Changes in hemodynamic and neurohumoral control cause cardiac damage in one-kidney, one-clip hypertensive mice

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Submitted 13 February 2008; accepted in final form 28 September 2008


First published October 1, 2008; doi:10.1152/ajpregu.00107.2008.—Sympathovagal balance and baroreflex control of heart rate (HR) were evaluated during the development (1 and 4 wk) 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 ANG 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 they exhibited elevated sympathetic and reduced vagal tone. The intrinsic HR was elevated only 1 wk after clipping. Systolic AP variability was elevated, while HR variability and baroreflex sensitivity were reduced 1 and 4 wk after clipping. Renal ANG II staining and PRA were elevated only 1 wk after clipping. Concentric cardiac hypertrophy was observed at 1 and 4 wk, while cardiac fibrosis was observed only at 4 wk after clipping. In conclusion, these data further support previous findings in the literature and provide new features of neurohumoral 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 nondependent ANG II hypertension in transgenic mice.

renovascular hypertension; sympathetic tone; vagal tone; intrinsic heart rate; baroreflex

THE UNDERSTANDING OF THE PATHOPHYSIOLOGY of hypertension has been greatly advanced because of 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 have 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, ANG II, has important hemodynamic effects, leading to rise in arterial pressure (AP) (9). Studies from our laboratory (24, 26) demonstrated that the 1st wk 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 1st wk after clipping and a reduced cardiac vagal activity after 4 wk of the onset of 1K1C hypertension (4). It has also been demonstrated that the baroreflex gain was reduced 1 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, 36, 39, 40). 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 et al. (45) adapted the 1K1C hypertensive model to the mouse and demonstrated that, similar to 1K1C hypertension in the rat, the elevation of AP was rapid and progressive. In addition, renal renin expression and plasma renin activity (PRA), markers of RAS activity, were not different in the 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 ANG II was also evaluated (31). It is well documented that augmented plasma ANG II elicits increased staining of this hormone within the kidneys (31, 46). Because we hypothesized that plasma ANG II generation may be augmented in the early phase of 1K1C hypertensive model, renal ANG II staining in the kidney was used as another marker of RAS activity, to provide an indirect inference of plasma ANG II combined with the evaluation of PRA.

Furthermore, it has been hypothesized that sympathetic tone is elevated in 1K1C hypertensive mice, contributing to the

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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 have 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 used 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, the 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, in line with other species, whether 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, weighing 29–31 g, supplied by the Animal Facility of the School of Medicine of Ribeirão Preto, University of São Paulo, São Paulo, Brazil. Mice were housed in a temperature- and humidity-controlled chamber (Alesco Indústria e Comércio Ltda, model 9902-001, Monte Mor, São Paulo, Brazil) with a 12:12-h light-dark cycle. Animals were allowed free access to water and standard chow (Nuvilab CR-1, Nuvital, Colombo, Paraná, Brazil).

Renovascular Hypertension Surgery

Mice were anesthetized by tribromoethanol (250 μg/g ip) 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, São Paulo, 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 im; Veterinary Pentabiotic, Fort Dodge, Campinas, São Paulo, Brazil) and returned to the temperature- and humidity-controlled chamber for full recovery for 1 or 4 wk.

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), kept at 31–33°C, and received an integrated sensor-cuff occluder (model B60–1/4, IITC Life Science) 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 before and weekly after clipping surgery.

Direct measurement. Experiments were carried out 1 or 4 wk after renovascular hypertension or sham surgery. At least 24 h before the experiments, mice were anesthetized with tribromoethanol (250 μg/g ip) and polyethylene catheters (Intramedic; Clay Adams, Parsippany, NJ) 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 Statham transducer (model P23 Db). Pulsatile AP was continuously sampled (4 kHz) using an IBM computer equipped with an analog-to-digital interface (220; Dataq, Akron, OH).

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 iv; Sigma Chemical, St. Louis, MO) was administered. After 15 min, a bolus of a solution with propranolol (4 mg/kg iv; Sigma Chemical) 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 (1 and 4 wk 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 ANG II), and the heart was 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). 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 an 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. 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. 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/~hardal/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 of 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-phenantrolin (30 mM). The blood was centrifuged at 4°C and 3,000 rpm for 15 min. Thirty microliters of plasma plus 970 μl Trix–HCl (50 mM, pH 7.5) and 20 μl of tetradecapeptide (2.0 mmol/ml) were incubated for 2 h. 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 reverse-phase HPLC using an aquaporine ODS 300 column equilibrated with 0.1% phosphoric acid containing 5% acetonitrile (vol/vol). ANG 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 (vol/vol) 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 ANG 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 ANG II

