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Arterial baroreflex function is an important determinant of acute cerebral ischemia in rats with middle cerebral artery occlusion

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ABSTRACT

Aims: To clarify whether arterial baroreflex function is an important determinant of acute cerebral ischemia in rats.
Main methods: Three animal models were used in this study. In the first, saponin conjugated with substance P (SP-SAP) was injected into the nucleus tractus solitarii (NTS) of Sprague–Dawley (SD) rats to block the central baroreflex arc. In the second model, sinoaortic denervation (SAD) was performed to destroy the peripheral baroreflex arc in SD rats. In the third model, SD rats were divided into two groups according to their naturally occurring BRS values. After determining hemodynamic indexes and baroreflex sensitivity (BRS), we subjected the animals to middle cerebral artery (MCA) occlusion. Levels of interleukin (IL)-1β and IL-6 were detected both in SAD/sham operation groups and low/high BRS groups.
Key findings: In all three animal models, baroreflex dysfunction significantly increased the infarct volume and weight. The levels of inflammatory factors were markedly elevated in SAD and low BRS groups.
Significance: These results demonstrate that the function of arterial baroreflex is an important determinant of acute cerebral ischemia in rats with MCA occlusion. Inflammation might be an important mechanism for the arterial baroreflex dysfunction-induced increase in brain damage in rats with MCA occlusion.

Introduction

Arterial baroreflex is one of the most important mechanisms regulating cardiovascular activity. Its function can be expressed as baroreflex sensitivity (BRS). Over the past 20 years, it has been reported that arterial baroreflex function is significantly related to the prognosis of acute cardiovascular infarction, arrhythmias, heart failure and stroke in humans (La Rovere et al., 1998, 2001; Mortara et al., 1997; Appenzeller and Descarries 1964; Robinson et al., 1997, 2003). Clinical observations indicate that patients with a lower BRS exhibit shorter survival times with these diseases. Recently, we reported that arterial baroreflex function plays an important role in the pathogenesis and prognosis of hypertension, atherosclerosis, aconitine-induced arrhythmia and LPS-induced shock (Cai et al., 2005; Shu et al., 2004; Shen et al., 2004; Liu et al., 2007); however, there is still a lot that is unknown about the pathological importance of arterial baroreflex. Apart from systemic circulation, autonomic nervous system control is less important in cerebral circulation, and it is not clear whether the arterial baroreflex plays an important role in the acute cerebral ischemia as it does in other cardiovascular diseases. Additionally, the cause and effect relationship between arterial baroreflex function and the prognosis of many cardiovascular diseases remains unclear. Finally, the mechanism by which the dysfunction of arterial baroreflex causes a poor prognosis for acute cerebral ischemia is unknown. We undertook the following study to address these issues.

To study arterial baroreflex function, we usually use sinoaortic denervation (SAD) to interrupt the baroreflex arc. In addition, research has shown that the nucleus tractus solitarii (NTS) and a certain receptor within it are very important for the modulation of baroreceptor reflex of rats (Matsumura et al., 1998). The injection of a cellular toxin, saponin, conjugated with substance P (SP-SAP) into the NTS was recently proposed to be able to block the baroreflex arc (Riley et al., 2002). Also, we showed in our previous study that a portion of normotensive Sprague–Dawley (SD) rats spontaneously exhibit a lower BRS (Cai et al., 2005). Therefore, in the present work we chose to use three different models of baroreflex dysfunction: SAD, NTS injection of SP-SAP and spontaneously lower BRS rats.

We also observed how arterial baroreflex function affects the levels of inflammatory factors in the brain to help determine the mechanism underlying arterial baroreflex dysfunction leading to a poor prognosis in acute cerebral ischemia.

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Methods

Animals

Male Sprague–Dawley (SD) rats were purchased from Sino-British SIPPR/BK Lab Animal Ltd (Shanghai, China). They were housed under controlled temperature (23–25 °C) and a 12:12 light/dark cycle with free access to standard food and drinking water. Animal protocols were approved by the Medical Ethics Committee of the Second Military Medical University, adopting NIH guidelines for health and care of experimental animals.

