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Determination of arterial baroreflex-blood pressure control in conscious rats[1]

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KEY WORDS anesthesia; baroreflex; blood pressure; blood pressure variability; inbred SHR rats

ABSTRACT

AIM: To study the determination of arterial baroreflex-blood pressure control (ABR-BP) in conscious rats. **METHODS**: Blood pressure was continuously recorded with a computerized system in conscious freely moving rats. The principle of ABR-BP measurement is to compare the pressor response to a vasoactive drug (angiotensin Ⅱ) before and after the interruption of this reflex. **RESULTS**: (1) ABR-BP values revealed by angiotensin Ⅱ were closely correlated with those by phenylephrine. Doses of angiotensin Ⅱ did not influence the results within certain range. (2) ABR-BP was well correlated with arterial baroreflex-heart period control (ABR-HP). (3) Anesthesia inhibited ABR-BP. There existed a circadian variation of ABR-BP in WKY rats. (4) Blood pressure variability was closely related to ABR-BP, but not to ABR-HP. (5) ABR-BP was impaired in hypertensive rats. **CONCLUSION**: ABR-BP is an important parameter to reflect the function of ABR. The present work makes it possible to determine ABR-BP in conscious rats. ABR-BP plays an important role in maintaining blood pressure stability and it is impaired in hypertension.

INTRODUCTION

Arterial baroreflex (ABR) plays a crucial role in the regulation of cardiovascular activity. Its main function is to maintain the stability of blood pressure (BP)[1,2]. Most studies to the function of ABR have been performed in a variety of anesthetized animal species. It is well known that general anesthesia affects the function of ABR and there is increasing interest to study the role of ABR in conscious animals[3,4]. From beginning of 1980s, several methods have been available for measuring the function of ABR in conscious freely moving rats. The classic method widely used was proposed by Smyth *et al* for human[5-8]. A dose of phenylephrine (Phe) or angiotensin Ⅱ (Ang Ⅱ) is injected iv to produce an increase in BP. Arterial baroreceptors are activated to return the increased BP to initial level. Vasodilatation and decrease in cardiac output are involved in this reflex response. However, the only detectable parameter in conscious animals is the decrease in heart rate or the prolongation of heart period (HP). HP is plotted against with systolic blood pressure (SBP) for linear regression analysis, the slope of SBP-HP is defined as baroreflex sensitivity[7]. We use the term of arterial baroreflex-heart period control (ABR-HP), in lieu of baroreflex sensitivity, to describe the relationship between the increase in BP and the prolongation of HP. Although there are many methods for measuring ABR function in conscious animals, the function measured by these methods is always ABR-HP[6-9]. In the present study, we established a new method to determine the arterial baroreflex-blood pressure control (ABR-BP).

MATERIALS AND METHODS

Animals and animal preparations Spontaneously hypertensive rats (SHR) and Wistar Kyoto (WKY) rats (10-15 weeks old, 250-320 g, Grade Ⅱ, Certificate № 0566) were provided by Shanghai Institute of Hypertension Research. Male animals were used in all experiments and females were used only to study the sex-related difference in ABR-BP. Hybrid offspring rats (16 weeks old) were the first generation (F1) from SHR and WKY rats. Renovascular hypertension was prepared with classic 2K1C method in male WKY rats[10]. All animals used in this work were

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housed under controlled conditions (temperature: 21 ℃ ± 1 ℃ and lighting: 8:00 – 20:00) with food and water *ad libitum*[7].

BP measurement SBP, diastolic BP (DBP), and HP were continuously recorded using a previously described technique with some modifications[7,11]. Firstly, rats were anesthetized with a combination of ketamine (40 mg/kg) and diazepam (6 mg/kg). A floating polyethylene catheter was inserted into the lower abdominal aorta via femoral artery for BP measurement, and another catheter was brought into the jugular vein for iv injections. After a 2-d recovery period, the animals were placed in individual cylindrical cages containing food and water. The aortic catheter was connected to a BP transducer via a rotating swivel that allowed the animals to move freely in the cage. The BP signal was digitized by a microcomputer, and SBP, DBP, and HP values were determined beat by beat on line. In the study of the relationship between blood presure variability (BPV) and ABR function, the standard deviation over the mean of 24 h SBP levels was calculated with our previously described method and defined as the quantitative parameter of BPV[11~15].

