Arterial baroreflex function in conscious rats[1]

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ABSTRACT

Arterial baroreflex (ABR) is a very important mechanism in the regulation of cardiovascular activities. As ABR function is largely inhibited by anesthesia, its measurement in conscious animal becomes important. The present review summarizes the works concerning ABR function in conscious rats completed in our department in the last 10 years. Firstly, a new method was established to measure arterial baroreflex-blood pressure control (ABR-BP). ABR-BP and baroreflex sensitivity measured with classic method are two different parts of the ABR function. Secondly, it was proposed that ABR function predicted the end-organ damage in hypertension. Thirdly, interruption of ABR induced severe end-organ damages. Increased blood pressure variability (BPV) and activation of renin angiotensin system were involved in the mechanisms underlying organ damages in sinoaortic denervation (SAD) rats. Fourthly, we propose that amelioration of ABR function may serve as a new strategy for improving the prognosis of cardiovascular diseases. Ketanserin improved the impaired ABR function in SHR. Finally, the possibility to develop a strain of rats with spontaneous deficiency on ABR function is mentioned.

INTRODUCTION

Arterial baroreflex (ABR) is a very important mechanism in the regulation of cardiovascular activities. ABR function was impaired in hypertension and many other cardiovascular diseases[1-4]. Recently, it was reported that ABR function was related to sudden death during a period of 2 years after the first attack of acute myocardial infarction[5-7].

What is the function of ABR? The main ABR function is to maintain blood pressure stable. The principle of the measurement of ABR function in conscious animals or in human is to observe the prolongation of the heart period (HP) in response to an increase in blood pressure (BP). 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 HP. HP is plotted against with systolic blood pressure (SBP) for linear regression analysis and the slope of SBP/HP

is defined as baroreflex sensitivity (BRS, ms/mmHg)[4,8-10]. 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 in this paper. The signification of BRS or ABR-HP is how many milliseconds of HP are prolonged by an increase in SBP of 1 mmHg.

As ABR function is largely inhibited by anesthesia[11,12], its measurement in conscious state becomes important. The present review summarizes the works concerning ABR function in conscious rats completed in our department in the last 10 years.

IMPORTANCE TO MEASURE ARTERIAL BAROREFLEX-BLOOD PRESSURE CONTROL (ABR-BP)

Blood pressure is not stable and there exists a spontaneous variation. This variation was defined as blood pressure variability (BPV). The main function of ABR is to maintain the stability of blood pressure, or to limit BPV to a given extent. If we destroy this system, we will find a great variation in blood pressure. Such an experiment is called "sinoaortic denervation" (SAD). It is an interruption of ABR by destroying the afferent fibers of baroreceptors. In these SAD animals, average blood pressure level during 24 h is normal, but BPV is markedly increased[13-15].

It is reasonable to expect a negative correlation between blood pressure variability and ABR-HP. However, the lack of such a linear correlation between ABR-HP and BPV was reported previously by some clinical observations and animal studies[16,17]. Furthermore, it was found that (i) BPV was not increased by atropine which blocked more than 80 % of ABR-HP[16,18]; (ii) BPV was markedly increased in chronically sympathectomized rats, but ABR-HP was normal[17]. These facts make us believe that ABR-HP cannot represent the totality of ABR function and it is important to find a method to complement this defect of ABR-HP.

In our department, we have established a new method for measuring arterial baroreflex-blood pressure control (ABR-BP)[19]. The principle is to compare the pressor responses to angiotensin II (Ang II) with and without ABR. The responses are expressed as the area under the curve (AUC, A). A_1 is the response to Ang II with intact ABR function, and A_2 is the response to Ang II without ABR function. The interruption of ABR function was realized by blocking the efferent pathway of baroreflex, that is, blocking sympathetic and parasympathetic nerves by using guanethidine and methylatropine, respectively. ABR-BP was calculated by the formula: ABR-BP (%)=$(A_2-A_1)/A_2 \times 100$ %. The signification of ABR-BP is what percentage of pressor response is buffered by ABR.

With this new method, it was found that BPV in WKY rats was not related to ABR-HP measured with classic method, but it was significantly related to ABR-BP. This finding provided 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 is a main subject to be investigated.

