Experimental Methods

Antihypertensive Agents Have Different Ability to Modulate Arterial Pressure and Heart Rate Variability in 2K1C Rats

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We examined the effect of chronic (15 days) administration of antihypertensive agents, from different pharmacologic classes, on arterial pressure (AP) and heart rate variability in two-kidney, one-clip hypertensive (2K1C) rats. The 2K1C rats received by gavage one of the following: water, ramipril, losartan, atenolol, amlodipine, or hydrochlorothiazide. Sham-operated normotensive rats received water. After 15 days of treatment AP was continuously sampled from an indwelling catheter in awake rats during a 2-h period and systolic AP and pulse interval (PI) were submitted to autoregressive spectral analysis with oscillatory components quantified in low (LF: 0.25 to 0.75 Hz) and high (HF: 0.75 to 3.0 Hz) frequency bands. The AP measured by tail-cuff was 170 ± 2 mm Hg in 2K1C and 131 ± 3 mm Hg in normotensive rats. Pooled data indicated that all antihypertensive agents reduced the AP of 2K1C rats to 127 ± 2 mm Hg, whereas 2K1C rats treated with water remained hypertensive (206 ± 11 mm Hg). Variance of systolic AP was found increased in 2K1C rats treated with water (34 ± 2 mm Hg²), whereas 2K1C rats treated with ramipril, atenolol, amlodipine, or hydrochlorothiazide presented AP variance similar to normotensive rats (16 ± 2 mm Hg²). Losartan normalized AP of 2K1C rats but variance of systolic AP remained increased (34 ± 7 mm Hg²). The 2K1C rats treated with water had increased LF of systolic AP, whereas 2K1C rats treated with losartan showed higher LF of systolic AP and PI. Atenolol presented lower LF and higher HF of PI. In conclusion, losartan normalized AP but did not reduce AP variability, suggesting an autonomic imbalance characterized by higher sympathetic modulation of the cardiovascular system.


Key Words: Antihypertensive treatment, hypertension, arterial pressure variability, heart rate variability, rat.

In addition to blood pressure (BP)-lowering properties, different medications commonly used in antihypertensive treatment do have cardiovascular or systemic effects. Autonomic modulation of the cardiovascular system can be differently influenced by antihypertensive medications, and may affect the prognosis of hypertensive patients. Cholinergic/adrenergic receptors blockade, as well as measurements of neural activity and baroreflex sensitivity, are useful tools for the assessment of autonomic influence on the heart and vasculature; however, these procedures are invasive and difficult to standardize.

An advance in the study of autonomic function has been provided by the development of methods for the evaluation of heart rate variability (HRV) in both time and frequency domain. Heart rate (HR) fluctuates at regular frequencies, and the magnitude of each oscillation can be accurately quantified by the use of power spectral analysis. Using a simple electrocardiographic (ECG) recording and processing of the HR data collected under resting condition, important information regarding the autonomic influences to the heart can be drawn.

Although BP fluctuations over time have been documented since the 18th century, the clinical importance of this phenomenon has been recognized only recently, making BP variability (BPV) a new concept in cardiovascular medicine. The BPV arises from the development of techniques designed for continuous BP monitoring, and it has been proposed that instability of BP may produce organ damage that is associated with severity of underlying diseases. In hypertension, BPV increases with increasing BP and correlates closely with target organ damage, independently of the absolute level of arterial pressure (AP). This
nformation has important outcomes for treatment of hypertensive patients. In the past antihypertensive treatment focused primarily on the reduction of the absolute value of AP. Experimental evidence suggests that drugs capable of buffering or reducing BPV may provide additional benefits on target organ protection. It is now accepted that the goal of antihypertensive treatment should consider not only the reduction of the absolute level of AP, but also its variability.

It is well documented that BPV is high in experimental models of hypertension, particularly in renovascular hypertensive rats. Studies evaluating the effect of different classes of antihypertensive agents on cardiac autonomic modulation, as well as in BPV in experimental hypertension, were not found in the literature. Therefore, the aim of the present study was to evaluate the effect of chronic administration of antihypertensive agents, from different pharmacologic classes, on BPV and HRV in two-kidney, one-clip hypertensive (2K1C) rats.

**Methods**

Male Wistar rats (170 to 190 g) were anesthetized with trichlorethanol (2.5 mg/kg, intraperitoneally) and 2K1C hypertension was surgically induced by means of partial constriction of the main left renal artery with a silver clip with a 0.20-mm gap. Sham-operated normotensive rats had their left renal artery isolated without receiving the clip.