ABR-HP measurement A bolus injection of Ang Ⅱ (Sigma) was used to induce an elevation of BP, and its dose was adjusted to raise SBP between 20 – 40 mmHg. HP was plotted against with SBP with 5 shifts (ie. SBP_1/HP_6, SBP_2/HP_7, *etc*) for linear regression analysis, and the slope of SBP-HP was expressed as ABR-HP[7,11].

ABR-BP measurement The principle of the measurement of ABR-BP is to compare the pressor response to a vasoactive drug before and after the interruption of the baroreflex. The pressor response was expressed as the area under pressor curves (AUC, mmHg · s), and the interruption was realized by means of blocking the baroreflex efferent pathway by using guanethidine (Gua, Sigma, 10 mg/kg, iv, and a semi-dose was given 45 min late) and methyl-atropine (MA, Sigma, 1 mg/kg, iv, 20 min after the second dose of guanethidine)[16]. The protocols are showed in Fig 1. The pressor responses (AUC) to a bolus iv injection of Ang Ⅱ (20 ng/kg) were determined before (A_1) and after (A_2) blockade of the efferent pathway. ABR-BP was calculated by the formula: ABR-BP (%) = $(A_2 - A_1)/A_2 \times 100$. The venous catheter should be filled with Ang Ⅱ solution before injection and then a fixed volume of Ang Ⅱ was injected with a constant velocity. The AUC (A_1 or A_2) was calculated by computer.

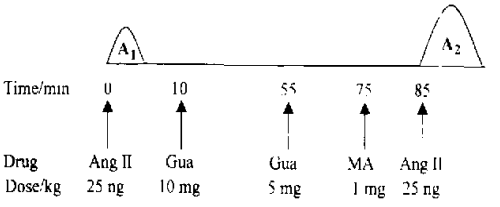

Fig 1. Schematic of protocols of determining arterial baroreflex-blood pressure control (ABR-BP). A_1 and A_2 indicate area under the curve (AUC) of pressor response to Ang Ⅱ.

ABR-BP values revealed by different vasoactive drugs and doses Firstly, three vasoactive drugs, Ang Ⅱ, Phe (Sigma), and sodium nitroprusside (SNP, Sigma) were used for measuring ABR-BP in 10 WKY rats. Secondly, ABR-BP was determined with 5 different doses of Ang Ⅱ (2, 5, 10, 20, and 50 ng/kg) and 5 doses of Phe (0.5, 1, 2, 5, and 10 ug/kg) in adult WKY rats ($n = 6$ and 10 respectively for Ang Ⅱ and Phe).

Relationship between ABR-BP and ABR-HP Three groups of rats were used: SHR, WKY rats, and the hybrid offspring of SHR and WKY rats. They were undergone both ABR-BP and ABR-HP measurements.

Factors influencing ABR-BP values

(1) Influence of anesthesia on ABR-BP: Urethane (1.0 g/kg) or pentobarbital (45 mg/kg) was used as anesthetic. Rats were divided into three groups: one in conscious state and the others in anesthetic condition. (2) The circadian variation of ABR-BP in WKY rats: Thirty-six male WKY rats were divided into six time groups ($n = 6$). ABR-BP was measured at 7:00, 11:00, 15:00, 19:00, 23:00, and 3:00. (3) Sex-related difference in ABR-BP: ABR-BP was measured in male ($n = 13$) and female ($n = 11$) WKY rats. All these measurements were carried out at the same time (10:00 – 12:00).

Relationship between ABR function and BPV BP and HP were recorded for 24 h. After 24 h (8:00 – 8:00), ABR-BP and ABR-HP were determined (10:00 – 12:00). The linear correlation was calculated between BPV and ABR-BP or ABR-HP.

