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Blood pressure variability is increased in genetic hypertension and *L*-NAME-induced hypertension[1]

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KEY WORDS hypertension; blood pressure; nitric oxide; enzyme inhibitors; rats

ABSTRACT

AIM: To examine whether the blood pressure variability (BPV) is increased in spontaneously hypertensive rats (SHR) and *L*-NAME-induced hypertensive rats (NHR). **METHODS**: BPV was recorded with continuous hemodynamic monitoring in conscious unrestrained rats. Time course of *L*-NAME-induced hypertension was measured by the tail-cuff method. Plasma NO concentration was determined by the method of nitric acid reductase. **RESULTS**: In both SHR and NHR, systolic and diastolic BPV were significantly increased when compared with their respective controls. In SHR, increase in diastolic BPV was predominant, whereas in NHR, increase in systolic BPV was predominant. Moreover, increase in systolic BPV in NHR (102 %) was obviously higher than that in SHR (28 %). Chronic administration of *L*-NAME 1 g/L in drinking water caused a progressive increase in arterial blood pressure in rats. All rats were hypertensive at 4 weeks after treatment. Plasma NO level was decreased in NHR. **CONCLUSION**: Increased BPV is a general phenomenon in hypertension. NO is involved in the regulation of BPV.

INTRODUCTION

Blood pressure variability (BPV) is a new concept in cardiovascular medicine, arising from the development of techniques designed for continuous blood pressure (BP) monitoring. BPV is higher in hypertensive than normotensive patients[1]. This increased BPV has been linked with the severity of the underlying disease. For example, among hypertensive patients with similar BP levels, the level of hypertensive cardiovascular structural damage has been shown to be more advanced in those with the highest levels of BPV[2]. BPV is also higher in genetically hypertensive (LH) rats of the Lyon strain when compared with normotensive (LN) and low BP (LL) rats of the Lyon strain[3]. So we hypothesized that high BPV might be a general phenomenon in hypertension. To verify the hypothesis, we observed BPV in the other two kinds of hypertensive models, a genetic model of spontaneously hypertensive rats (SHR) and an experimental model of hypertension induced by chronic inhibition of nitric oxide (NO) synthesis in rats[4].

MATERIALS AND METHODS

Materials Twenty-six-week-old male SHR and Wistar-Kyoto (WKY) rats were provided by the Animal House of our Department. Systolic BP was measured by the tail-cuff method[5]. Only those SHR with systolic BP more than 21.3 kPa (160 mmHg) were used in the study. Body weight was lower in SHR (331 g ± 18 g, $n = 8$) than in WKY rats (439 g ± 60 g, $n = 5$). Ten-week-old male Sprague-Dawley (SD) rats weighing 307 g ± 8 g ($n = 16$) were purchased from Sino-British SIPPR/BK Lab Animal Ltd (Certificate 02-25-3). N^{ω}-nitro-*L*-arginine methyl ester (*L*-NAME), an inhibitor of nitric oxide synthase (NOS), was purchased from Sigma, St Louis, USA.

Preparation of *L*-NAME-induced hypertension in rats (NHR) Sixteen SD rats were randomly divided into two groups. Control group received no treatment. *L*-NAME group received *L*-NAME 1 g/L in drinking water for 4 weeks. Systolic BP and heart rate (HR) were measured every week by the tail-cuff method[5]. At 4 weeks after treatment, body weight was lower in *L*-NAME group (366 g ± 40 g, $n = 8$) than in control group (403 g ± 18 g, $n = 8$).

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Hemodynamic monitoring in conscious unrestrained rats The rat was anaesthetized with a solution of ketamine (50 mg/kg) and diazepam (5 mg/kg), ip. A polyethylene catheter (PE-10 connectee to PE-50) was chronically placed into the lower abdominal aorta via the left femoral artery for measurement of BP and HR. The catheter was tunneled subcutaneously, exteriorized between the scapulae, and fixed on the saddle. More than 24 h later, the rat was placed in a cylindrical cage with controlled temperature (23 ℃ ± 1 ℃) and lighting conditions. The aortic catheter was connected to a BP transducer coupled to a recorder by means of a three-way stopcock that allows continuous perfusion of the arterial catheter with heparinized (0.02 IU/L) isotonic glucose (0.5 mL/h). With the swivel, the rat could freely move. Then BP was continuously recorded over a 3-h period with a computerized technique[3,6]. Briefly, the BP signals, transmitted to the electric signals by a transducer, were digitized and processed by a personal computer, which calculated on line the BP and HR. These values were sampled beat to beat and stored on hard disk. In off-line analysis, the means and standard deviations of BP and HR over the last 1-h period were calculated. BPV was expressed by the standard deviation of BP recorded during the last 1-h period.