It is well known that ABR-HP is impaired in hypertension[1-4]. But there was no convincible information about ABR-BP in conscious animals because of lacking effective method. We studied ABR function in normotensive WKY rats, SHR, and renovascular hypertensive rats (RVHR). It was found that, both ABR-HP and ABR-BP was significantly decreased in SHR and RVHR[19,20]. So, it is concluded that ABR-BP was also impaired in hypertension although the impairment of ABR function was more severe on ABR-HP than on ABR-BP. This impairment seems to be secondary to the elevation of blood pressure.

An obvious limitation for this new method is the time required for measurement. It takes about 2 h for ABR-BP while only several minutes for ABR-HP. Usually it needs a comparison between groups for ABR-BP study.

ABR FUNCTION PREDICTED ORGAN DAMAGES IN HYPERTENSION

More than thirty years ago, SAD animal was used as animal model of neurogenic hypertension. With the development of the technique for continuous blood pressure recording in conscious animals, it was found that BPV was markedly increased, but 24-h average BP level was normal in SAD animals, including rats, rabbits, monkeys, and dogs[1,13-15]. It is well accepted that baroreflex dysfunction is not the cause of hypertension. However, it is not clear whether ABR function predicts the end-organ damage (EOD) in hypertension.

A study was carried out in 15 WKY rats and 40 SHR aged about 50 weeks[20]. Compared with WKY rats, SHR exhibited an increase in SBP and diastolic blood pressure (DBP), a decrease in ABR-HP and ABR-BP. The score of EOD is significantly higher in SHR. Tab 1 summarizes the linear regression coefficients between EOD score and cardiovascular variables. It was found that blood pressure level, BPV, and ABR function were all related to EOD score. ABR function is one of the independent variables related to EOD score in multiple regression analysis.

Tab 1. Linear regression coefficients between EOD score and cardiovascular variables in conscious SHR (extracted from Reference 20).

	Coefficient (r)		Coefficient (r)
SBP	0.572**	DBP	0.563**
SBPV	0.506*	DBPV	0.569**
ABR-HP	−0.650**	ABR-BP	−0.620**

$*P<0.05$, $**P<0.01$.

It was concluded that ABR dysfunction was not the cause of hypertension, but ABR function predicted end-organ damage in hypertension.

INTERRUPTION OF ABR INDUCED SEVERE ORGAN DAMAGES

Interruption of ABR was completed by SAD operation. A series of studies had been carried out in our department. The effects of SAD on organ damage were studied at 2, 4, 8, 16, and 32 weeks after SAD, using histopathological technique and computer image analysis. The following results were obtained[21-25].

Myocardial damage Compared with sham-operated rats, SAD rats exhibited an increased heart weight. In left ventricular tissues, there were cardiomyocyte swell and necrosis, mononuclear cell infiltration, interstitial fibrosis, and thickening of the wall, narrowing of the lumen, and increase of the perivascular collagen in myocardial coronary arterioles after SAD. A focal fibrillar scar that replaced the damaged cardiomyocytes was found in SAD rats 16 or 32 weeks after operation[21,24].

Renal lesions There existed an increase in mesangial matrix associated with focal proliferation, marked glomerular collapse, and fibrohyalinosis, and thickened basement membrane of Bowman's capsule in SAD rats. The thickening and hyalinization of the media, narrowing of the lumen, and progressive perivascular fibrosis, and mononuclear cell infiltration were found in intrarenal small arteries and arterioles after SAD. Tubular epithelial damages and cast were also observed in SAD rats. Focal fibrillar scarring, which replaced the damaged cells, occurred 16 and 32 weeks after SAD[21,25].

Arterial remodeling The aortic structural remodeling developed progressively at 4, 8, 16, and 32 weeks after SAD. In geometric morphology, it was characterized by increases in wall thickness, wall area, and wall thickness to internal diameter ratio. The relative area of smooth muscle cells was increased, but the nucleus number of smooth muscle cells remained unchanged. The aortic contraction elicited by norepinephrine was found progressively increased at 8, 16, and 32 weeks after SAD. The aortic relaxation elicited by acetylcholine was depressed from 8 weeks after SAD. In the group of rats after 16 weeks of SAD, vascular structural changes of different arteries were measured. Compared with sham-operated rats, SAD rats exhibited an increase in wall thickness, wall area, and wall thickness to internal diameter ratio in all arter-

ies studied[21,22].

