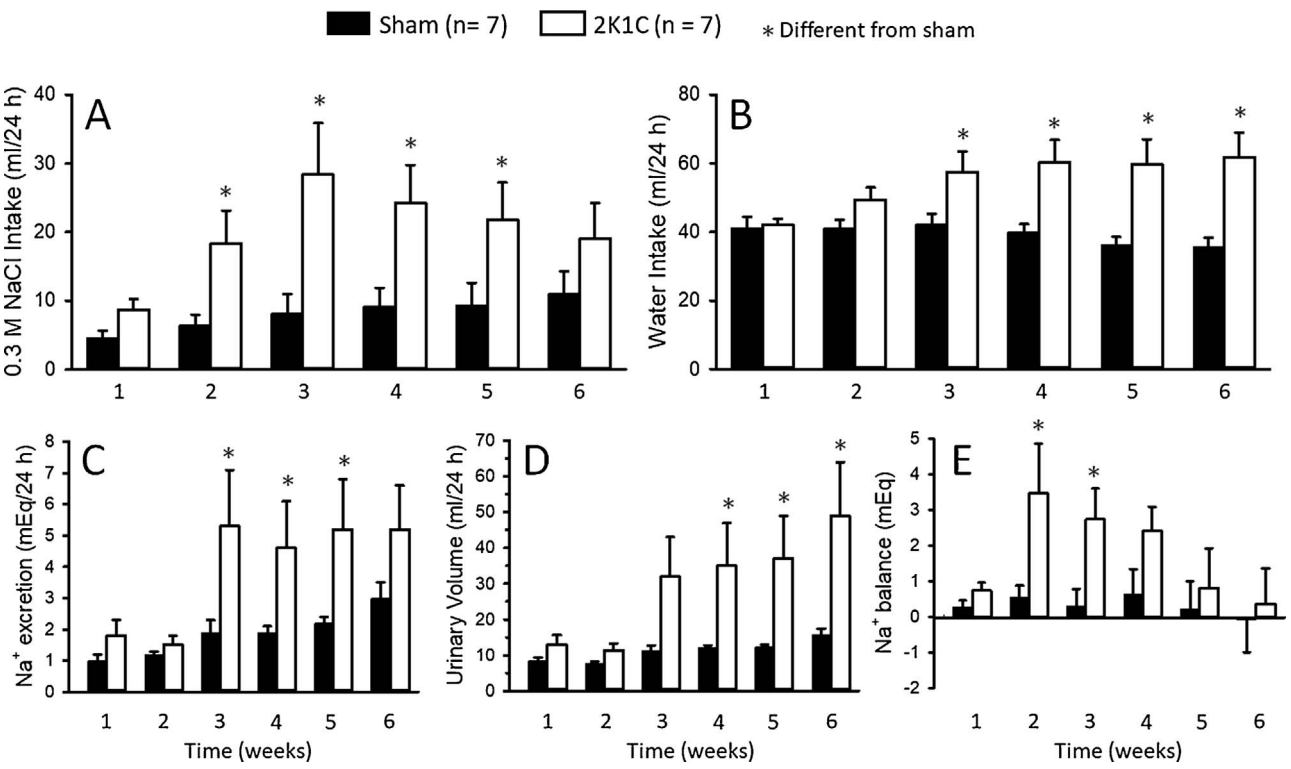

Fig. 1. Average daily (A) 0.3 M NaCl intake, (B) water intake, (C) sodium excretion, (D) urinary volume, and (E) sodium balance by 2K1C and sham rats during the first six weeks of renovascular hypertension development. Values are reported as means ± SEM; n = number of animals; two-way ANOVA followed by Student-Newman-Keuls test, $P < 0.05$.

deprivation – WD). After this period, food was removed and rats were allowed access to a burette containing water (PR). Cumulative intake was recorded at every 30 min for 120 min. At the end of PR, rats were immediately offered a burette containing 0.3 M NaCl (salt appetite test) and cumulative intake of both water and 0.3 M NaCl was recorded for an additional 120 min period at every 30 min. As a control, animals were also tested in water replete (WR) condition. One week later, MAP and HR were recorded in conscious rats before and 24 h after water and 0.3 M NaCl removal.