Plasma NO measurement More than 24 h after BP measurement, the rats from *L*-NAME group and corresponding control group were killed by decapitation. Blood sample was collected in prechilled tube containing ethylenediaminetetraacetic acid-disodium salt (Na_2-edetic acid; 1 g/L whole blood) and centrifuged for 15 min at 3200 × *g* and 4 ℃. Plasma was stored at −20 ℃ before assay. Plasma samples were sent to Nanjing Biomedical Engineering Institute for measurement of NO concentration. Plasma NO concentration was determined by the method of nitric acid reductase.

Data analysis All data are reported as $\bar{x} \pm s$. Statistical significance was judged at $P < 0.05$. Statistical analysis was performed with unpaired *t*-test using statistical program, SAS.

RESULTS

BP and BPV in SHR and NHR Hemodynamic parameters obtained continuously in conscious unrestrained rats are summarized in Tab 1 and Tab 2. In both SHR (Tab 1) and NHR (Tab 2), systolic and diastolic BP and BPV were significantly increased when compared with their respective controls. There were no significant differences in HR between hypertensive and normotensive rats. In SHR, systolic and diastolic BPV were increased by 28 % and 61 %, respectively, and in NHR, they were increased by 102 % and 77 %, respectively. That is, in SHR increase in systolic BPV was lower than increase in diastolic BPV, whereas in NHR increase in systolic BPV was higher than increase in diastolic BPV. In addition, increase in systolic BPV in NHR was obviously higher than that in SHR.

Tab 1. Hemodynamic data in genetic hypertension. $n = 5$ **in each group.** $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* **WKY.**

	WKY	SHR	SHR/WKY
Systolic BP (kPa)	18.8 ± 1.1	24.0 ± 2.0^c	1.28
Diastolic BP (kPa)	13.0 ± 0.7	18.2 ± 1.8^c	1.40
Systolic BPV (kPa)	1.09 ± 0.22	1.40 ± 0.20^b	1.28
Diastolic BPV (kPa)	0.84 ± 0.13	1.4 ± 0.5^b	1.61
HR (beats/min)	353 ± 53	334 ± 24	0.95

Tab 2. Hemodynamic data in *L*-NAME-induced hypertension. $n = 6$ **in each group.** $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* **control.**

	Control	*L*-NAME	*L*-NAME/ Control
Systolic BP (kPa)	19.4 ± 1.1	26.4 ± 2.0^c	1.36
Diastolic BP (kPa)	13.5 ± 1.8	19.0 ± 2.9^c	1.41
Systolic BPV (kPa)	0.85 ± 0.09	1.7 ± 0.6^c	2.02
Diastolic BPV (kPa)	0.74 ± 0.12	1.3 ± 0.4^b	1.77
HR (beats/min)	375 ± 23	378 ± 29	1.01

Development of hypertension and plasma NO level in *L*-NAME-treated rats To observe the development of hypertension induced by *L*-NAME, we examined systolic BP and HR weekly by the tail-cuff method. Chronic oral administration of *L*-NAME 1 g/L in drinking water caused a time-dependent increase in systolic BP in SD rats (Fig 1). HR remained unchanged during the treatment period (data not shown). *L*-NAME-induced hypertension occurred at 2 weeks after treatment, and progressively developed at 3 and 4 weeks after treatment. Moreover, all treated rats were hypertensive after 4 weeks of treatment, ie, their systolic BP were more than 21.3 kPa (160 mmHg). At the end of the experiment, plasma NO levels were determined in *L*-NAME-treated rats and untreated SD rats. There was a significant decrease in plasma NO concentration after chronic administration of *L*-NAME (Fig 2).

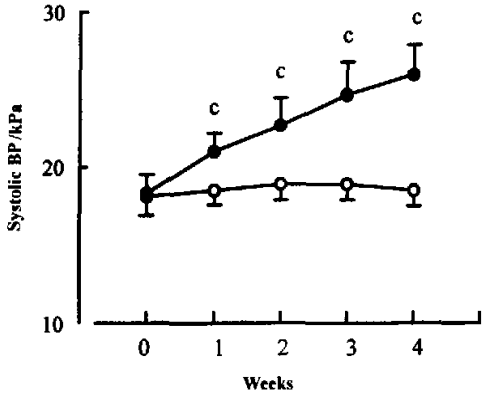

Fig 1. Time-related changes in systolic blood pressure in SD rats (○, Control) and *L*-NAME-treated rats (●, *L*-NAME 1 g/L in drinking water). $n = 8$ in each group. $\bar{x} \pm s$. $^cP < 0.01$ *vs* control.

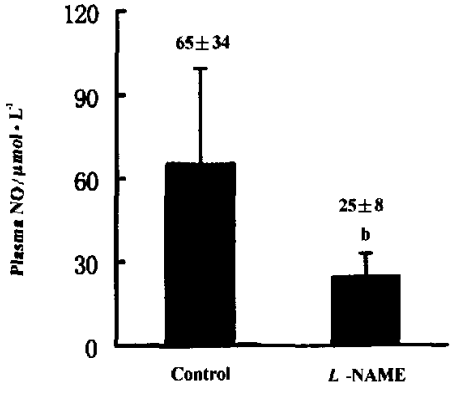

Fig 2. Plasma NO concentration in SD rats (Control) and *L*-NAME-treated rats (*L*-NAME). $n = 5$ in each group. $\bar{x} \pm s$. $^bP < 0.05$ *vs* control.

