Changes in hemodynamic and neuro-humoral control cause cardiac damage in 1 kidney, 1 clip hypertensive mice

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Running Head: Sympathovagal balance and cardiac damage in 1K1C mice

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ABSTRACT

Sympathovagal balance and baroreflex control of heart rate (HR) were evaluated during the development (one and four weeks) of one kidney, one clip (1K1C) hypertension in conscious mice. The development of cardiac hypertrophy and fibrosis was also examined. Overall variability of systolic arterial pressure (AP) and HR in the time domain and baroreflex sensitivity were calculated from basal recordings. Methyl atropine and propranolol allowed the evaluation of the sympathovagal balance to the heart and the intrinsic HR. Staining of renal angiotensin II in the kidney and plasma renin activity (PRA) were also evaluated. One and four weeks after clipping, the mice were hypertensive and tachycardic, and exhibited elevated sympathetic and reduced vagal tone. The intrinsic HR was elevated only one week after clipping. Systolic AP variability was elevated while HR variability and baroreflex sensitivity were reduced one and four weeks after clipping. Renal angiotensin II staining and PRA were elevated only one week after clipping. Concentric cardiac hypertrophy was observed one and four weeks, while cardiac fibrosis was observed only at 4 weeks after clipping. In conclusion, these data further support previous findings in the literature and provide new features of neuro-humoral changes during the development of 1K1C hypertension in mice. In addition, the 1K1C hypertensive model in mice can be an important tool for studies evaluating the role of specific genes relating to dependent and non-dependent angiotensin II hypertension in transgenic mice.

Key Words: renovascular hypertension, sympathetic tone, vagal tone, intrinsic heart rate, baroreflex.
INTRODUCTION

The understanding of the pathophysiology of hypertension has been greatly advanced due to studies performed in several species including dog, rabbit and rat (9; 12; 14). Among the experimental models of hypertension, the renovascular model has brought considerable insights to studies of the pathophysiology of hypertension (4; 33; 45). In particular, it is well recognized that the onset and development of one-kidney, one clip (1K1C) hypertension has complex mechanisms involving humoral and autonomic aspects.

The renin-angiotensin system (RAS) plays a key role in the onset of 1K1C hypertension, and its biologically active hormone, angiotensin II, has important hemodynamic effects, leading to rise in arterial pressure (AP) (9). Studies from our laboratory (24; 26) demonstrated that the first week of 1K1C hypertension in rats is accompanied by a transient tachycardia and increased intrinsic heart rate (HR). Other studies performed on 1K1C hypertensive rats have indicated that sympathetic drive is involved in the development and maintenance of this model of hypertension (4; 17). It was observed that 1K1C hypertension is accompanied by an increased cardiac sympathetic drive from the first week after clipping and a reduced cardiac vagal activity after four weeks of the onset of 1K1C hypertension (4). It has also been demonstrated that the baroreflex gain was reduced one day after clipping, while the major baroreflex impairment occurred after 30 days (30). A number of studies have revealed that hypertension is associated with the development of myocardial remodeling and cardiac end-organ damage such as left ventricular hypertrophy (23; 28). The development of organ damage is closely related to an increased risk factor for cardiovascular morbidity and mortality (32;
Since the 1980s, the development of organ damage has been related to high levels of AP (32). More recently, it has been well accepted that the increase in AP variability and decreased baroreflex modulation can also lead to end-organ damage (27; 28; 36; 39; 40). Additionally, low HR variability has been shown to be a powerful predictor of cardiac events, indicating decreased autonomic modulation (18; 19; 44).

With advances in genomic studies, the mouse is gaining special attention due to its capacity for genetic manipulation (2; 11), including gene engineering related to molecular mechanisms involved in arterial hypertension (5; 21). There is very sparse literature concerning the pathophysiological mechanisms involved in the development of 1K1C hypertension in mice. Wiesel and coworkers (45) adapted the 1K1C hypertensive model to the mouse and demonstrated that, similar to 1K1C hypertension in rat, the elevation of AP was rapid and progressive. In addition, renal renin expression and plasma renin activity (PRA), markers of RAS activity, was not different in chronic phase of clipping between 1K1C hypertensive and sham mice, indicating that RAS activity may not play a role in this phase of hypertension (45). In the current study, in addition to the characterization of the hemodynamics (AP and HR) and PRA measures in 1K1C hypertensive mice, the renal staining for angiotensin II was also evaluated (31). It is well documented that augmented plasma angiotensin II elicits increased staining of this hormone within the kidneys (31; 46). Since we hypothesized that plasma angiotensin II generation may be augmented in the early phase of 1K1C hypertensive model, renal angiotensin II staining in the kidney was used as another marker of RAS activity, in order to provide an indirect inference of plasma angiotensin II combined with the evaluation of PRA.
Furthermore, it has been hypothesized that sympathetic tone is elevated in 1K1C hypertensive mice contributing to the elevation of AP in this model. The quantification of sympathetic and vagal tone by means of intravenous propranolol and methyl atropine has been extensively used to evaluate the autonomic control of the HR (4; 7). In the present study, this approach facilitated assessment of not only the sympathetic and vagal tone, but also the intrinsic HR when both drugs were given simultaneously (24; 25; 33). The small size of the mouse and its low blood volume has hampered reliable measurements of baroreflex sensitivity by means of infusion of vasoactive drugs. The baroreflex sequence method is an approach that measures spontaneous beat-to-beat AP fluctuations and their related HR changes without the influence of drugs (3; 27). Thus, the sequence method (3; 25) was employed in the present study to investigate spontaneous baroreflex sensitivity in 1K1C hypertensive mice.