2.8.2. Tests in rats that received icv ANG II

In another group of 2K1C and sham rats, ANG II (25 ng/l μl) or saline were injected into the LV. Immediately after, water and 0.3 NaCl burettes were offered to the animals and the volumes were measured at 15, 30 and 60 min after icv injection of ANG II. The effects of icv ANG II on MAP and HR were tested in these animals one week after the intake tests.

2.8.3. Blood biochemistry analysis

In a different group of 2K1C and sham animals, serum sodium concentration, total serum protein, plasma osmolality and PRA were analyzed.

2.9. Euthanasia

At the end of the experiments, animals were deeply anesthetized with sodium thiopental (70 mg/kg of body wt, ip). Kidneys were removed and weighed to verify the ratio between left kidney to the right kidney [3,41]. For trunk blood collection, a group of animals were euthanized by decapitation and serum concentration of sodium, serum total protein, plasma osmolality, and PRA were determined as mentioned above. Rats with LV cannula implant received a 2% Evans blue solution (1 μl) injection into the LV and then were perfused transcardially with 10% buffered formalin. Brains were removed, fixed in 10% formalin, frozen and cut in coronal sections (50-μm thickness) on a cryostat (Leica, CM1850 UV, Wetzlar, Hesse, Germany), stained with Giemsa stain and analyzed by light microscopy to confirm the injections into the LV.

2.10. Statistical analysis

Results are reported as means ± SEM. Two-way ANOVA followed by Student-Newman-Keuls or Student's *t*-tests were used for comparisons. Differences were considered significant at $p < 0.05$.

3. Results

3.1. Daily water and 0.3 M NaCl intake and urine output in 2K1C and sham rats

Compared to sham rats, daily 0.3 M NaCl intake increased from the 2nd to the 5th week in 2K1C rats, peaking at the 3rd week, and returning to levels comparable to sham in the 6th week [$F(1,72) = 24.61$; $P < 0.05$] (Fig. 1A). In contrast, water intake remained elevated in 2K1C rats compared to sham from the 3rd week until the end of the recording period at the 6th week [$F(1,72) = 37.24$; $P < 0.05$] (Fig. 1B). In 2K1C rats, sodium excretion also increased from the 3rd to the 5th week [$F(1,72) = 14.69$; $P < 0.05$] (Fig. 1C) and urinary volume increased from the 4th week until the end of the test [$F(1,72) = 18.25$; $P < 0.05$] (Fig. 1D). Sodium balance also increased in 2K1C rats in the 2nd and 3rd week [$F(1, 72) = 9.71$; $P < 0.05$] (Fig. 1E).

3.2. Water and 0.3 M NaCl intake and cardiovascular changes in the WD-PR protocol in 2K1C and sham rats

After 24 h of water deprivation and previously to the salt appetite test, 2K1C and sham rats ingested similar amount of water (2K1C: 15.9 ± 4.1 and sham: 14.8 ± 1.2 ml/120 min). In contrast, in the salt appetite test, 2K1C rats ingested an increased amount of 0.3 M NaCl [F