Thirty days after surgical procedures AP was indirectly measured (tail-cuff method) to monitor the development of hypertension. Only 2K1C rats with AP more than 150 mm Hg were included in the protocol and received by gavage (1 mL/d), for 15 days, one of the following treatments: tap water (n = 8), ramipril (2 mg/kg/d, n = 8), losartan (10 mg/kg/d, n = 8), amlodipine (10 mg/kg/d, n = 7), or hydrochlorothiazide (20 mg/kg/d, n = 8). One group (n = 13) of sham-operated normotensive rats received tap water by gavage (1 mL/d). The treatments were carried out always between 8 and 9 AM.

After 15 days of treatment, the animals were anesthetized (trichloroethanol, 2.5 mg/kg intraperitoneally) and a polyethylene catheter was inserted into the femoral artery, and exteriorized in the back to allow direct measurement of AP in the conscious state. On the following day, after receiving the corresponding treatment, the rats were taken to the recording room at least 1 h before the beginning of the experiment, and a quiet environment was maintained to avoid stress. The arterial catheter was connected to a pressure transducer (Statham P23Gb, Hato Rey, Puerto Rico) and pulsatile AP was continuously sampled (1 kHz), during 2 h, on an IBM/PC equipped with an analog-to-digital interface (DI220 Dataq, Akron, OH). The files were stored and the data were analyzed later.

Pulsatile AP recordings were analyzed by means of customized computer software designed to detect inflection points of a periodic wave. The graphic interface of the analysis software allowed visual inspection of AP recordings and manual editing of spurious events. Beat-by-beat time series of systolic AP were generated. In addition, series of pulse interval (PI) were obtained by measurement of the intervals between consecutive systolic AP values.

Because we did not perform ECG recordings, PI was used as a measure of HR and its variability. Series of systolic AP and PI were divided into contiguous segments of 300 beats overlapped by 50% and visually inspected to exclude nonstationary changes in systolic AP and PI (eg. slow trends and sharp changes). All stationary segments of time series had their mean and variance calculated and submitted to a model-based autoregressive spectral analysis as described elsewhere. Briefly, autoregressive spectral analysis was performed with parameters estimated with Levinson-Durbin recursion, with model order chosen according to Akaike’s criterion. The power of each relevant oscillatory component in absolute, as well as in normalized units, was quantified in low (LF: 0.25 to 0.75 Hz) and high (HF: 0.75 to 3.0 Hz) frequency bands. The normalization procedure was performed by dividing the power of the LF or HF component by the total spectral power, from which the power of oscillations slower than 0.25 Hz (very low frequency) had been subtracted.

**Statistical Analysis**

Results are presented as mean ± SEM. Baseline values of systolic AP and PI were compared, between groups, by one-way analysis of variance. If differences were found, a Bonferroni-corrected test was used to determine the differences between groups. Variance, LF, and HF power of systolic AP and PI were compared, between groups, using the nonparametric one-way analysis of variance on ranks.

If differences were found the post hoc Mann-Whitney test was applied. Differences were considered statistically significant at P < .05.

**Results**

Systolic AP measured indirectly 30 days after surgery to induce arterial hypertension was 170 ± 2 mm Hg in 2K1C (n = 40) and 131 ± 3 mm Hg in sham-operated rats (n = 13).

Basal levels and overall variance of systolic AP and PI are shown in Fig. 1. All antihypertensive drugs reduced AP of 2K1C rats to normotensive levels, providing values similar to that found in sham-operated rats, whereas 2K1C rats treated with water remained hypertensive. Only 2K1C rats treated with atenolol presented lower basal HR. Variance of systolic AP was found increased in 2K1C rats treated with water, whereas 2K1C rats treated with ramipril, atenolol, amlodipine, or hydrochlorothiazide presented AP variance similar to sham-operated normotensive rats. Nevertheless, despite that losartan was able to normalize the AP of 2K1C rats, the variance of systolic AP of these animals remained high, showing values similar to those
found in 2K1C rats treated with water. Variance of PI did not differ among the groups studied.

Data from spectral analysis of systolic AP and PI of the different groups are presented in Table 1. The 2K1C rats treated with water presented increased power of LF oscillations of systolic AP as compared to sham-operated normotensive rats. The 2K1C rats treated with losartan also showed higher LF power not only of systolic AP but also of PI variability as compared to sham-operated normotensive or to 2K1C rats treated with the other antihypertensive agents. The HF power of systolic AP was similar among groups. The 2K1C rats treated with atenolol presented lower LF and higher HF power of PI as compared to the other groups. In terms of normalized units 2K1C rats treated with losartan also showed lower HF power of PI oscillations.

**Discussion**

The 2K1C rats treated with water were remarkably hypertensive on the day of the experiment. However, chronic treatment with any antihypertensive agent normalized AP of 2K1C rats. The HR was found similar in 2K1C rats and sham-operated normotensive rats. The β-adrenergic blocker atenolol was the only antihypertensive agent that changed HR, producing resting bradycardia in 2K1C rats.