Because the heart is one of the primary end-organs of hypertension exhibiting structural alterations (23; 28), in the present study the following parameters were evaluated: the minor diameter of myocytes, myocardium wall thickness, and presence of fibrosis. Cardiac mass alterations in 1K1C hypertensive mice have already been quantified indirectly using cardiac weight index (45), while more accurate morphological studies, i.e. quantification of the minor diameter of myocytes, myocardium wall thickness, and the presence of fibrosis, have not yet been performed.

A number of studies in several species such as dog, rabbit and rat (8) have examined the hemodynamics, PRA, role played by the autonomic nervous system, baroreflex regulation of HR, and cardiac end-organ damage during the development of 1K1C hypertension. Nevertheless, no study has examined these
issues in 1K1C hypertensive mice. Therefore, the present study was designed to verify if, in line with other species, 1K1C hypertensive mice also have an imbalance in cardiac autonomic control, impaired baroreflex control of HR as well as altered RAS activity and cardiac end-organ damage.

MATERIALS AND METHODS

All experimental procedures were carried out in accordance with the Guide for the Care and Use of Laboratory Animals [DHEW Publication No. (NIH) 85-23, Revised 1985; Office of Science and Health Reports, DRR/NIH, Bethesda, MD, 20892] approved by the Ethics in Animal Research Committee of the School of Medicine of Ribeirão Preto, University of São Paulo, SP, Brazil (protocol 054/2006).

Animals

Experiments were performed on male Swiss mice, weighting 29-31 g, supplied by the Animal Facility of the School of Medicine of Ribeirão Preto, University of São Paulo, SP, Brazil. Mice were housed in a temperature and humidity controlled chamber (Alesco Indústria e Comércio Ltda, model 9902-001, Monte Mor, SP, Brazil) with a 12-hour light/dark cycle. Animals were allowed free access to water and standard chow (Nuvilab CR-1, Nuvital, Colombo, PR, Brazil).

Renovascular Hypertension Surgery

Mice were anesthetized by tribromoethanol (250 µg/g, i.p.) and, through a flank incision the right renal artery was carefully isolated and received a rigid silver
clip (0.15 mm internal gap), as described by Wiesel et al. (45). The renal artery clip had its lumen size carefully adjusted under a surgical microscope (DF Vasconcellos S.A., model MC-A186, São Paulo, SP, Brazil). The left kidney was removed, leaving the adrenal gland intact. Normotensive control (NC) mice underwent unilateral nephrectomy without clipping the remnant right renal artery (sham surgery). At the end of surgery the incisions were sutured and the mice received a single dose of antibiotic (160 mg/kg, i.m., Veterinary Pentabiotic, Fort Dodge, Campinas, SP, Brazil) and returned to the temperature and humidity controlled chamber for full recovery for one or four weeks.

**Arterial Pressure Measurement**

*Indirect Measurement:* the development of 1K1C hypertension was accompanied by a tail-cuff method for indirect AP measurement. Animals were placed in a restrainer (model 84 mouse restrainers, IITC Life Science, Woodland Hills, CA), put into a warm chamber (model 306, IITC Life Science, Woodland Hills, CA) kept at 31-33°C, and received an integrated sensor-cuff occluder (model B60-1/4, IITC Life Science, Woodland Hills, CA) around their tail. The sensor cuff was inflated until the disappearance of the tail pulse and systolic AP was determined by the return of tail pulsations during the deflation of the cuff. Systolic AP was measured one day before and weekly after clipping surgery.

*Direct Measurement:* experiments were carried out one or four weeks after renovascular hypertension or sham surgery. At least 24 h before the experiments, mice were anesthetized with tribromoethanol (250 µg/g, i.p.) and polyethylene catheters (Intramedic, Clay Adams, Parsippany, NJ, EUA) were inserted into the left carotid artery and right jugular vein for direct AP recording and drug
administration, respectively. The catheters were exteriorized on the nape of the mice and incisions were sutured. Mice were maintained in individual cages for recovery for a minimum of 24 h. On the day of the experiment, the mice were taken to the recording room at least 30 min before the beginning of the experiment, and a quiet environment was maintained to minimize stress. The arterial catheter was connected to a pressure transducer (model P23 Gb; Statham, EUA). Pulsatile AP was continuously sampled (4 kHz) using an IBM computer equipped with an analog-to-digital interface (220; Dataq, Akron, OH, EUA).