MECHANISMS UNDERLYING ORGAN DAMAGE IN SAD RATS

Results of increased BPV The most important characteristic of SAD rats is the increased BPV. Higher BPV may produce a greater variation in tissue perfusion. This may be harmful to some sensitive tissues. Cellular metabolism may be disturbed by such a variation. Furthermore, a large variation of BP level may produce a direct lesion on vascular endothelial cells. In any case, the function of endothelium was changed in SAD rats although we were not sure that it was a direct lesion[21,26]. In one of our recent studies, linear regression analysis was performed to study the correlation between haemodynamic variables and ventricular or aortic hypertrophy induced by SAD. It was found that left ventricular hypertrophy or aortic hypertrophy was significantly related to BPV, but not to BP level. These results suggest that increased BPV after SAD is involved in organ damages.

Role of neurohumoral activation In this aspect, activation of renin-angiotensin system (RAS) may play the most important role. It is well known that angiotensin II possesses the actions of vasoconstriction, cell growth and proliferation, cardiovascular hypertrophy, angiogenesis, and augmentation of sympathetic activity. Most of these actions of angiotensin II may contribute to organ damages. It was found that plasma renin activity was increased in acute phase of SAD[27]. Using radioimmunoassay and reverse transcription-polymerase chain reaction (RT-PCR) analysis, we found that aortic angiotensin II level was increased 10 and 16 weeks after SAD and AT_1 receptor mRNA expression in left ventricle and aorta was upregulated 16 weeks after SAD (unpublished data). Theorically, a preventive effect on organ damage will be expected by blocking RAS when considering the role of RAS activation in SAD rats. In accordance with this hypothesis, it was found that candesartan effectively prevented cardiac hypertrophy, renal damage, and vascular remodeling. Chronologically, increased BPV was occurred immediately after SAD and existed permanently. Aortic hypertrophy appeared

from 2 weeks after SAD, and aortic angiotensin II increased from 10 weeks after SAD. It is concluded that SAD induced increased BPV, which activated RAS. The activation of RAS will accelerate EOD.

Involvement of myocardial apoptosis The study on apoptosis was performed in rats 16 weeks after SAD. It was found that apoptotic cells increased in ventricles of SAD rats. The mRNA and protein of bcl-2 decreased, fas, fas-L and bas increased (unpublished data). It is known that bcl-2 is an inhibiting factor for apoptosis while fas, fas-L, and bas are the promoting factors. However, it is only descriptive result and we do not know what is its role and how is its importance in the SAD-induced organ damage.

KETANSERIN IMPROVED ABR FUNCTION IN SHR

Ketanserin is an antihypertensive drug. This compound possesses a special property. It blocks $5-HT_{2A}$ receptor and in higher concentration also blocks α_1 receptor. This drug can stabilize blood pressure, that is, it can decrease BPV, in different animal models. It was found that ketanserin ameliorated impaired baroreflex function in SHR. It increased ABR-HP and ABR-BP, when it was administered whether intravenously (iv) or intracerebroventricularly (icv). However, prazosin, α_1 receptor blocker, had no obvious effect on ABR function. Ritanserin, a $5-HT_{2A}$ receptor blocker enhanced ABR-HP and ABR-BP only when it was administered icv as ritanserin is difficult to penetrate the blood-brain barrier (Tab 2). These results suggest that the effects of ketanserin on ABR function is mainly mediated by central $5-HT_{2A}$ receptor.

It is important for an antihypertensive drug to stabilize blood pressure. We have recently observed the effects of long-term treatment of ketanserin on the organ damage in SHR. Ketanserin 10 mg·kg^{-1}·d^{-1} was administered through food for 5 months. It was found that ketanserin decreased BP and BPV. BRS was significantly higher in ketanserin-treated [(0.64±0.14) ms/mmHg, n=7] than untreated [(0.30±0.17) ms/mmHg, n=7, P<0.01] SHR. At the same time, it was found that the parameters reflecting left ventricular hyper-

Tab 2. Effects of ketanserin, prazosin, and ritanserin on ABR-BP and ABR-HP in SHR. [b]$P<0.05$, [c]$P<0.01$ *vs* solvent control.

Drugs	Route	n	ABR-BP (%)	ABR-HP (ms/mmHg)
Saline	iv	8	51±12	0.24±0.13
Ketanserin	iv	11	74±8[c]	0.9±0.5[c]
Me$_2$SO	iv	7	52±11	0.46±0.14
Prazosin	iv	8	49±14	0.40±0.17
Ritanserin	iv	8	56±4	0.43±0.17
Saline	icv	8	56±8	0.32±0.11
Ketanserin	icv	8	68±6[c]	0.7±0.3[c]
Me$_2$SO	icv	8	57±12	0.35±0.13
Prazosin	icv	8	57±14	0.53±0.12[b]
Ritanserin	icv	8	71±9[b]	0.73±0.25[c]

Me$_2$SO: dimethyl sulfoxide, solvent for prazosin and ritanserin. Saline: solvent for ketanserin.

trophy, renal damage, and vascular remodeling were lower in ketanserin-treated than untreated rats.