In the present study, as expected, variance of systolic AP was found increased in 2K1C rats. Taking into consideration that previous observations indicated that BPV is elevated in clinical and experimental hypertension, increased BPV seems to be a very common phenomenon in arterial hypertension. Normalization of AP was accompanied by reduction of BPV in all antihypertensive treatment...
groups, except losartan. Although losartan normalized the AP of 2K1C rats, the variance of AP of these subjects remained as high as the variance of AP of 2K1C rats treated with water.

Spectral analysis showed two major oscillatory components in distinct frequencies for either systolic AP or PI spectra. The 2K1C rats treated with water showed increased power of AP oscillations at LF ranges as compared to normotensive sham-operated normotensive rats. The 2K1C rats treated with losartan presented not only an augmented variance, but also a higher power of LF oscillations of systolic AP. The HF power of systolic AP did not differ among groups. The AP oscillations at the respiratory frequency (HF power) are linked with rhythmic changes of intrathoracic pressure due to respiratory mechanics.10 The LF fluctuations of AP, often identified as Mayer’s waves, are the major contributor to the overall variance of this parameter and are associated with efferent sympathetic activity of the vasculature.10 The involvement of the sympathetic nervous system on the genesis of LF power of BPV is widely accepted, as high spinal cord transection, or ganglionic blockade abolish these oscillations.11,12 In line with the results obtained in the present study Ponchon and co-workers13,14 demonstrated that losartan decreased systolic AP of 2K1C rats, without affecting the increased oscillatory component of AP in the range of 0.2 to 0.6 Hz. Despite clinical and experimental evidence indicating that blockade of AT1 receptors reduces sympathetic activity,15 maintenance of a higher power of LF oscillations of systolic AP in 2K1C rats after chronic treatment with losartan is at least intriguing. Nevertheless, when the effects of several AT1 receptor antagonists (ie, losartan, valsartan, ibesartan, and eprosartan) were evaluated on pressor responses evoked by activation of the sympathetic outflow through spinal cord stimulation in pithed rats, eprosartan was the only AT1 receptor antagonist that inhibited the pressor response.16 On the other hand, eprosartan did not blunt sympathetic activation caused by lower body negative pressure or mental stress in young male subjects.17 These findings clearly show the diversity of effect of AT1 receptor antagonists, which might explain, at least partially, the lack of effect of losartan on the increased LF power of systolic AP in 2K1C rats.

The HRV examined by spectral analysis showed no difference when 2K1C rats treated with water was compared with sham-operated normotensive rats. Chronic treatment of 2K1C rats with atenolol reduced the LF power of PI spectra in both absolute values (in milliseconds squared) and normalized units, whereas it increased the HF power of PI only in normalized units. Losartan did the opposite, that is, increased the LF power of PI in absolute and normalized units and decreased the HF power of PI only in normalized units. The interpretation of HRV spectra is more complex than BPV spectra because the first takes into account the interplay of sympathetic and vagal influence on the heart. It is accepted that respiratory sinus arrhythmia (HF power) depends on vagal activity with a very small influence of the sympathetic drive.18 On the other hand, LF oscillations of PI certainly involves a major participation of the sympathetic drive,18,19 but vagal modulation of the heart should be also taken into account.20,21 Accordingly, the baroreflex control of HR does also contribute to the power of LF oscillations of PI.22

Despite difficulties in the assessment of BPV, particularly with noninvasive techniques,23 strong evidence points to an independent and positive relationship between the extent of target organ damage and the magnitude of BPV in essential hypertension.24 Moreover, BPV has been taken as an independent predictor for cardiovascular mortality in the general population.25 Interestingly, Mancia et al26 reported data of intra-arterial pressure monitoring, indicating that hypertensive patients present steeper fast- and short-duration beat-to-beat AP changes when compared with normotensive subjects. Experimental studies have suggested that the traumatic effect of intravascular pressure on the vessel wall, resulting in vascular remodeling and atherosclerosis, may be more closely associated with oscillatory than steady laminar shear stress.27 Therefore, the goal of antihypertensive treatment should consider not only the reduction of mean AP but
also its variability, and antihypertensive therapy should induce smooth and sustained AP control throughout the 24-h dosing interval.\textsuperscript{28,29}

In conclusion, the data obtained in the present study from both BPV and HRV allow speculation that 2K1C rats treated chronically with losartan present an autonomic imbalance characterized by higher sympathetic modulation of the cardiovascular system. With respect to chronic treatment with the other antihypertensive agents, data from BPV and HRV preclude the possibility of an autonomic imbalance. Further experiments are necessary to elucidate why the blockade of angiotensin AT\textsubscript{1} receptors with losartan normalized BP, but failed to decrease BP variability of 2K1C rats.

References