ABR-BP and hypertension ABR-BP and ABR-HP were measured in three group rats: SHR, renohypertensive rats, and WKY normotensive rats. In

addition, BP values and ABR function were studied in the group of 19 hybrid offspring rats.

Statistical analysis Data were expressed as $\bar{x} \pm s$. Comparisons between 2 groups were made by unpaired t-test and one-way ANOVA analysis was used when comparisons were made among more than 2 groups. $P < 0.05$ was considered statistically significant.

RESULTS

ABR-BP values revealed with different vasoactive agents and different doses It was found that ABR-BP values revealed with Phe were the highest (82 % ± 4 %) and those with SNP were the lowest (60 % ± 6 %). ABR-BP values revealed with Ang Ⅱ (77 % ± 6 %) were slightly lower than those with Phe ($P < 0.05$) and higher than those with SNP ($P < 0.01$). There existed a close correlation between ABR-BP values revealed with Phe and Ang Ⅱ ($r = 0.796$, $P < 0.01$). However, ABR-BP values revealed with SNP related neither to those with Phe nor to those with Ang Ⅱ (Fig 2). In another experiment, ABR-BP values obtained with the first 4 doses of Ang Ⅱ (2 – 20 ng/kg) were very similar and those with the fifth dose (40 ng/kg) were slightly lower (Tab 1). A similar result was obtained when Phe was used as vasoactive agent.

Tab 1. ABR-BP values measured with different doses of Angiotensin Ⅱ (Ang Ⅱ, $n = 6$) and phenylephrine (Phe, $n = 10$) in WKY rats. $\bar{x} \pm s$.

Ang Ⅱ / ng·kg^{-1}	ABR-BP / %	Phe / μg·kg^{-1}	ABR-BP / %
2	74 ± 8	0.5	89 ± 5
5	73 ± 15	1	86 ± 9
10	72 ± 13	2	85 ± 4
20	75 ± 9	5	86 ± 7
40	64 ± 14	10	83.4 ± 2.5

Relationship between ABR-BP and ABR-HP In a group of consisting with SHR and WKY rats, there existed a significantly positive correlation between ABR-BP and ABR-HP ($n = 17$, $r = 0.858$, $P < 0.01$). However, if the linear correlations were analyzed separately to SHR and WKY rats, no significant correlation could be found between ABR-BP and ABR-HP ($n = 10$, $r = 0.234$, $P > 0.05$ in WKY rats group; $n = 7$, $r = 0.466$, $P > 0.05$ in SHR group). In the group of hybrid offspring of SHR and WKY rats, there also existed a significant correlation between ABR-BP and ABR-HP ($n = 19$, $r = 0.626$, $P < 0.01$) (Fig 3).

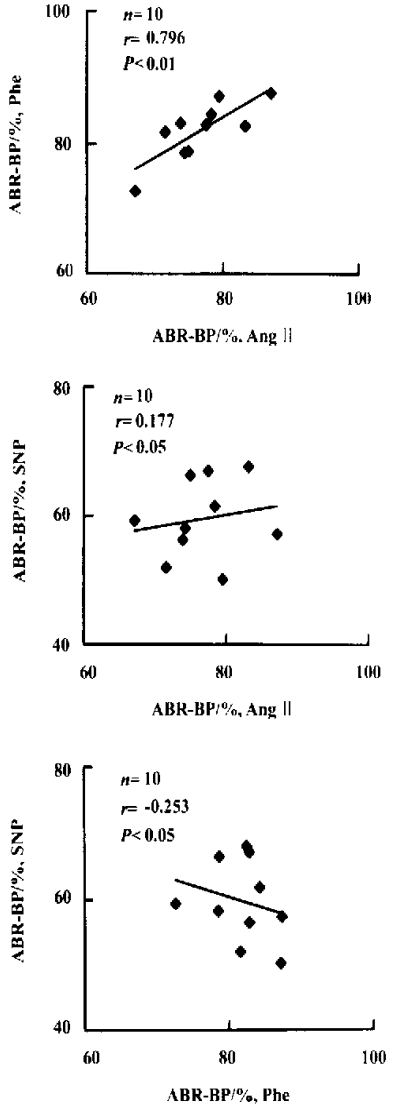

Fig 2. Relationships between ABR-BP values revealed with different vasoactive agents: angiontensin Ⅱ (Ang Ⅱ), phenylephrine (Phe), and sodium nitroprusside (SNP).