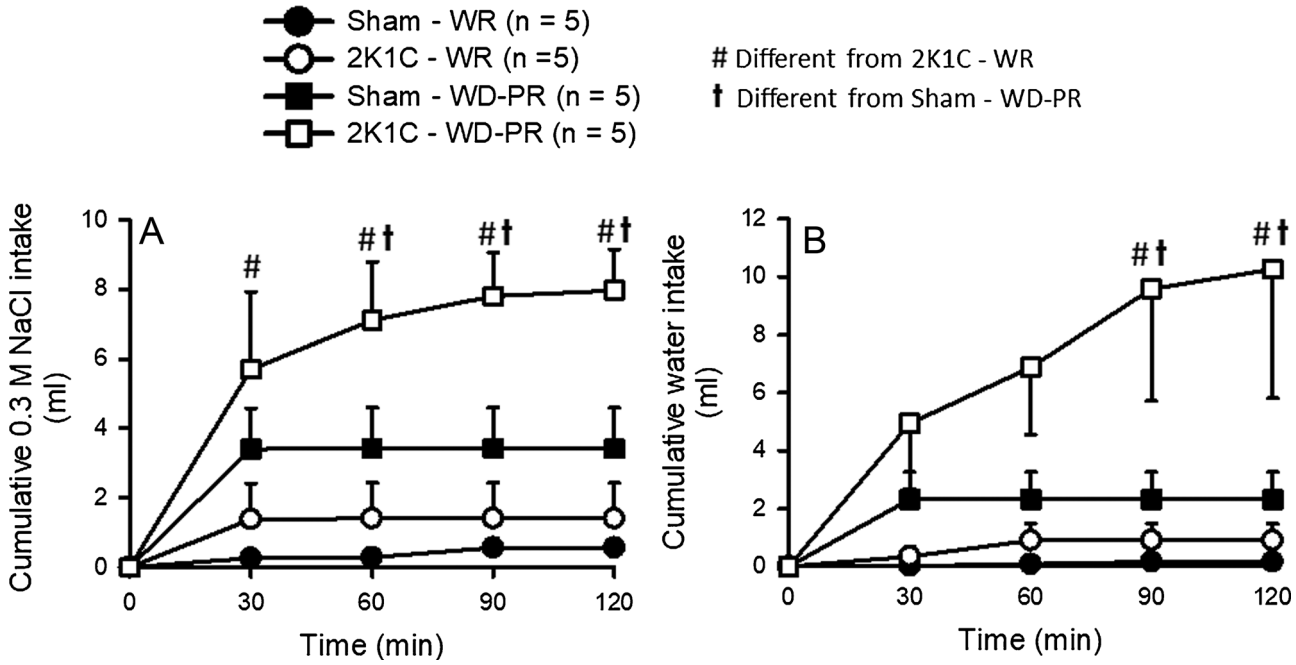

Fig. 2. Cumulative (A) 0.3 M NaCl and (B) water intake induced by 24 h of water deprivation (WD) and partial rehydration (PR) protocol or in water replete condition (WR) in 2K1C and sham rats. Values are reported as means ± SEM; n = number of animals; two-way ANOVA followed by Student-Newman-Keuls test; $P < 0.05$.

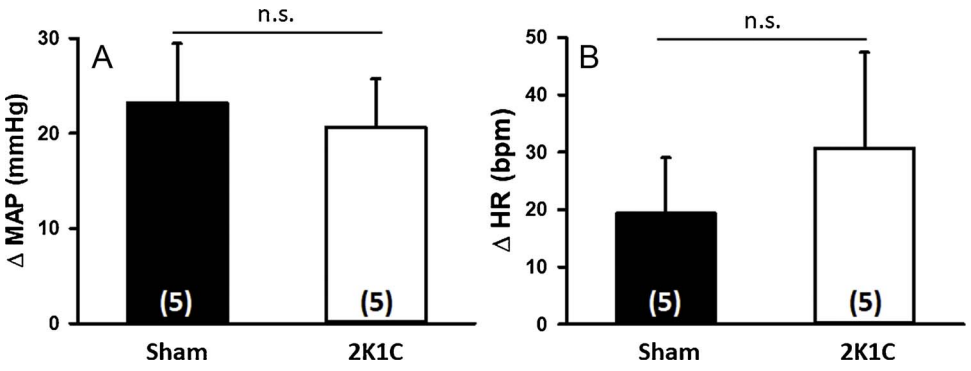

Fig. 3. Change in (A) mean arterial pressure (MAP) and (B) heart rate (HR) after 24 h of water deprivation (WD). Values are reported as means ± SEM; number of animals is given in parenthesis; Student *t* test, $P < 0.05$.