Another long-term treatment study was carried out in myocardial infarction (MI) rats. Sprague-Dawley rats were divided into three groups: sham-operated (sham) group, MI group, and ketanserin-treated MI (MI+ketanserin) group. Compared with sham group, BRS was markedly reduced, and left ventricular hypertrophy index and occurrence of ventricular arrhythmia were increased in MI group. After oral treatment with ketanserin 8 mg·kg^{-1}·d^{-1} for 4–5 weeks, ABR function was significantly improved, left ventricular hypertrophy was reduced, and occurrence of ventricular arrhythmia was decreased by 15 %, when compared with untreated MI group. There were no significant differences in baroreflex function and left ventricular hypertrophy between sham and ketanserin-treated MI rats.

Based on these preliminary results from ketanserin treatment studies in SHR and MI rats, we propose that amelioration of ABR function may serve as a new strategy for improving the prognosis of some cardiovascular diseases.

PERSPECTIVES

As ABR is a very important subject to be studied, it will be very interesting to have a strain of rats possessing spontaneous deficiency of ABR function. This is possible. It is well known that lower BRS (ABR-HP) in SHR is due to hypertension and genetics[29,30]. Increased blood pressure will inhibit ABR function. So ABR function is impaired in almost all models of hypertension. It was reported that partial deficiency of ABR function was seen before the elevation of blood pressure in SHR and in young normotensive persons whose parents are hypertensive. So we decided to isolate the genetic component of ABR deficiency from SHR.

In recent 3 years, we have paid our efforts to develop such a strain of rats. A hybrid of SHR and SD was made. In the first generation of the hybrid offspring, some of them are hypertensive and some of them are normotensive. Only the normotensive rats were used. Their SBP is lower than 140 mmHg. In these rats, those with a BRS lower than 0.3 ms/mmHg were selected as arterial baroreflex-deficient rat (ABR-DR) and those BRS higher than 0.7 ms/mmHg were selected as arterial baroreflex-normal rat (ABR-NR). These selected rats were defined as G0 of ABR-DR or ABR-NR. Fig 1 shows the BRS obtained from a ABR-NR and a ABR-DR.

There exist many difficulties for this work. First, BRS measurement is not an easy job; it needs the measurements in conscious rats with computerized BP monitoring system. Taking account from the day implanting the arterial catheter, 4 days are required for the BRS measurement for one rat. Second, we have to keep rats living after measurement of BRS. It is not easy to keep them living, and furthermore to make them to be able to produce the next generation. Third, a genetically pure strain requires about 20 generations. The quantity of work is huge.
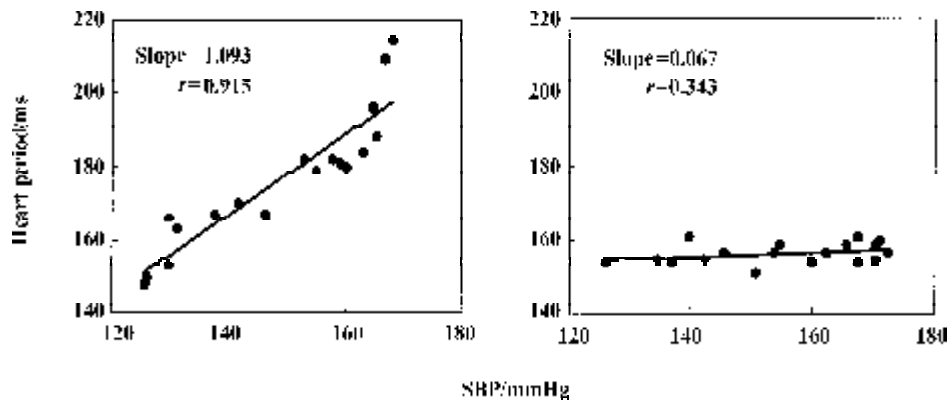
Fig 1. Schematic presentation of baroreflex sensitivity obtained from ABR-NR (left) and ABR-DR (right). Slope of the regression is expressed as baroreflex sensitivity.

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