Factors influencing ABR-BP values

Influence of anesthesia on ABR-BP Anesthesia depressed ABR-BP. This influence was greater in rats anesthetized with urethane than that with pentobarbital.

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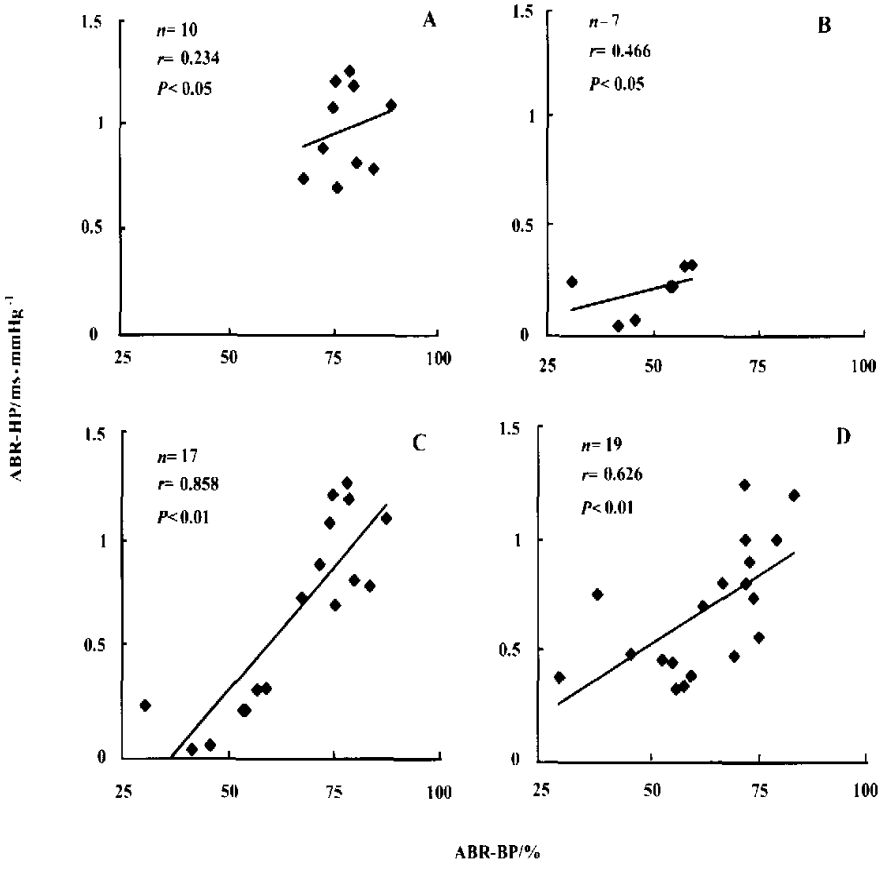

Fig 3. Relationships between ABR-BP and ABR-HP in WKY rats (A), SHR (B), SHR and WKY rats (C), and hybrid offspring of SHR and WKY rats (D).

ABR-BP values were as follows: (73 ± 7)% in conscious control rats (n = 8), (32 ± 6)% in rats anesthetized with urethane (P < 0.01 *vs* control), and (55 ± 6)% in rats anesthetized with pentobarbital (P < 0.01 *vs* control).

Circadian variation of ABR-BP in WKY rats As showed in Fig 4, there existed a circardian variation of ABR-BP in WKY rats. The ABR-BP values were higher in daytime (sleeping time for rats) measurements (11:00, 15:00, and 19:00) and lower in nighttime (active time for rats) measurements (23:00, 3:00, and 7:00). The highest value of ABR-BP was observed at 15:00 and the lowest value at 3:00 and 7:00.

ABR-BP in male and female WKY rats ABR-BP was found only slightly higher in female rats (80 % ± 9 %) than that in male rats (72 % ± 9 %), and this difference was not significant (P > 0.05).