(3, 64) = 27.40; $P < 0.05$] and water compared to sham rats [F(3, 64) = 16.16; $P < 0.05$] (Fig. 2).

Baseline MAP in satiated and normohydrated condition in 2K1C and sham rats was 183 ± 4 and 105 ± 4 mmHg, respectively, and baseline HR was 375 ± 20 and 345 ± 9 mmHg, respectively. After 24 h of water deprivation, baseline MAP increased in 2K1C [t(4) = 4.076; $P < 0.05$] and sham rats [t(4) = 3.748; $P < 0.05$] compared to the satiated and normohydrated condition, without significant changes in HR (Fig. 3). However, the increase in MAP after 24 h of water deprivation was similar in 2K1C (21 ± 5 mmHg) and sham rats (23 ± 6 mmHg) [t(8) = 0.323; $P > 0.05$], (Fig. 3).

3.3. Water and 0.3 M NaCl intake and cardiovascular responses induced by icv ANG II in 2K1C and sham rats

ANG II (25 ng/1 μl) icv induced significant 0.3 M NaCl intake in 2K1C rats (4.3 ± 1.2, vs. saline: 1.5 ± 0.6 ml/60 min), but not in sham rats (2.0 ± 0.6, vs. saline: 0.4 ± 0.3 ml/60 min; $P > 0.05$) (Fig. 4A). ANG II-induced 0.3 M NaCl intake in 2K1C rats was significantly different from sham rats [F(3, 72) = 11.52; $P < 0.05$] (Fig. 4A). ANG II icv induced similar water intake in 2K1C rats and sham rats (14.7 ± 2.1 and 13.4 ± 2.6 ml/60 min, respectively) that was significantly different from icv saline (3.1 ± 0.6 and 0.2 ± 0.1 ml/60 min, respectively) [F(3, 72) = 55.41; $P < 0.05$] (Fig. 4B).

ANG II-induced pressor response was similar in 2K1C (21 ± 4 mmHg) and sham rats (25 ± 4 mmHg) [t(12) = 0.671; $P > 0.05$] (Fig. 5). Baseline MAP of 2K1C and sham rats was 190 ± 7 mmHg and 108 ± 4 mmHg, respectively.

3.4. Body mass and left/right kidney ratio in 2K1C and sham rats

There was no difference in body mass of 2K1C hypertensive rats [t(57) = 1.01; $P > 0.05$]. The ratio of left kidney/right kidney weight in 2K1C rats decreased when compared with that observed in sham rats [t(57) = 16.63; $P < 0.05$] (Table 1).

3.5. Blood biochemistry analysis in 2K1C and sham rats

At the 6th week of 2K1C renovascular hypertension, PRA increased by 3.6-fold in comparison to rats submitted to sham surgery [t(10) = 3.14; $P < 0.05$]. Serum sodium concentration [t(10) = 1.89; $P > 0.05$], total serum protein [t(10) = 1.37; $P > 0.05$] and plasma osmolality [t(10) = 1.03; $P > 0.05$] were not different comparing 2K1C and sham rats (Table 2).