Middle cerebral artery (MCA) occlusion in rats

Rats were anesthetized with chloral hydrate (300 mg/kg, ip). Focal cerebral ischemia was induced by occlusion of the MCA, using the intraluminal filament technique according to the previously described method (Shimamura et al., 2006). Briefly, after a midline neck incision had been made, the left common and external carotid arteries were isolated and ligatured. A nylon monofilament (Ethilon) coated with silicon resin was introduced through a small incision into the common carotid artery and advanced to a position 18 mm distal from a carotid bifurcation for occlusion of the MCA.

Brain samples were harvested 24 h after MCA occlusion. Coronal sections 2 mm in thickness were immediately stained with 2% 2,3,5-triphenyltetrazolium chloride (TTC) as previously described (Bederson, 1986). The infarct region looked pale, while the normal region looked red. The infarct area and hemisphere areas of each section (both sides) were traced and quantified by an image analysis system (Microsystems Type DM LB2, Leica, Germany). The possible interference of a brain edema in assessing the infarct volume was corrected for with a standard method of subtracting the volume of the nonischemic ipsilateral hemisphere from the contralateral hemisphere volume. The infarct volume was expressed as a percentage of the contralateral hemisphere. The weights of the infarct tissue and the hemisphere were measured in a similar manner. The infarct weight was expressed as the percentage of hemisphere weight.

Blood pressure and heart period measurements

Systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart period were continuously recorded using our previously described technique (Liu et al., 2007) Briefly, rats were anesthetized with a combination of ketamine and diazepam (i.p.). A polyethylene catheter was inserted into the lower abdominal aorta via the left femoral artery for blood pressure measurement. Another catheter was inserted into the left femoral vein for phenylephrine administration. The catheters were exteriorized through the interscapular skin. After a one-day recovery from anesthesia, the animals were placed in individual cylindrical cages containing food and water for blood pressure recording. The aortic catheter was connected to a blood pressure transducer via a rotating swivel that allowed the animals to move without constraint in the cage. After 4 h habituation, the blood pressure signal was digitized by a microcomputer. SBP, DBP and heart period were recorded by remote-controlled computerized transducers for 1 h. The mean values of these parameters during 1 h were calculated.

Determination of BRS

In the above-mentioned condition for recording blood pressure, BRS was measured in the conscious rat using a method described previously (Liu et al., 2007). Briefly, a bolus injection of phenylephrine was used to induce a temporary elevation of blood pressure. The dose of phenylephrine (5–10 μg/kg) was adjusted to raise the SBP about 30 mm Hg. There was a delay (about 1 s) between the elevation of blood pressure (stimulus) and the prolongation of heart period (response) for arterial baroreflex. In rats, the heart rate is about 5 or 6 beats per second. Heart period was plotted against SBP for linear regression analysis for 2–8 shifts (calculated by computer). The slope with the largest correlation coefficient (r) between heart period and SBP was expressed as BRS (ms/mm Hg). Data were derived from the average of two measurements in each animal.

Preparation of sinoaortic denervation (SAD)

SAD was performed in SD rats according to the procedure described by Krieger (1964) with minor modifications (Shen et al., 2006). Briefly, rats were anesthetized with a mixture of ketamine and diazepam (i.p.) and medicated with atropine sulfate (0.5 mg/kg, ip) and procaine benzylpenicillin (60,000 U, i.m.). Bilateral aortic baroreceptor denervation was performed by cutting the superior laryngeal nerves near the vagi, removing the superior cervical ganglia, including a small section of the sympathetic trunk, and sectioning the aortic depressor nerves. The carotid sinus baroreceptors were denervated bilaterally by stippling the carotid bifurcation and its branches followed by application of 10% phenol (in 95% ethanol) to the external, internal and common carotid arteries as well as the occipital artery. Sham operation included a midline neck incision and bilateral isolation of the neck muscles.