**Experimental Protocol**

Hemodynamic recordings were carried out in conscious freely moving mice placed in individual cages. Basal pulsatile AP was recorded for 30 min and a bolus of a solution with methyl atropine (2 mg/kg, i.v., Sigma Chemical Co., St. Louis, MO, USA) was administrated. After 15 min, a bolus of a solution with propranolol (4 mg/kg, i.v., Sigma Chemical Co., St. Louis, MO, USA) was given to the mice and the AP was continuously recorded for another 15 min. The sequence of drug administration was chosen randomly. After the hemodynamic recordings, the mice were killed by an overdose of anesthesia (sodium thiopental), and the heart and kidney were rapidly removed, rinsed in ice-cold 0.9% NaCl solution (saline) and weighed. The heart weight index was calculated by dividing the heart weight by total body weight, while the kidney weight index was calculated by dividing the kidney weight by total body weight.

In separate groups of NC and 1K1C hypertensive mice, after the recording of AP to verify the development (one and four weeks after clipping) of hypertension, the animals were killed by decapitation, blood was immediately
collected for measurement of plasma renin activity (PRA), the kidney was collected for immunohistochemistry (renal angiotensin II), and the heart collected for morphological analysis (cardiac hypertrophy and fibrosis).

Data Analysis

Basal Hemodynamic Parameters: Pulsatile AP recordings were analyzed by a computer software designed to detect inflection points of a periodic wave (Advanced CODAS, Dataq Instruments, OH, USA). A graphic interface on the analysis software allowed visual inspection and manual editing of erroneously detected events. Beat-by-beat time series of systolic, diastolic and mean AP were generated. HR was measured from successive diastolic pulse intervals. The overall variability of systolic AP and HR was calculated by means of average standard deviation from the beat-by-beat time series of systolic AP and HR.

Autonomic Tone and Intrinsic HR: Sympathetic and vagal tones were assessed by autonomic blockade produced by injection of propranolol and methyl atropine, respectively. The difference between HR calculated at the end of 15 min after propranolol administration and basal HR was considered as the sympathetic tone. On the other hand, the difference between HR calculated at the end of 15 min after methyl atropine administration and basal HR was considered as the vagal tone. The HR calculated at the end of 15 min when both autonomic blockers were administered was considered as the intrinsic HR.

Baroreflex Sensitivity (BRS): The baroreflex control of HR was assessed through spontaneous changes in AP and pulse interval (PI) by the sequence method described by Bertinieri and coworkers (3). Ramps of progressive increases and decreases in systolic AP were automatically detected in $10^4$ beats pulsatile AP
recordings using the freely available HemoLab computer software (http://www.intergate.com/~harald/HemoLab/Hemolab.html). Sequences defined ramps of four or more systolic AP values associated with parallel changes in pulse interval (PI), i.e. systolic AP increases and PI lengthenings as well as systolic AP decreases and PI shortenings. The spontaneous BRS was calculated from the slope (ms/mmHg) of linear regression lines between the systolic AP and the subsequent PI. Only regression lines with a correlation coefficient higher than 0.85 were considered. The average of the slopes of all individual regression lines was then used as an index of BRS. The baroreflex effectiveness index (BEI), which provides information on the baroreflex function that is complementary to BRS was also calculated (6). It is defined as the ratio between the number of systolic AP ramps followed by the respective reflex changes in PI, and the total number of systolic AP ramps (independently on whether they are or not accompanied by the corresponding reflex PI ramps) observed over the time window studied.

**Plasma Renin Activity Analysis**

Blood was collected in cold tubes containing EDTA (7.5%) and a cocktail of enzyme inhibitors: p-OHHBz (1 mM), PMSF (1 mM), pepstatin (1 mM), and O-Phenantroline (30 mM). The blood was centrifuged at 4°C and 3000 rpm for 15 min. Thirty μL of plasma plus 970 μL Tris/HCl (50 mM, pH 7.5) and 20 μL of tetradecapeptide (2.0 nmol/mL) were incubated for 2h. Aliquots were collected at 0 min, 2 h and 24 h. The reaction was interrupted with 10 μL of orthophosphoric acid (50%) and subjected to HPLC. Substrate hydrolysis was analyzed by reversed phase HPLC using an aquapore ODS 300 column equilibrated with 0.1% phosphoric acid containing 5% acetonitrile (v/v). Angiotensin I was separated from
tetradecapeptide by isocratic elution for 5 min followed by a 20 min linear gradient of 5–35% acetonitrile in 0.1% phosphoric acid (v/v) at 1.5 mL/min. The chromatographic profile of each sample was compared with that obtained for standard samples containing tetradecapeptide (retention time = 21.70 min) and angiotensin I (retention time = 18.98 min) at an absorbance of 240 nm. Peptide fragments were identified by elution position and quantified by integration area using repeated injections of standard peptide solution to correct for small differences in retention time (<6%) and peak height.