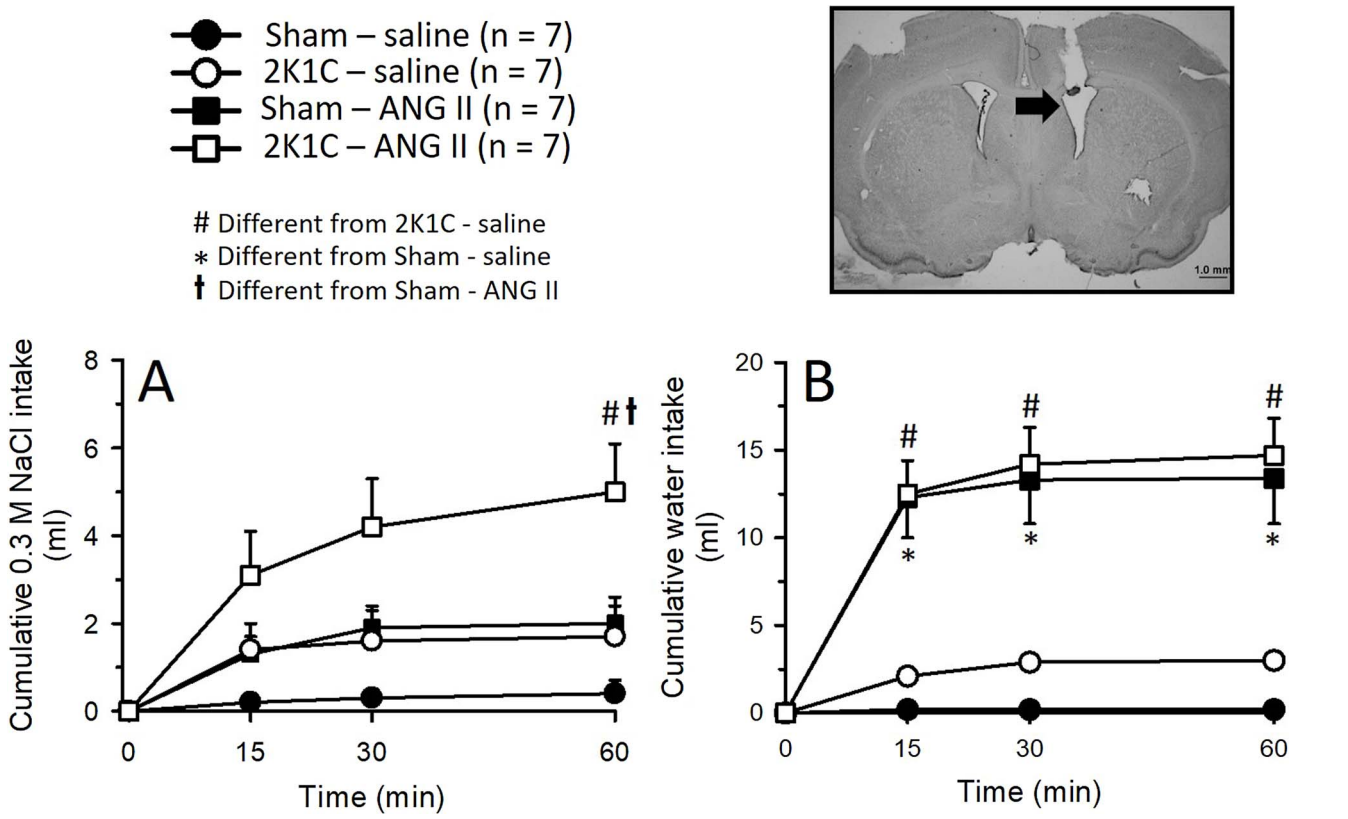

Fig. 4. Cumulative (A) 0.3 M NaCl and (B) water intake induced by angiotensin II (ANG II; 25 ng/1 µl) or saline (0.15 M NaCl; 1 µl) injected into the lateral ventricle (LV) of 2K1C and sham rats. Inset: photomicrograph of a coronal brain section from a rat representative of those tested, showing (arrow) the typical site of injection into the LV. Values are reported as means ± SEM; n = number of animals; two-way ANOVA followed by Student-Newman-Keuls test; $P < 0.05$.