NTS injection

Rats were anesthetized with a combination of ketamine and diazepam (i.p.). The dorsal surface of the brainstem was exposed as previously described (La Rovere et al., 1998). A glass micropipette filled either with SP-SAP (18 ng; supplied by Advanced Targeting Systems, San Diego, CA, USA) or vehicle alone (Artificial CSF) was stereotaxically placed in the NTS. After injection of 100 nL over more than 15 min, the pipette was left in place for 15 min to limit the efflux of the injectate from the pipette track. In all animals, injections were made bilaterally. After withdrawal of the pipette, wounds were closed with silk suture; buprenorphine (0.1 mg/kg) was administered for analgesia, and halothane was discontinued. Once they fully recovered from anesthesia, the animals treated with SP-SAP were returned to the animal care facility, where they were carefully observed at least twice a day (morning and night) for 2 weeks.

Determination of inflammatory factors in the brain

Rats were deeply anaesthetized with a combination of ketamine (50 mg/kg) and diazepam (5 mg/kg). Brain samples were removed. The proteins in the brains were extracted by tissue protein extraction solution (Kangcheng Bio-Tech, Shanghai, China) according to the manufacturer's protocol. All samples were stored at −80 °C until analysis. Interleukin (IL)-1β and IL-6 levels were detected by enzyme linked immunosorbent assay (ELISA) kits (Shanghai Transhold Tech. Dev. Co. Ltd, Shanghai, China).

Protocol

Effects of SP-SAP injection into NTS on the cerebral ischemia in rats

Rats were randomly divided into two groups (10 in the control group and 12 in the SP-SAP group). SP-SAP or CSF (100 nL) were injected into NTS of treated and control rats, respectively. Then, rats were returned to the animal care facility. After 3 weeks, the blood pressure, heart period and BRS were measured in a conscious state. Then, the rats were subjected to MCA occlusion. The infarct volume and weight of the brain were measured at 24 h after operation.

The effect of SAD on the cerebral ischemia in rats

Rats were randomly divided into two groups (n=10 in each group). SAD and sham operations were performed. To confirm the success of the SAD operation, SBP and BRS were measured in all rats in a