**Immunohistochemistry Analysis for Renal Angiotensin II**

After decapitation and blood collection, the kidney was removed and submitted to immunohistochemistry analysis for tissue angiotensin II staining. Next, it was cut into transverse sections and stored in a solution of 60% methanol, 30% chloroform, and 10% acetic acid. After 12h the tissue was placed in 70% alcohol solution for paraffin inclusion. Sections were incubated overnight at 4°C with a 1:500 dilution of an angiotensin II polyclonal antibody (Peninsula Laboratories Inc, San Carlos, USA). The reaction product was detected with an avidin-biotin-peroxidase complex (Vector Laboratories, Burlingame, CA, USA). The color reaction was developed with 3,3'-diaminobenzidine (Sigma Chemical Co., St. Louis, MO, USA), and the material was counterstained with methylgreen, dehydrated and mounted. Nonspecific protein binding was blocked by incubation with 20% goat serum in PBS for 20 min. Negative controls consisted of a replacement of primary antibody with equivalent concentrations of normal rabbit IgG. To quantify the mean number of infiltrating angiotensin II cells in renal cortical
tubulointerstitium, grid fields measuring 0.245 mm\(^2\) each were evaluated and mean counts per kidney were calculated.

**Morphological Analysis**

The hearts were rapidly removed, rinsed in ice-cold 0.9% NaCl solution (saline), cut transversely and fixed in phosphate-buffered 10% formalin, for morphological studies. The ventricles were isolated and submitted to paraffin inclusion. Each block was serially cut at 5 µm from the midventricular surface, either to the base or to the apex. The sections were stained with hematoxylin-eosin or picrosirius red. For morphometric analysis, hearts stained with hematoxylin-eosin were used. The absolute thickness of the left ventricular and septum wall were measured using the public-domain software NIH ImageJ (developed by U.S. National Institute of Health and available on the internet site http://rsb.info.nih.gov/nih-image/). The minor diameter of myocytes in the left ventricle and septum was measured using video microscopy Leica Qwin (Leica Imaging Systems Ltd, Cambridge, England). Approximately 30 values were obtained per region, per mouse. The mean value was then calculated. All measures were done in the mid myocardium portion. To estimate the volume fraction (%) of fibrosis in picrosirius red-stained sections, quantitative examination of the left ventricular and septum myocardium was carried out on a medium power light-microscopic field (x400). For each heart, approximately 15 fields per region, per mouse, were randomly selected and analyzed using Leica Qwin software (Leica Imaging Systems Ltd, Cambridge, England). The mean value was subsequently calculated.
**Statistical Analysis**

All data are presented as mean ± SE. Averages of systolic AP indirect measurement comparisons were performed using the two-way analysis of variance (Two way ANOVA) for repeated measures followed by Tukey post-test. Averages of basal AP, HR, sympathetic tone, vagal tone, intrinsic HR, systolic AP and HR variability, renal cortex angiotensin II staining, PRA, weights and histological analysis comparisons were performed using two way analysis of variance (Two way ANOVA), followed by Tukey post-test. The HR responses to methyl atropine or propranolol administration were compared using the paired Student t test. Differences were considered statistically significant for $P < 0.05$.

**RESULTS**

**Development of 1K1C Hypertension**

The development of hypertension was verified by the significant increase in systolic AP, which was measured by the tail cuff method before and after clipping. The systolic AP attained a plateau at $159 ± 4$ mmHg on the 3rd week after clipping, and was maintained at $159 ± 3$ mmHg on the 4th week. NC mice displayed stable normotensive levels within $107 ± 1$ and $115 ± 2$ mmHg throughout the protocol. Figure 1 shows typical tracings of direct recordings of AP and HR from NC and 1K1C hypertensive mice, four weeks after surgery. A conspicuous hypertensive level and tachycardia were observed in 1K1C hypertensive mice. The average mean AP (MAP) and pulsatile AP (PAP) from all groups are presented in Table 1. Basal MAP in both 1K1C hypertensive groups was higher than that for NC groups. In addition, the MAP of four weeks 1K1C hypertensive mice was higher than the
MAP of one week 1K1C hypertensive mice. The PAP from 1K1C hypertensive mice one and four weeks after surgery was also elevated. Table 1 shows that the 1K1C hypertensive and NC mice exhibited similar body weight during the development of hypertension. However, one and four weeks after clipping, both groups of 1K1C hypertensive mice showed a marked increase in cardiac weight index. Renal weight index was not different between 1K1C hypertensive and NC groups.

**Sympathovagal Balance and Heart Rate Control**

As shown in Figure 2, basal HR was elevated one and four weeks after clipping, as compared to NC groups, even though a smaller tachycardia was detected four weeks after clipping. Figure 2 also shows, by means of the bradycardia elicited by propranolol, that 1K1C hypertensive or NC mice have an increased sympathetic tone to the heart. Moreover, the bradycardic response caused by propranolol shows that 1K1C hypertensive mice have a sympathetic tone greater than that of the NC mice. Propranolol injection elicited only a transient (1-2 min of duration) increase, approximately 10 mmHg, in MAP which did not induce any change in HR. On the other hand, the tachycardic response caused by methyl atropine indicates that the parasympathetic tone is reduced one and four weeks after clipping. The combined administration of methyl atropine and propranolol revealed that the intrinsic HR of 1K1C hypertensive mice was elevated only one week after clipping.