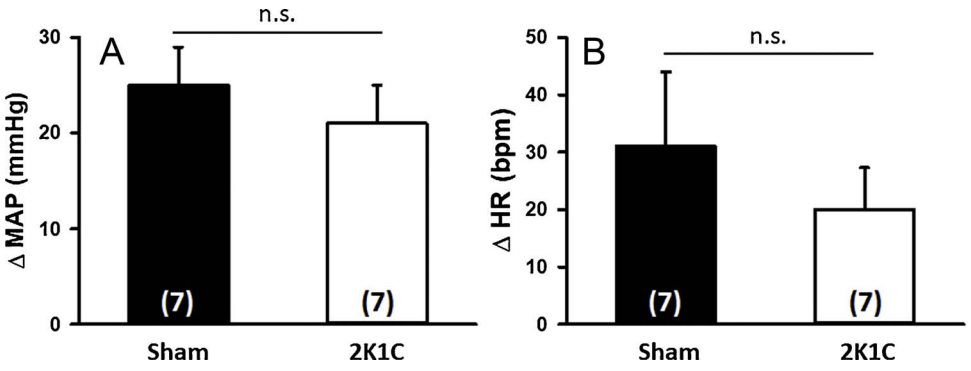

Fig. 5. Change in (A) mean arterial pressure (MAP) and (B) heart rate (HR) induced by angiotensin II (ANG II; 25 ng/1 µl) or saline (0.15 M NaCl; 1 µl) injected into the lateral ventricle (LV) of 2K1C and sham rats. Values are reported as means ± SEM; number of animals is given in parenthesis; Student *t* test, $P < 0.05$.

Table 1
Body mass and left kidney (LK)/right kidney (RK) ratio in 2K1C and sham rats.

Group	n	Body mass (g)	LK/RK
Sham	30	372.4 ± 6.1	0.97 ± 0.01
2K1C	29	364.3 ± 5.2	0.61 ± 0.02*

Values are reported as means ± SEM; n = number of animals.
* Different from sham; Student *t* test; $p < 0.05$.

Table 2
Serum sodium concentration, total serum protein, plasma osmolality, and plasma renin activity in 2K1C and sham rats.

Group	n	S_{Na} (mEq/l)	TP (g/%)	P_{osm} (mOsm/kg)	PRA (ng/ml/h)
Sham	7	140 ± 1	6.6 ± 0.1	294.9 ± 1.9	1.6 ± 0.3
2K1C	5	136 ± 2	6.2 ± 0.3	297.4 ± 1.2	5.8 ± 1.6*

Values are reported as means ± SEM; n = number of animals.
* Different from sham; Student *t* test; $p < 0.05$. S_{Na}, serum sodium concentration; TP, total serum protein; P_{osm}, plasma osmolality; PRA, plasma renin activity.

4. Discussion

The present results show that sodium intake induced by WD-PR or by icv ANG II increased in hypertensive 2K1C rats, suggesting that 2K1C rats are more sensitive to the natriorexigenic effects of ANG II. Water intake also increased in 2K1C rats submitted to WD-PR, whereas the pressor responses after water deprivation or icv ANG II were not different comparing 2K1C hypertensive and sham normotensive rats. Similar to previous studies [28,40], the present data also demonstrated increased daily water and 0.3 M NaCl intake in the first weeks of the development of 2K1C hypertension. On the 6th week after the renal surgery, water intake is still high, while daily 0.3 M NaCl intake and sodium balance returned to levels comparable to the normotensive rats. The results show a clear sensitization in the natriorexigenic effects of ANG II in 2K1C hypertensive rats at the time that daily sodium intake

returned to normal levels. Although the sensitization of the pressor response to ANG II was demonstrated in rats previously exposed to subpressor doses of ANG II [46], this effect was not detected in the present study for icv ANG II in 2K1C hypertensive rats.