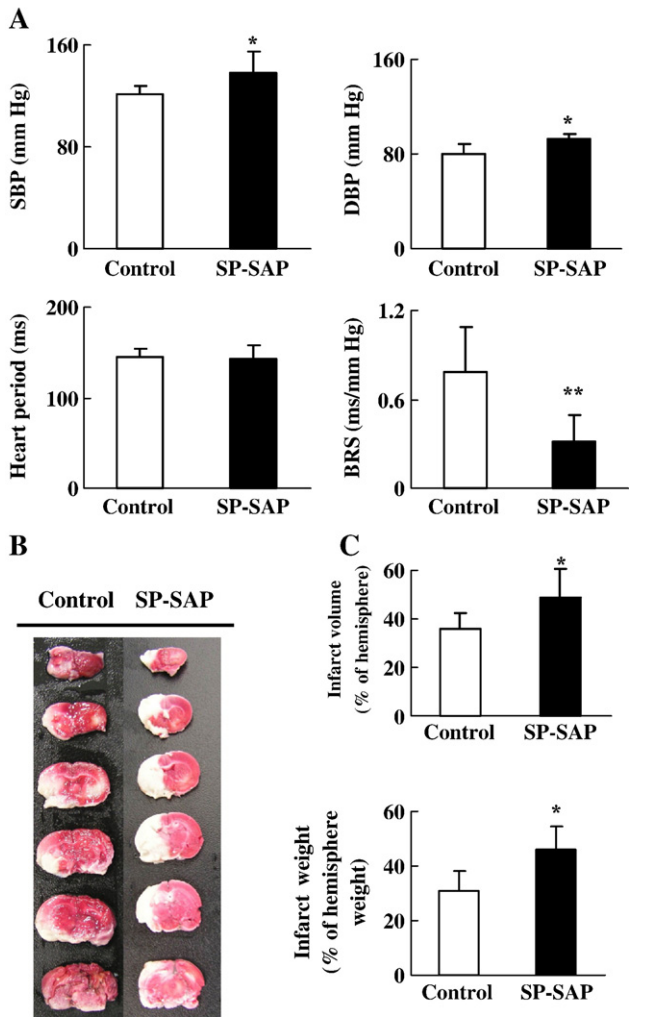

Fig. 1. The effect of saponin conjugated with substance P (SP-SAP) injection into NTS on the hemodynamic indexes and cerebral ischemia induced by middle cerebral artery (MCA) occlusion in SD rats. A, the effect of SP-SAP on SBP, DBP, heart period and BRS in conscious animals. B, Representative images of triphenyltetrazolium chloride (TTC)-stained brain sections. The white-colored area represents the infarct region. C, Summary of cerebral infarct volume and weight in brains from control and SP-SAP groups. Data were presented as mean±SD. The infarct volume was expressed as a percentage of the contralateral hemisphere. *$P<0.05$ vs. control group. $n=10$ in control group and $n=9$ in SP-SAP group.

conscious state at four weeks after surgery. The animals were then subjected to MCA occlusion. The infarct volume and weight of the brain were measured at 24 h after operation.

The cerebral ischemia rats with spontaneously lower BRS

Rats weighing about 250 g were used in this experiment. SBP and BRS were determined with the above mentioned method in 40 rats. They were divided into two groups according to their mean BRS values (0.69±0.36 ms/mm Hg). Then, half of the animals ($n=10$ in each group) were subjected to MCA occlusion; the rest were used in experiment four. The infarct volume and weight of the brain were measured at 24 h after operation.

The expression of IL-1β and IL-6 in rats with lower BRS

Rats weighing about 250 g were used in this experiment. First, 18 animals were randomly divided into two groups ($n=9$ in each group). SAD and sham operations were performed. Then, IL-1β and IL-6 levels in the brain were detected at four weeks after operation. Second, the other 20 animals from experiment three were used. After determination of BRS, the brain content of IL-1β and IL-6 levels were detected in rats with lower or higher BRS.

Statistical analysis

Investigators were blind to the procedures during blood pressure recording, BRS determination, measurement and infarct size examination. Data are expressed as the mean±SD. The comparisons between two groups were made with unpaired *t*-tests; the comparisons between three or four groups were made by one-way analysis of variance (ANOVA). Statistical significance was judged at $P<0.05$ level.

Results

Effect of SP-SAP injection into the NTS on the cerebral ischemia in rats

The effects of SP-SAP injection on blood pressure in conscious rats are summarized in Fig. 1A. Compared to the control group, the level of SBP in the SP-SAP group was increased by 17 mm Hg, and the DBP increased by 12 mm Hg. There was no difference in heart period