Relationship between ABR function and BPV BPV in WKY rats (n = 13) was closely related to ABR-BP measured with this new method (r = 0.895, P < 0.01), but not to ABR-HP measured with classic

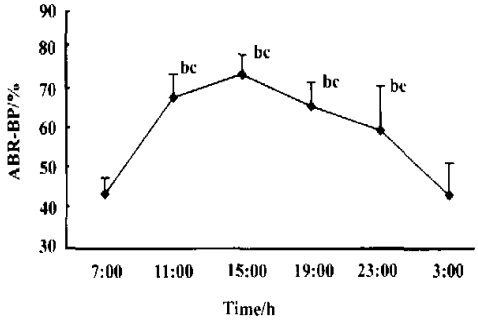

Fig 4. The Circadian variation of ABR-BP in WKY rats (n = 6 in each time group). The daytime (sleeping time for rats) is between 8:00 and 20:00. The nighttime (active time for rats) is between 20:00 and 8:00. $\bar{x} \pm s$. ^bP < 0.05 *vs* 7:00. ^cP < 0.05 *vs* 3:00.

method ($r = 0.245$, $P > 0.05$).

ABR-BP and hypertension In SHR, both ABR-BP and ABR-HP markedly decreased. This impairment of ABR function was also seen in renovascular hypertensive rats but in a less important manner (Tab 2). In hybrid offspring rats, the mean values were as follows: (166 ± 12) mmHg for SBP, (62 ± 14) % for ABR-BP and (0.68 ± 0.28) ms/mmHg for ABR-HP. There existed a significant and negative correlation between ABR-BP and SBP ($r = 0.44$, $P < 0.05$) as well as between ABR-HP and SBP ($r = 0.59$, $P < 0.01$).

Tab 2. ABR-BP and ABR-HP in SHR and renovascular hypertensive rats (RVHR). $\bar{x} \pm s$. $^cP < 0.01$ *vs* WKY rats.

Rats	n	ABR-BP/%	ABR-HP/ms·mmHg^{-1}
WKY	12	73 ± 11	1.04 ± 0.23
SHR	8	51 ± 12^c	0.24 ± 0.13^c
RVHR	8	59 ± 9^c	0.44 ± 0.14^c

DISCUSSION

The main function of ABR is to maintain the stability of blood pressure, and this concept is consistent with the finding in sinoaortic denervated (SAD) animals [1,2]. In SAD animals, the mean BP level over 24 h was remained normal and BPV was markedly increased. Therefore, we could expect an inverse relationship between BPV and baroreflex sensitivity (or ABR-HP, measured with classic method). However, the lack of such a linear correlation between ABR-HP and BPV was reported previously by some clinical observations and animal studies[11,17]. Furthermore, it was found that (i) BPV was not increased by atropine which blocks more than 80% of ABR-HP[11,18]; (ii) BPV was markedly increased in chronically sympathectomized rats, but ABR-HP was normal[17]. These facts make us believe that ABR-HP can not represent the totality of ABR function, and it is important to find a method to complement this defect of ABR-HP.

It is reasonable to compare the pressor response to a vasoactive drug before and after the interruption of the baroreflex for discovering ABR function in BP control. The pressor response was expressed as AUC that took into account not only the peak but also the duration of increase in BP. The interruption of the baroreflex may be performed by: (i) a sinoaortic denervation to block the afferent pathway; (ii) an electronic destruction of nucleus fractus solitarii (NTS) to destroy the center of this reflex; (iii) a chemical blockade of the efferent pathway. The first two methods require a complex manipulation and can not be used for ABR-BP measurement. In the present study, the third method was applied. Methyl-atropine and guanethidine were used to block the efferent pathway of baroreflex[16].