The present and previous studies [10,22,31] have shown that renovascular 2K1C hypertensive rats have an increase in RAS activity, which is an important stimulus for water and NaCl intake [reviewed by [12,36]. The present results show that daily sodium intake increased in 2K1C rats from the 2nd to the 5th week after the surgery, daily sodium excretion increased from the 3rd to the 5th week, daily water intake increased from the 3rd week and urinary volume from the 4th week until the end of the recording period (6th week). The increase in NaCl intake preceded the increase in sodium excretion, suggesting that the increase in NaCl intake at the 2nd week was not consequence of sodium loss. This suggestion is reinforced by the increase in positive sodium balance in 2K1C rats on the 2nd and 3rd weeks. Sodium retention was temporary and sodium balance was similar in 2K1C and sham rats from the 4th to the 6th week after the surgery. When the experimental protocols to test water and sodium intake started (6–7 weeks after renal surgery) plasma osmolality, total serum protein and serum sodium concentration were similar in 2K1C and sham rats. Body weight was also similar between 2K1C and sham rats. A previous study demonstrated a reduction in body weight in hypertensive animals only if they have no access to hypertonic saline to ingest [40].

In the WD-PR protocol, during the salt appetite test, besides the increase in 0.3 M NaCl intake, 2K1C hypertensive rats also ingested an increased amount of water. The increased plasma osmolarity due to the ingestion of hypertonic NaCl may stimulate ingestion of water when water and 0.3 M NaCl are simultaneously available (two bottle test). Thus, the increase in water intake during the sodium appetite test in the WD-PR protocol might be consequence of the increase in the osmolarity produced by the increased of ingestion of 0.3 M NaCl intake. The increase of MAP produced by water deprivation, a response dependent mainly on hyperosmolality-induced sympathoexcitation [14], was similar in 2K1C and normotensive rats. In addition, water deprivation-induced water intake, a response that depends on hyperosmolality and hypovolemia [9], was also similar in 2K1C and normotensive rats during the PR that preceded salt appetite test. However, 2K1C rats ingested significantly more 0.3 M NaCl during the salt appetite test, which is an ANG II-dependent response. Therefore, it seems that 2K1C rats have normal responses to hyperosmolality, but more pronounced responses to RAS activation, such as salt appetite.

The increased 0.3 M NaCl and water intake induced by WD-PR in 2K1C hypertensive rats is similar to that shown in SHR [33,34]. In the SHR, forebrain sites rich in ANG II receptors involved with sodium and water intake, particularly the SFO, presented increased activity after WD-PR compared to normotensive strains [34]. A similar mechanism might also occur during the WD-PR protocol in 2K1C hypertensive rats in the present study. However, a difference between the two models is that SHR shows a continuous increase in daily hypertonic sodium and water intake [33], whereas 2K1C hypertensive rats show only a transitory increase in daily sodium intake, although they are sensitized for ANG II-induced sodium intake later.

ANG II injected icv immediately produces pressor and dipsogenic responses [5,8,26,43], whereas the induction of NaCl intake depends on the dose of ANG II used and the concentration of NaCl solution [reviewed in [7]. In the present study, icv ANG II in the dose tested (25 ng/1 μl) significantly increased 0.3 M NaCl in 2K1C rats, but not in sham rats, which together with WD-PR data suggest that 2K1C rats are more responsive to ANG II-induced salt appetite. Differently from sodium intake, however, the pressor and dipsogenic responses to icv ANG II were similar in 2K1C and sham rats. A previous study demonstrated that icv ANG II produced a more pronounced effect in MAP in 2K1C rats [47], however, the dose of ANG II used in that study was 1000 times greater than in the present study and rats were anesthetized and not conscious as in the present study.