DISCUSSION

The major novel finding of the present study is that BPV was increased in both genetic (SHR) and experimental (NHR) hypertensive rats. Combined with previous results that BPV is higher in hypertensive patients[1] and LH rats[3], it is concluded that increased BPV is a general phenomenon in hypertension. This conclusion is very important for further study of the mechanisms for target organ damage in hypertension, since some clinical studies have shown that the severity of target organ damage in hypertensive patients correlates positively with both BP and BPV[2,7,8]. Comparing the extent of increase in systolic and diastolic BPV, we found that in SHR increase in diastolic BPV was predominant, whereas in NHR increase in systolic BPV was predominant. Moreover, increase in systolic BPV in NHR was obviously higher than that in SHR. These differences may have two possible explanations: (1) They may be the characteristic differences between genetic and experimental models of hypertension. It has been reported that increase in diastolic BPV is predominant in genetically LH rats[3]. (2) The evolution with age (or the time after treatment) of BPV may occur in hypertensive rats.

The mechanism by which BPV is increased in hypertension is not very clear at the present time. Earlier studies have shown that impaired baroreflex function is related to increased BPV in essential hypertension[9]. On the other hand, many evidences have demonstrated that BPV is not dependent on the BP level. First, chronic sinoaortic denervation in rats can produce a substantial increase in BPV with no change in the mean level of BP[10]. Second, rabbit exhibits a lower BP level and a higher BPV in physiological condition, when compared with rat[11]. Third, BPV can be attenuated by adenosine infusion even when BP is maintained at control levels by simultaneous infusion of phenylephrine[12]. This effect of adenosine on BPV is mediated by adenosine A_{2a} receptor[13]. Fourth, the effects of some agents on BPV are clearly dissociated from their effects on BP. For example, parachloroamphetamine raised BP level but reduced BPV[14]. On the contrary, hydralazine decreased BP level but increased BPV[15]. In the present study, chronic oral administration of *L*-NAME, an inhibitor of nitric oxide synthase (NOS), increased BPV in rats. Nafz *et al*[16] reported that BPV was increased by acute intravenous bolus of *L*-NAME in rats. These indicate that endogenous NO is involved in the regulation of BPV. Recently, Stauss *et al*[17] found that blood pressure buffering effect of endogenous NO is mediated by the endothelial isoform of NOS. It is well known that shear stress is a potent stimulus for the formation and release of NO. It seems, therefore, possible that a transient increase in BP, which leads to a concomitant rise in endothelial shear stress, stimulates NO release. The latter induces a relaxation of blood vessels that, in turn, counteracts the initial increase in BP. Thus this chain of events may constitute a negative feedback loop reducing BPV. The present study supports this hypothesis, since in *L*-NAME-treated rats, the loop was damaged by inhibition of NO synthesis, and an enhanced BPV was observed at the same time. Also, we confirmed that

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chronic administration of *L*-NAME caused systemic arterial hypertension in rats. It is reasonable to assume that the arterial hypertension observed in these rats was due to the decreased NO synthesis, because this study found a decrease in plasma NO level in the *L*-NAME-treated rats, and a previous study showed that the *L*-NAME-induced hypertension in rats was reversed or prevented by short-term adminsitration of excess *L*-arginine but not by *D*-arginine[18]. In our study, *L*-NAME 1 g/L in drinking water for 4 weeks produced a 100 % success in preparation of hypertensive rats. By comparison, for preparation of DOCA-salt hypertensive rats and renovascular hypertensive rats, the methods were more complex, and only about 70 % of rats were hypertensive[5]. Obviously, NHR as a new model of hypertension possesses two advantages: (1) It is easy to prepare. (2) The incidence of hypertension is very high.

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遗传性高血压大鼠和 *L*-NAME 性高血压大鼠的血压波动性升高[1]

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关键词 高血压;血压;一氧化氮;酶抑制剂;大鼠

目的: 观察自发性高血压大鼠(SHR)和 *L*-NAME 性高血压大鼠(NHR)的血压波动性(BPV). **方法**: 清醒自由活动大鼠连续血压监测技术测定 BPV. 尾套法观察 *L*-NAME 引起的升压过程,硝酸还原酶法测定 NO 浓度. **结果**: 两种高血压大鼠的收缩压波动性(SBPV)和舒张压波动性(DBPV)均升高. 在 SHR,DBPV 升高较多. 而在 NHR,SBPV 升高较多. 而且,NHR 的 SBPV 升高(102 %)明显高于 SHR(28 %). 饮用水中加 *L*-NAME 1 g/L 使大鼠血压进行性升高,4 周时 100 %造成高血压. NHR 的血浆 NO 水平下降. **结论**: BPV 升高是高血压的普遍现象. NO 参与 BPV 的调控.

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