After decapitation and blood collection, the kidney was removed and submitted to immunohistochemistry analysis for tissue ANG II staining. Next, it was cut into transverse sections and stored in a solution of 60% methanol, 30% chloroform, and 10% acetic acid. After 12 h, 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 ANG II polyclonal antibody (Peninsula Laboratories, San Carlos, CA). The reaction product was detected with an avidin-biotin-peroxidase complex (Vector Laboratories, Burlingame, CA). The color reaction was developed with 3,3'-diaminobenzidizine (Sigma Chemical), and the material was counterstained with methyl green, 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 ANG II cells in renal cortical tubulointerstitium, grids fields measuring 0.245 mm² were measured, 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-and-eosin or picrosirius red. For morphometric analysis, hearts stained with hematoxylin-and-eosin were used. The absolute thickness of the left ventricular and septum wall was measured using the public-domain software NIH ImageJ (developed by U.S. National Institutes 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, Cambridge, UK). 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 (×400). For each heart, ~15 fields per region per mouse were randomly selected and analyzed using Leica Qwin software (Leica Imaging Systems). The mean value was subsequently calculated.

Statistical Analysis

All data are presented as means ± SE. Averages of systolic AP indirect measurement comparisons were performed using the two-way ANOVA for repeated measures followed by the Tukey post-test. Averages of basal AP, HR, sympathetic tone, vagal tone, intrinsic HR, systolic AP, HR variability, renal cortex ANG II staining, pRA, weights, and histological analysis comparisons were performed using two-way ANOVA, followed by the Tukey post-test. The HR responses to methyl atropine or propranolol administration were compared using the paired Student’s 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 wk after clipping and was maintained at 159 ± 3 mmHg on the 4th wk. 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, 4 wk 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 4-wk 1K1C hypertensive mice was higher than the MAP of 1-wk 1K1C hypertensive mice. The PAP from 1K1C hypertensive mice 1 and 4 wk 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, 1 and 4 wk 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 Fig. 2, basal HR was elevated 1 and 4 wk after clipping, compared with NC groups, even though a smaller tachycardia was detected 4 wk 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 in MAP, ~10 mmHg, 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 1 and 4 wk after clipping. The combined administration of methyl atropine and propranolol revealed that the intrinsic HR of 1K1C hypertensive mice was elevated only 1 wk after clipping.

Baroreflex Sensitivity

The spontaneous changes in AP were of small amplitude (<10 mmHg) within the periods of observation. Despite the fact that the mean level of AP was higher in 1K1C hypertensive compared with 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 (Fig. 3A) and the baroreflex gain (Fig. 3B), as determined by the sequence method, were significantly reduced in both groups 1 and 4 wk after clipping compared with their NC counterparts.

Arterial Pressure and Heart Rate Variability in Time Domain

Systolic AP variability was elevated in 1K1C hypertensive mice 1 wk (4.3 ± 0.3 vs. 3.1 ± 0.3 mmHg) and 4 wk (5.4 ± 0.5 vs. 3.9 ± 0.2 mmHg) after clipping, compared with NC mice. In addition, 4 wk after clipping, the systolic AP variability was significantly greater than in 1 wk 1K1C hypertensive mice. Nevertheless, 1K1C hypertensive mice presented decreased HR variability 1 wk (11.1 ± 0.7 vs. 16.2 ± 2.1 bpm) and 4 wk (10.3 ± 0.8 vs. 17.7 ± 1.5 bpm) after clipping as well.

PRA

PRA was elevated in 1-wk 1K1C hypertensive mice compared with their NC counterparts (0.53 ± 0.09 vs. 0.25 ± 0.06 nmol·mL⁻¹·h⁻¹). However, 4 wk after clipping, PRA was not different between the 1K1C hypertensive and NC mice (0.22 ± 0.04 vs. 0.30 ± 0.04 nmol·mL⁻¹·h⁻¹).

Immunohistochemistry of ANG II in the Renal Cortex

Immunohistochemical studies (Fig. 4) showed increased ANG II staining in the tubular compartment from the renal cortex of 1K1C hypertensive mice 1 wk after clipping. There was no significant difference between staining for ANG II in the renal cortex of 4 wk 1K1C hypertensive mice compared with their NC counterparts.

Morphometric Analysis

The average minor diameter of the myocytes from left ventricle and septum 1 and 4 wk after clipping was significantly greater than the average minor diameter of their NC counterparts (Table 2 and Fig. 5). The left ventricle and septum free wall thickness were also increased 1 and 4 wk after clipping (Table 2). Picrosirius red-stained sections showed left ventricular myocardial fibrosis only 4 wk after clipping (Fig. 5, bar graphs). Figure 6 illustrates a remarkable concentric hypertrophy in the left ventricle of 1K1C hypertensive mice 1 and 4 wk after clipping.

DISCUSSION

The pathophysiological mechanisms involved during the development of 1K1C hypertension in mice are poorly under-
stood. To our knowledge, this is the first study to examine autonomic function during the development (1 and 4 wk) 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 1st wk after clipping, attaining a plateau during the 3rd and 4th wk. 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 4 wk after clipping. In the present study, PRA and renal staining for ANG II were found to be increased only 1 wk after clipping, suggesting that the RAS is not overactive in chronic 1K1C hypertensive mice. Despite the well-known fact that ANG 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 others (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 1 and 4 wk after clipping. In the present study, the double pharmacological blockade of the autonomic nervous system of 1-wk 1K1C hypertensive mice revealed an increased intrinsic HR. Therefore, this phenomenon might be associated with the higher basal HR observed during this time frame (1 wk). 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 significant decrease in vagal tone to the heart was found in both groups (1 and 4 wk) 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 1-wk 1K1C hypertensive mice may be explained by the increased intrinsic HR during the first wk of 1K1C hypertension.