**Baroreflex Sensitivity**
The spontaneous changes in AP were of small amplitude (<10 mmHg) within the periods of observation. Despite the mean level of AP was higher in 1K1C hypertensive as compared to NC mice, no differences were found in the magnitude of spontaneous change in AP among the groups studied. Estimates for the number of baroreflex sequences (Figure 3A) and the baroreflex gain (Figure 3B) determined by the sequence method were significantly reduced in both groups one and four weeks after clipping as compared to their NC counterparts.

**Arterial Pressure and Heart Rate Variability in Time Domain**

Systolic AP variability was elevated in 1K1C hypertensive mice one week (4.3 ± 0.3 vs 3.1 ± 0.3 mmHg) and four weeks (5.4 ± 0.5 vs 3.9 ± 0.2 mmHg) after clipping, as compared to NC mice. In addition, four weeks after clipping the systolic AP variability was significantly greater than in one week 1K1C hypertensive mice. Nevertheless, 1K1C hypertensive mice presented decreased HR variability one week (11.1 ± 0.7 vs 16.2 ± 2.1 bpm) and four weeks (10.3 ± 0.8 vs 17.7 ± 1.5 bpm) after clipping as well.

**Plasma Renin Activity**

PRA was elevated in one week 1K1C hypertensive mice as compared with their NC counterparts (0.53 ± 0.09 vs 0.25 ± 0.06 nmol/mL/h). However, four weeks after clipping PRA was not different between the 1K1C hypertensive and NC mice (0.22 ± 0.04 vs 0.30 ± 0.04 nmol/mL/h).

**Immunohistochemistry of Angiotensin II in the Renal Cortex**
Immunohistochemical studies (Figure 4) showed increased angiotensin II staining in the tubular compartment from the renal cortex of 1K1C hypertensive mice one week after clipping. There was no significant difference between staining for angiotensin II in the renal cortex of four weeks 1K1C hypertensive mice as compared to their NC counterparts.

**Morphometric Analysis**

The average minor diameter of the myocytes from left ventricle and septum one and four weeks after clipping was significantly greater than the average minor diameter of their NC counterparts (Table 2 and Figure 5). The left ventricle and septum free wall thickness were also increased one and four weeks after clipping (Table 2). Picrosirius red-stained sections showed left ventricular myocardial fibrosis only four weeks after clipping (Figure 5 – bar graphs). Figure 6 illustrates a remarkable concentric hypertrophy in the left ventricle of 1K1C hypertensive mice one and four weeks after clipping.

**DISCUSSION**

The pathophysiological mechanisms involved during the development of 1K1C hypertension in mice are poorly understood. To our knowledge, this is the first study to examine autonomic function during the development (one and four weeks) of 1K1C hypertension in conscious mice. The indirect AP measurement, by means of the tail cuff method, showed a prompt and continuous increase in systolic AP during the first week after clipping, attaining a plateau during the 3rd
and 4th weeks. These data are consistent with studies performed in other species such as dog, rabbit, rat and mouse (4; 9; 26; 45).

It is well known that the RAS plays a significant role in the onset of renovascular hypertension in several species (5; 9). In mice, Wiesel et al. (45) have shown that this system is not stimulated four weeks after clipping. In the present study, PRA and renal staining for angiotensin II was found to be increased only one week after clipping, suggesting that the RAS is not overactive in chronic 1K1C hypertensive mice. Despite the well known fact that angiotensin II causes an increased sympathetic drive, particularly by means of central mechanisms (1; 10; 15; 43), changes in autonomic function during the onset, and development, of 1K1C hypertension in mice have not yet been examined.

The predominance of the sympathetic over the vagal tone observed in the present study in NC mice corroborates studies from other (16) and our laboratory (7). Moreover, the bradycardia caused by propranolol indicates that 1K1C hypertensive mice present a greater sympathetic tone than their NC counterparts. Accordingly, this sympathetic overactivity might be responsible for the tachycardia observed one and four weeks after clipping. In the present study the double pharmacological blockade of the autonomic nervous system of one week 1K1C hypertensive mice revealed an increased intrinsic HR. Therefore, this phenomenon might be associated with the higher basal HR observed during this time frame (one week). This hypothesis is based on the observation that during the onset of 1K1C hypertension in rats, the development of tachycardia was associated with an increased intrinsic HR (24; 26). Furthermore, in the current study, a significantly decrease in vagal tone to the heart was found in both groups (one and four weeks) of 1K1C hypertensive mice. A previous study in rats (4) has
demonstrated a close relationship between tachycardia, increased sympathetic tone, and decreased vagal tone in 1K1C hypertensive rats. Thus, it is hypothesized that the enhanced cardiac sympathetic activity and a reduced cardiac vagal activity found in 1K1C hypertensive mice may contribute to the tachycardia observed in both periods of hypertension. In addition, the greater magnitude of this tachycardia in one week 1K1C hypertensive mice may be explained by the increased intrinsic HR during the first week of 1K1C hypertension.