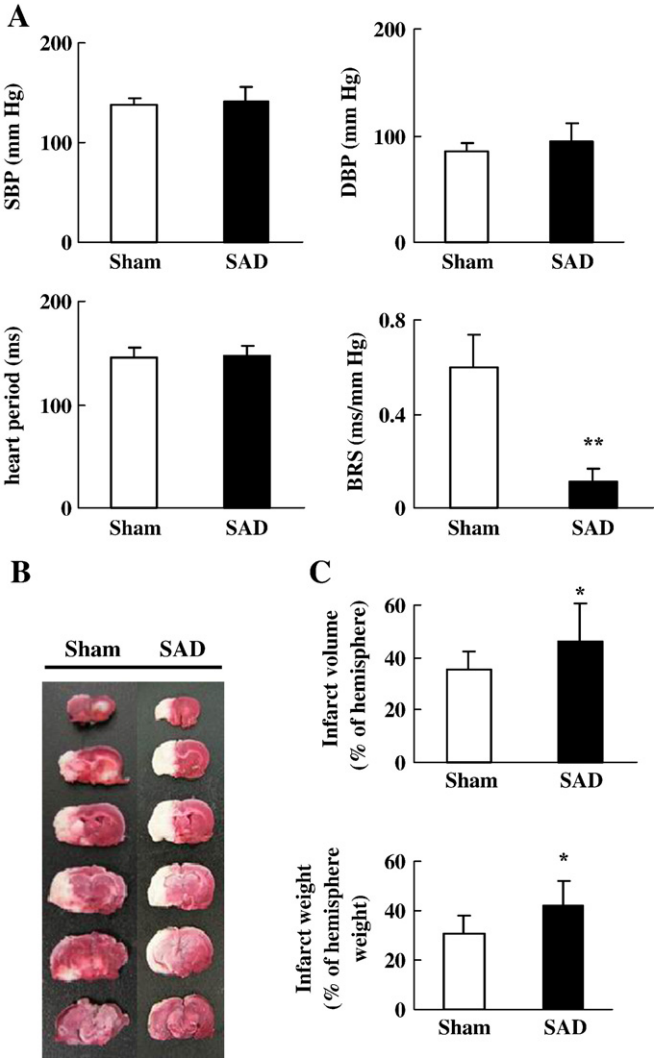

Fig. 2. The effects of SAD on the hemodynamic indexes and cerebral ischemia induced by MCA occlusion in SD rats. A, the effect of SAD on SBP, DBP, heart period and BRS in conscious animals. B, Representative images of TTC-stained brain sections. The white-colored area represents the infarct region. C, Summary of cerebral infarct volume and weight in brains from Sham ($n=10$) and SAD ($n=8$) groups. Data were presented as mean±SD. The infarct volume was expressed as a percentage of the contralateral hemisphere. *$P<0.05$ vs. Sham group.

between the two groups. Compared with the control group, the BRS was significantly lower in the SP-SAP group in a conscious state (0.31 ± 0.18 vs. 0.79±0.30 ms/mm Hg). In the SP-SAP group, the infarct volume and infarct weight were both larger/heavier than the control group; these differences were statistically significant (Fig. 1B).

Effect of SAD on the cerebral ischemia in rats

The blood pressure and heart period in SAD rats were similar to those in sham-operated rats. The BRS in the SAD group was significantly lower than that in the sham-operated rats (Fig. 2A). Focal brain ischemia was induced by MCA occlusion. SAD significantly increased both the infarct volume and weight (Fig. 2B).

Cerebral ischemia in rats with spontaneously lower BRS

According to the mean BRS value (0.69 ms/mm Hg), 40 SD rats were divided into two groups: group BRS-low with BRS <0.6 ms/mm Hg (n=18) and group BRS-high with BRS >0.8 ms/mm Hg (n=18). The other four rats with BRS between 0.6 and 0.8 ms/mm Hg were discarded. There were no differences in blood pressure levels and heart periods between these two groups, but the BRS values were significantly different (0.39±0.18 vs. 0.99±0.23 ms/mm Hg). In the BRS-low group, both the infarct volume and weight were significantly higher than the BRS-high group (Fig. 3).