Phe and Ang Ⅱ are widely used vasoactive agents in ABR-HP determination. Eventually, we used Ang Ⅱ as vasoactive agent for the determination of ABR-BP. This is due to the super-sensitization of α-receptor after guanethidine administration and the pressor effect of Phe, α_1-receptor agonist, will be exaggerated. Concerning the ABR-BP measured with SNP, the values are significantly lower than those with pressor agents. This phenomenon is quite different from ABR-HP, which is higher when measured with nitroglycerin than with Phe in our previous works[7,11]. These results indicate that ABR-BP is more sensitive to pressor variation and ABR-HP is more sensitive to depressor variation. Although it is well known that to measure ABR function against depressor stimulation is equally important to measure ABR function against pressor stimulation, the measurement of ABR function against depressor stimulation is much less frequently used. The most important cause is probably due to the difficulty of manipulation: animal often moves when BP decreases rapidly.

It is well known that there exists a circadian variation of BP, but little information about circadian variation of ABR function is described. It was showed that ABR-HP increased in nighttime (during sleep) in human[19] and ABR-HP decreased in daytime (rest period for rats) and increased in nighttime (active period) in rats[20]. The present study obtained a great variation of ABR-BP in WKY rats similar to the previously reported study on ABR-HP in Sprague-Dawley rats[20]. These results indicate that the measurements of ABR function, ABR-BP or ABR-HP, should be taken in a fixed time.

BPV was closely and negatively correlated with ABR-BP measured with this new method but not with ABR-HP measured with classic method. This finding provides an evidence for the validity and significance of this new method, and suggests that BP variation comes largely from the variation of the vascular tension rather than from the variation of cardiac output or heart rate. It will be better to measure ABR-BP in a study where BPV

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is a main subject to be investigated.

Many works have demonstrated that ABR-HP was impaired in hypertensive human and animals, but there was no convincible information about ABR-BP in conscious animals because of lacking effective method. The present study showed that ABR-BP was impaired in both SHR and renohypertensive rats. This impairment of ABR-BP seems to be secondary to the elevation of BP. In hybrid offspring rats, both ABR-BP and ABR-HP significantly correlated with BP level. Furthermore, BP level was more closely related to ABR-HP than to ABR-BP. These results consist well with the findings showing that the impairment of ABR-HP was more severe than that of ABR-BP in hypertensive rats (Tab 2).

The results from WKY rats alone and SHR alone showed no correlation between ABR-BP and ABR-HP. It is suggested that ABR-BP does not reflect the same function as ABR-HP. This was also seen in the study of its relation with BPV. The differences for these two parameters are evident. However, there exist many similarities between ABR-BP and ABR-HP. For example, both of them exhibited a great circadian variation, which were inhibited by anesthesia and impaired in hypertension. As both of them were impaired in hypertensive rats, a significant correlation between ABR-BP and ABR-HP was found when analysis was done in a group rats consisting with SHR and WKY. A similar result was obtained in hybrid offspring rats, the ABR-BP and ABR-HP values in these rats were normal distributions.

In conclusion, the present study established a new method to quantitatively determine ABR-BP. ABR-BP plays an important role in maintaining blood pressure stability, and it is impaired in hypertension.

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清醒大鼠动脉压力感受性反射-血压控制的测定[1]

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关键词　麻醉；压力感受性反射；血压；血压波动性；近交 SHR 大鼠

目的：研究清醒大鼠动脉压力感受性反射（ABR）-血压控制（ABR-BP）的测定．**方法**：用计算机化清醒自由活动大鼠血压连续监测技术，记录动物的血压．ABR-BP 测定的原理是比较阻断 ABR 前后机体对血管活性物质反应的差异．**结果**：（1）用血管紧张素-Ⅱ测得的 ABR-BP 值与用新福林测得的 ABR-BP 值密切相关，在一定范围内药物的剂量不影响结果．（2）ABR-BP 与 ABR-心动间期控制（ABR-HP）显著相关．（3）麻醉抑制 ABR-BP，ABR-BP 本身存在昼夜节律性变化．（4）血压波动性与 ABR-BP 相关，而与 ABR-HP 不相关．（5）高血压时 ABR-BP 受损．**结论**：本项工作使 ABR-BP 的测定成为可能．ABR-BP 在维持血压的稳定性中起重要作用，在高血压时其功能受损．

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