Even though our methodological approach does not provide a mechanism for the increase in the intrinsic HR, data from the literature have demonstrated that an overactivity of the RAS was associated with an increase in intrinsic HR in one week 1K1C hypertensive rats (26). Furthermore, Machado and coworkers (24; 25) showed that the infusion of angiotensin II elicited tachycardia associated with an elevated intrinsic HR in conscious normotensive rats. Moreover, the present study showed increased angiotensin II in the kidney and increased PRA in 1 week 1K1C hypertensive mice. Therefore, it might be suggested that an overactivity of the RAS is responsible for the increased intrinsic HR and the greater magnitude of tachycardia in this period of hypertension. Importantly, Bealer (1) has demonstrated that the development of 1K1C hypertension in rats is characterized by tachycardia induced by the chronotropic effects and increased intrinsic HR induced by angiotensin II acting in the tissue within the AV3V region of the hypothalamus. Moreover, a number of studies have demonstrated that angiotensin II could elicit a chronotropic effect acting through stimulation of sympathetic pathways (8; 15), reduction in vagal tone (22; 35), central suppression of the baroreflex (12; 34), and a direct action on the AT₁ receptors in atrium myocardium.
The underlying intracellular mechanisms that are involved in the chronotropic action of angiotensin II in the brain are not well established. However, recent studies have shown that angiotensin II acts by means of mechanisms involving protein kinases cascades (41; 42). Overall, data from the literature combined with data from the present study suggest that an overactivity of the RAS in one week 1K1C hypertensive mice may lead to an increase in the intrinsic HR which may play a significant role in the higher magnitude of tachycardia in one week 1K1C hypertensive mice.

In the present study, a significant decrease in baroreflex control of HR was found from the first week of the development of 1K1C hypertension in mice. Baroreflex sensitivity was evaluated using the sequence method described by Bertinieri and coworkers (3; 27). The sequence analysis is a widely employed method to investigate spontaneous BRS over the years (6), providing information on the dynamic aspects of baroreflex control of HR during spontaneous behavior (20). This approach estimates baroreflex function under conditions where the baroreflex are not far from its baseline operation, since spontaneous AP changes are of small amplitude (< 10 mmHg). The slope of the regression line between spontaneous changes in AP and PI values is taken as an index of BRS, as done when AP and PI changes are induced by vasoactive drug injections (37). The baroreflex nature of the PI/AP sequences has been demonstrated in cats (3), rats (38) and mice (20). This technique is particularly suitable to mice because these animals are not amenable to receive multiple intravenous injections.

In the present study, a significant decrease, not only in BRS, but also in BEI was found from the first week of the development of 1K1C hypertension in mice. The low value of BEI observed in the present study is in accordance with previous
founds in humans (6) and mice (20), indicating that baroreflex induces changes in PI only in a minor fraction of systolic AP ramps. The baroreceptor itself is known to be affected at variable degrees by central inhibitory influences (6) and the cardiac rhythm is also controlled by other nonbaroreflex mechanisms (direct central neural control, respiratory activity, humoral substances, etc). Therefore, the information provided by these two indexes in not redundant but rather complementary.

It is well-established that the baroreflex is a powerful buffering mechanism that counteracts short-term fluctuations in AP, and that this cardiovascular reflex is impaired in experimental (13; 30; 33) and clinical (28) hypertension as well. The data from the present study confirm that 1K1C hypertensive mice also exhibit this hallmark of hypertension. The enhanced cardiac sympathetic activity and reduced cardiac vagal activity in 1K1C hypertensive mice observed during the development of hypertension (one and four weeks), may reflect the reduced baroreflex gain observed in both periods of hypertension. Moyses and coworkers (30) have examined the role played by cardiac vagal and sympathetic components in the reduced baroreflex gain of 1K1C hypertensive rats, and reached the conclusion that the progressive attenuation in baroreflex gain during the development of 1K1C hypertension appears to be mediated by overactivity of the sympathetic component, while the vagal component was attenuated afterwards. More recently, it was demonstrated in 2K1C hypertensive mice that baroreflex sensitivity is also chronically attenuated in this model (33). Furthermore, in the same study (33), resting tachycardia was observed appearing to be due to an increased predominance of the cardiac sympathetic tone over the cardiac vagal tone.