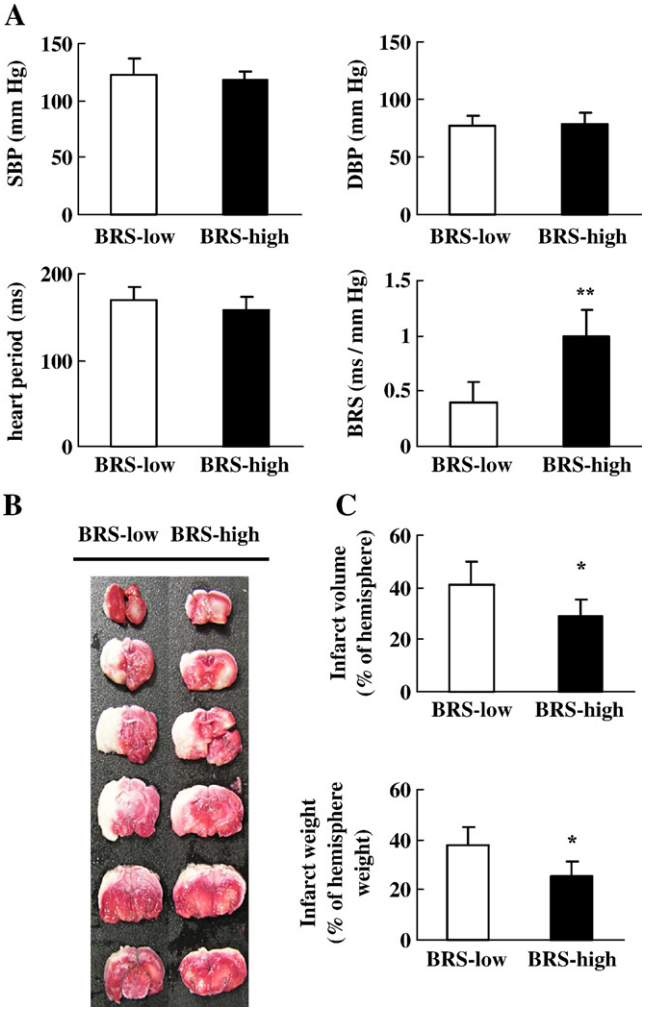

Fig. 3. Cerebral ischemia induced by MCA occlusion in rats with spontaneously low/high BRS. A, the difference in SBP, DBP, heart period and BRS values between 2 groups rats with spontaneously lower/higher BRS. B, Representative images of TTC-stained brain sections. The white-colored area represents the infarct region. C, Summary of cerebral infarct volume and weight in brains from BRS-low (n=9) and BRS-high (n=9) groups. Data were presented as mean±SD. The infarct volume was expressed as a percentage of the contralateral hemisphere. *P<0.05 vs. BRS-low group.

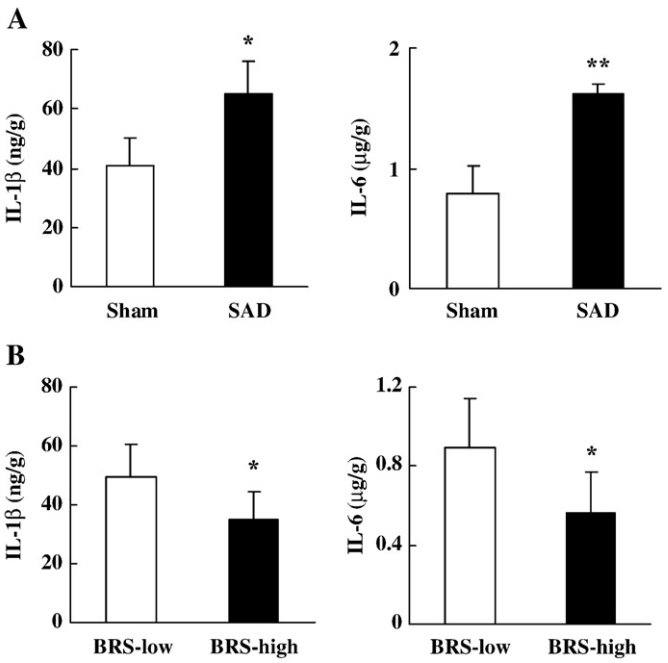

Fig. 4. Brain contents of IL-1β, IL-6 in SAD rats (A) and rats with spontaneously low/high BRS (B). Data were presented as mean±SD. *P<0.05 vs. sham or BRS-low group. n=8–10 in each group.

Levels of IL-1β and IL-6 in the brains of SAD rats and rats with spontaneously lower BRS

Compared to sham-operated rats, SAD significantly increased the levels of IL-1β and IL-6 in the brains of rats (Fig. 4A). Similar results were also obtained for spontaneously BRS-low rats compared to spontaneously BRS-high rats (Fig. 4B).