In the present study, the levels of renin were still high on the 6th week after the induction of 2K1C hypertension. As pointed out earlier, repeated injections or continuous icv infusion of ANG II may also sensitize sodium intake in normovolemic/normohydrated rats [4]. Thus, the increased sodium intake in 2K1C rats might be due to increased sensitivity to central ANG II action, in spite of normal daily 0.3 M NaCl intake, as observed in other models dependent of ANG II [19]. Icv infusion of a low (subpressor) dose of ANG II icv also causes an increased hypertensive response to a subsequent treatment with a high (pressor) dose of ANG II, suggesting that previous exposure to ANG II can sensitize the pressor response to a latter treatment with ANG II [46]. In the case of 2K1C rats, they have chronic increase of ANG II which may cause sensitization for the pressor response to ANG II, however, these rats were already hypertensive, which may affect an additional increase of arterial pressure by icv injection of ANG II. Perhaps the sensitization of the pressor response was not detected because the pressor mechanisms were already activated by the high RAS activity. Therefore, additional administration of ANG II in a condition in which RAS is activated and/or hypertension is present results in no additional pressor response. Differently from the pressor response, ANG II-induced sodium intake was enhanced, which suggests that in a condition of increased RAS activity, the natriorexigenic response to ANG II is facilitated. In this case, also differently from arterial pressure, daily basal sodium intake returned to normal, despite the increased RAS activity, which may create condition for the expression of sensitization for sodium intake, perhaps by a mechanism similar to that involved in the sensitization of the pressor response in a normotensive condition.

Sensitization occurs when repeatedly applied stimulus produces progressive increases in an observed response [19], such as the sodium sensitization observed with repeated episodes of sodium depletion [15,30,35,37,38]. Different studies have also suggested an important role of central neuroplasticity on sodium sensitization. It has been shown that sodium sensitization induced by repeated episodes of sodium depletion increases c-Fos and fos-B/Δfos-B expression in the SFO [17]. In addition, increases in the lamina terminalis mRNA expression for AT_1 receptor, mineralocorticoid receptor and serum-and-glucocorticoid-induced kinase (SGK) [17], this later a second messenger induced by mineralocorticoid receptor activation that seems to be involved in some forms of neuroplasticity [20], is also observed after multiples sodium depletions. In fact, studies suggest that ANG II acting at AT_1 receptors and aldosterone are critical for sodium appetite sensitization. For instance, peripheral administration of a high dose of the angiotensin converting enzyme (ACE) inhibitor captopril, which also blocks central ACE, combined with icv infusion of the mineralocorticoid receptor antagonist RU-28318, abolished sodium appetite and the sensitization produced by sodium depletion [37]. Moreover, intraperitoneal administration of losartan, an AT_1 receptor antagonist, blocks sodium sensitization induced by sodium depletion [35]. Lastly, the blockade of the glutamatergic N-methyl-D-aspartate (NMDA) receptors, which have been shown to mediate neuroplasticity [6], inhibits the sensitization of sodium intake induced by multiple sodium depletion and the increase of the mRNA for AT_1 receptor, mineralocorticoids receptor and SGK [17]. Rats with 2K1C hypertension have increased ANG II and mineralocorticoids [10,22–24,31] that are mechanisms previously shown to produce sodium sensitization. As suggested by the previous studies [16,35,38], the period of increased daily sodium intake is probably the moment of sensitization in 2K1C hypertensive rats. Therefore, the present study shows that a physiological phenomenon previously described in experimental models is also present in a pathophysiological condition like hypertension. Probably the mechanisms that cause sodium sensitization in 2K1C hypertensive rats are similar to those previously described in the repeated sodium depletion models. Future studies are necessary to confirm this suggestion.

Conflict of interest

The authors have no conflicts of interest.

Author contributions

Drs. Debora Colombari, José Menani and Camila Roncari wrote major portions of the paper. Dr. Camila Roncari and Rafaela Barbosa conducted most of the animal experiments. Dr. Camila Roncari analyzed most of the data. Dr. Regina Vendramini collected, analyzed and wrote the biochemistry data of the manuscript. Dr. Eduardo Colombari and Dr. Laurival De Luca critically discussed and analyzed the data. Dr. Debora Colombari supervised the experiments and edited the final version of the paper.

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