Another interesting finding of the present study was an increase in cardiac weight index of 1K1C hypertensive mice, indicating the development of cardiac
hypertrophy. In fact, the morphological studies revealed an early development of cardiac hypertrophy one and four weeks after clipping, expressed by the increase in the minor diameter of myocytes and increase in the wall thickness of the left ventricle and septum. Furthermore, a remarkable concentric hypertrophy characterizes the derangement of the heart (Figure 6) caused by 1K1C hypertension in mice. In addition, fibrosis was evident four weeks after clipping. It is well known that arterial hypertension is a common cause of cardiac end-organ damage, inducing morphological and functional modifications (28). Nevertheless, the AP levels are not the only determinant of organ damage. Observations from other forms of hypertension have suggested that increased AP variability is associated with organ damage (32; 39; 40). The presence of cardiac hypertrophy, fibrosis (after four weeks) and increased AP variability observed in the current study in 1K1C hypertensive mice is consistent with several models of hypertension (33; 39; 40; 45). Notably, the attenuation of baroreflex sensitivity, as observed in the present study, has been proposed as an independent variable related to cardiac end-organ damage, i.e. cardiac hypertrophy and fibrosis (28; 36). Furthermore, as illustrated in Figure 1 a remarkable lability of HR is observed in NC mice while it is reduced in both 1K1C hypertensive groups (one and four weeks). The decrease in HR variability is another outcome associated with hypertension and is related to increased cardiovascular morbidity and mortality (18; 19; 44). Assessment of HR variability throughout time domain variables, i.e. standard deviation, is a simple and practical method for assessing autonomic function. Its applicability has been incorporated in several clinical conditions related to prognostic outcomes (18; 19; 44). The development of 1K1C hypertension can be associated with a decrease in renal mass due to the
exaggerated reduction of renal flow which can lead to renal infarction (45). In the present study, the renal weight index was not different between 1K1C hypertensive and NC groups indicating that clipping did not induce exaggerated reduction of renal flow.

In conclusion, 1K1C hypertension in mice is characterized by a rapid and progressive rise in AP which is accompanied by tachycardia, increased sympathetic and decreased vagal tone to the heart, decreased baroreflex sensitivity and organ damage, i.e. cardiac concentric hypertrophy and fibrosis. Moreover, the initial onset (one week) of this model of hypertension is also characterized by elevated intrinsic HR associated with RAS overactivity. It is likely that the last two findings i.e. increased intrinsic HR and RAS overactivity, might be responsible for the greater magnitude of tachycardia observed in one week 1K1C hypertensive mice.

**PERSPECTIVES AND SIGNIFICANCE**

Most of our understanding about the complex mechanisms involved in arterial hypertension was obtained from experimental studies performed in several species such as dog, rabbit and rat. Now a day, with the advances in genomic sciences, the mouse is receiving special attention due their unique amenity to genetic manipulations. Nevertheless, much of the normal physiology as well as pathophysiologic aspects well established for other species remain unknown for the mouse. The present study provides valuable information regarding neurohumoral features of 1K1C hypertension in mice, which are certainly useful tool for future studies relating autonomic and renin-angiotensin system in genetically manipulated mice.
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GRANTS

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REFERENCES


of heart rate variability in patients with a recent acute myocardial infarction.  


42. **Sun C, Sumners C and Raizada MK.** Chronotropic action of angiotensin II in neurons via protein kinase C and CaMKII. *Hypertension* 39: 562-566, 2002.

43. **Tagawa T and Dampney RA.** AT(1) receptors mediate excitatory inputs to rostral ventrolateral medulla pressor neurons from hypothalamus. *Hypertension* 34: 1301-1307, 1999.


FIGURE LEGENDS

**Figure 1:** Typical tracings of basal arterial pressure and heart rate of a normotensive control (NC) and a 1K1C hypertensive mouse four weeks after clipping.

**Figure 2:** Bar graph showing basal heart rate (horizontal black line) and heart rate responses after propranolol or methyl atropine in normotensive control (NC) and 1K1C hypertensive mice, one and four weeks after clipping. Horizontal interrupted lines represent intrinsic HR after double blockade with propranolol and methyl atropine. Parentheses indicate the number of observations for sympathetic and vagal tone.

+P<0.001 compared to other three groups; *P<0.001 compared to vagal tone; **P<0.001 compared to NC mice.

**Figure 3:** Bar graph showing the average number of baroreflex sequences per 1000 beats (A), baroreflex sensitivity (gain, B) and baroreflex effectiveness index (BEI, C) from normotensive control (NC) and 1K1C hypertensive mice, one and four weeks after clipping. The number of observations is shown inside the bars.

*P<0.001 compared to NC mice.

**Figure 4:** Photomicrographs showing positive angiotensin II stained cells in the renal cortex from normotensive control (NC) and 1K1C hypertensive mice, one and four weeks after clipping. Bar graph shows the average number of positive angiotensin II stained cells per 15 fields in the renal cortex from NC and 1K1C
hypertensive mice, one and four weeks after clipping (magnification 280x). The number of observations is shown inside the bars. *P<0.001 compared to other groups.

**Figure 5:** Representative views of the minor diameter of myocytes from left ventricle from one and four weeks normotensive control (NC) and 1K1C hypertensive mice (black line represents 50 \( \mu \)m). Bar graphs represent the evaluation of the minor diameter of myocytes and the volume fraction of fibrosis from left ventricle from one and four weeks NC and 1K1C hypertensive mice. *P<0.05 compared to NC.