Fig. 2. Bar graph showing basal heart rate (horizontal black line) and heart rate responses after propranolol or methyl atropine in NC and 1K1C hypertensive mice, 1 and 4 wk 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 with other three groups; *P < 0.001 compared with vagal tone; **P < 0.001 compared with NC mice.
ways (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 cells (29). The underlying intracellular mechanisms that are involved in the chronotropic action of ANG II in the brain are not well established. However, recent studies have shown that ANG 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 1-wk 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 1-wk 1K1C hypertensive mice.

In the present study, a significant decrease in baroreflex control of HR was found from the 1st wk of the development of 1K1C hypertension in mice. Baroreflex sensitivity was evaluated using the sequence method described by Bertinieri et al. (3) and Martinka et al. (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 in which the baroreflex is 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 is 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 1st wk of development of 1K1C hypertension in mice. The low value of BEI observed in the present study is in accordance with previous findings in humans (6) and mice (20), indicating that the 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 is 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 (1 and 4 wk) may reflect the reduced baroreflex gain observed in both periods of hypertension. Moyses et al. (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 over-activity of the sympathetic component, while the vagal component was attenuated afterward. 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 1 and 4 wk after clipping, expressed by the increase in the minor diameter of myocytes and the increase in the wall thickness of the left ventricle and septum. Furthermore, a remarkable concentric hypertrophy characterizes the

![Fig. 3. Bar graph showing the average number of baroreflex sequences per 1,000 beats (A), baroreflex sensitivity (gain, B), and baroreflex effectiveness index (BEI; C) from NC and 1K1C hypertensive mice, 1 and 4 wk after clipping. The number of observations is shown inside the bars. *P < 0.001 compared with NC mice. **P < 0.001 compared to NC mice.](http://ajpregu.physiology.org/)

[Downloaded from http://ajpregu.physiology.org/ by 10.220.33.4 on October 14, 2017](http://ajpregu.physiology.org/)
derangement of the heart (Fig. 6) caused by 1K1C hypertension in mice. In addition, fibrosis was evident 4 wk 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 4 wk) 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 Fig. 1, a remarkable lability of HR is observed in NC mice, while it is reduced in both 1K1C hypertensive groups (1 and 4 wk). 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

**Table 2. Minor diameter of myocytes and wall thickness from left ventricle and interventricular septum from NC and 1K1C hypertensive mice, 1 and 4 wk after surgery**

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<th>One Week</th>
<th>Four Weeks</th>
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<tr>
<td></td>
<td>NC (5)</td>
<td>1K1C (7)</td>
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<td>Wall thickness, mm</td>
<td>LV</td>
<td>1.28±0.03</td>
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<tr>
<td></td>
<td>Septum</td>
<td>1.05±0.06</td>
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<td>1.23±0.06</td>
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<td>1.07±0.03</td>
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<td>Diameter of myocytes, μm</td>
<td>LV</td>
<td>13.6±0.47</td>
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<td></td>
<td>Septum</td>
<td>10.9±0.22</td>
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<td>13.4±0.3</td>
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<td>10.4±0.22</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE. Parentheses show the number of observations. LV, left ventricle. *P < 0.005 compared with NC mice.
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 (1 wk) 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 1-WK 1K1C hypertensive mice.

**Perspectives and Significance**
Most of our understanding about the complex mechanisms involved in arterial hypertension has been obtained from experimental studies performed in several species, such as dog, rabbit, and rat. Nowadays, with the advances in genomic sciences, the mouse is receiving special attention because of its unique amenity to genetic manipulations. Nevertheless, much of the normal physiology, as well as pathophysiological aspects well established for other species, remains unknown for the mouse. The present study provides valuable information regarding neurohumoral features of 1K1C hypertension in mice, which will be certainly useful in future studies relating to autonomic and RAS in genetically manipulated mice.

**ACKNOWLEDGMENTS**
The authors thank Drs. Dulce E. Casarini and Zaira Palomino for plasma renin activity measurement, as well as Dr. Tereza M. Coimbra for measuring of renal ANG II staining. The authors also appreciate the excellent technical assistance of Rubens F. de Melo, Cleonice G. da Silva, and Maria Elena Rial.

**GRANTS**
This work was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico, Fundação de Amparo à Pesquisa do Estado de São Paulo, Coordenação de Apequeroamento de Pessoal de Nível Superior, and Fundação de Apoio ao Ensino, Pesquisa e Assistência do Hospital das Clínicas da Faculdade de Medicina de Ribeirão Preto.

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