**Figure 6:** Representative photomicrographs of heart transverse sections of mice from normotensive control (NC) and 1K1C hypertension groups, one and four weeks after clipping (black line represents 2 mm).
Table 1: Mean arterial pressure (MAP; mmHg), pulsatile arterial pressure (PAP; mmHg), body weight (BW; g), cardiac weight index (CWI; mg/g) and renal weight index (RWI; mg/g) from normotensive control (NC) and 1K1C hypertensive mice, one and four weeks after surgery.

<table>
<thead>
<tr>
<th></th>
<th>1 Week</th>
<th>4 Weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NC (13)</td>
<td>1K1C (18)</td>
</tr>
<tr>
<td>MAP</td>
<td>107 ± 3</td>
<td>152 ± 2 **</td>
</tr>
<tr>
<td>PAP</td>
<td>121/94</td>
<td>175/133 **</td>
</tr>
<tr>
<td>BW</td>
<td>30 ± 0.5</td>
<td>31 ± 0.7</td>
</tr>
<tr>
<td>CWI</td>
<td>4.1 ± 0.13</td>
<td>5.3 ± 0.14 *</td>
</tr>
<tr>
<td>RWI</td>
<td>9.3 ± 0.40</td>
<td>9.8 ± 0.30</td>
</tr>
</tbody>
</table>

Values are mean ± SE. Parentheses show the number of observations in each group. *P<0.001 compared to NC mice; **P<0.001 compared to other three groups; +P<0.05 compared to their respective counterparts.
Table 2: Minor diameter of myocytes and wall thickness from left ventricle (LV) and interventricular septum from normotensive control (NC) and 1K1C hypertensive mice, one and four weeks after surgery.

<table>
<thead>
<tr>
<th></th>
<th>1 Week</th>
<th>4 Weeks</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NC (5)</td>
<td>1K1C (7)</td>
<td>NC (7)</td>
<td>1K1C (5)</td>
</tr>
<tr>
<td><strong>Wall Thickness</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LV</td>
<td>1.28±0.03</td>
<td>1.52±0.07*</td>
<td>1.23±0.06</td>
<td>1.49±0.08*</td>
</tr>
<tr>
<td>Septum</td>
<td>1.05±0.06</td>
<td>1.42±0.03*</td>
<td>1.07±0.03</td>
<td>1.40±0.08*</td>
</tr>
<tr>
<td><strong>Diameter of Myocytes (µm)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LV</td>
<td>13.6±0.47</td>
<td>15.3±0.41*</td>
<td>13.4±0.3</td>
<td>15.7±0.54*</td>
</tr>
<tr>
<td>Septum</td>
<td>10.9±0.22</td>
<td>13.1±0.34*</td>
<td>10.4±0.2</td>
<td>12.3±0.10*</td>
</tr>
</tbody>
</table>

Values are mean ± SE. Parentheses show the number of observations. * P<0.005 compared to NC mice.
Typical tracings of basal arterial pressure and heart rate of a normotensive control (NC) and a 1K1C hypertensive mouse four weeks after clipping.
Bar graph showing basal heart rate (horizontal black line) and heart rate responses after propranolol or methyl atropine in normotensive control (NC) and 1K1C hypertensive mice, one and four weeks after clipping. Horizontal interrupted lines represent intrinsic HR after double blockade with propranolol and methyl atropine. Parentheses indicate the number of observations for sympathetic and vagal tone. +P<0.001 compared to other three groups; *P<0.001 compared to vagal tone; **P<0.001 compared to NC mice.
Bar graph showing the average number of baroreflex sequences per 1000 beats (A), baroreflex sensitivity (gain, B) and baroreflex effectiveness index (BEI, C) from normotensive control (NC) and 1K1C hypertensive mice, one and four weeks after clipping. The number of observations is shown inside the bars. *P<0.001 compared to NC mice.

106x252mm (150 x 150 DPI)
Photomicrographs showing positive angiotensin II stained cells in the renal cortex from normotensive control (NC) and 1K1C hypertensive mice, one and four weeks after clipping. Bar graph shows the average number of positive angiotensin II stained cells per 15 fields in the renal cortex from NC and 1K1C hypertensive mice, one and four weeks after clipping (magnification 280x). The number of observations is shown inside the bars. *P<0.001 compared to other groups.
Representative views of the minor diameter of myocytes from left ventricle from one and four weeks normotensive control (NC) and 1K1C hypertensive mice (black line represents 50 µm). Bar graphs represent the evaluation of the minor diameter of myocytes and the volume fraction of fibrosis from left ventricle from one and four weeks NC and 1K1C hypertensive mice. *P<0.05 compared to NC.
Representative photomicrographs of heart transverse sections of mice from normotensive control (NC) and 1K1C hypertension groups, one and four weeks after clipping (black line represents 2 mm).

190x72mm (150 x 150 DPI)