Discussion

The main new findings of the present work may be summarized as follows: (1) as it does in many cardiovascular diseases, arterial baroreflex function plays an important and causal role in acute cerebral ischemia in rats; (2) an increased inflammatory reaction may be the main mechanism underlying how arterial baroreflex dysfunction leads to a poor prognosis in acute cerebral ischemia in rats.

It is well known that baroreceptor afferent terminals are most concentrated in the NTS (Ciriello, 1983). Electric lesions of NTS are sometimes used to interrupt the arterial baroreflex arc in studies concerning arterial baroreflex function. The main problem with the electric lesion of the NTS is that too many organisms may be affected by this operation. Neurons in the NTS that express certain receptors play a critical role in mediating baroreflex through the NTS. Additionally, neurokinin-1 (NK1) receptors, which bind peptide substance P (SP), are found in the NTS (Dixon et al., 1998). The neurons in the NTS that express SP receptors play a critical role in mediating baroreflex. These cells can be specifically targeted and selectively killed by SP-SAP (Yip and Chahl, 2001; Wiley and Lappi, 1997; Mantyh et al., 1997). Previous research has demonstrated that when the toxin is injected bilaterally into the NTS, the baroreflex gain is significantly reduced (Riley et al., 2002). It should be noted, however, that this study was performed on anesthetized animals, and

it is well recognized that anesthesia may blunt or abolish the lability of arterial pressure that follows chronic NTS lesions (Talman et al., 1980; Nathan et al., 1978). The BRS was also inhibited rapidly and dramatically (Rocchiccioli et al., 1989), with maximum depressions of 51–80% (Yi-Ming et al., 2004). In the present study, all the hemodynamic indexes and BRS values were measured in conscious animals. BRS values in SP-SAP treated rats were found to be significantly decreased, confirming that SP-SAP injections into the NTS sufficiently block the baroreflex arc.

As BRS is a continuous parameter, we can easily divide the animals into two groups according to their BRS values. There were no differences in blood pressure or heart period between rats with low BRS and those with high BRS; the only difference was arterial baroreflex function. Therefore, this may be a useful model for arterial baroreflex function studies.

Together with SAD, a widely used technique, we used three arterial baroreflex dysfunction models in this work to observe the role of arterial baroreflex function in cerebral damage induced by MCA occlusion in rats. Interestingly, the experiments with all three different animal models demonstrated that arterial baroreflex function is an important determinant of cerebral ischemia-induced brain damage.

Many researchers have confirmed that baroreflex dysfunction is associated with many diseases, including acute myocardial infarction, heart failure and stroke (La Rovere et al., 1998, 2001; Mortara et al., 1997; Appenzeller and Descarries, 1964; Robinson et al., 1997, 2003; Cai et al., 2005; Shu et al., 2004; Shen et al., 2004). Recently, we have demonstrated that BRS is an important factor in determining the survival time in stroke-prone SHR (SHR-SP). Lifespan is increased significantly in rats with high BRS compared to rats with low BRS (Liu et al., 2007) While the association between arterial baroreflex function and certain diseases has been well established, it is still not clear whether baroreflex dysfunction actually causes the poor prognosis in many diseases, including stroke. In this work, we used rats that were normal and healthy prior to the SAD operation, SP-SAP injection or group distribution by BRS values. We only observed a difference in arterial baroreflex dysfunction after the above-mentioned manipulations. Clearly, arterial baroreflex dysfunction is the main cause for the increased brain damage induced by MCA occlusion.

It should be noted that BRS may be influenced by the occlusion of the MCA. Clinically, Robinson et al. (2003) reported that BRS was significantly lower in stroke patients compared to in aged-matched controls. In rats, it was reported that MCA occlusion increases BRS by 10 days after operation (Saad et al., 1989). It will be interesting to measure in patients an evolution of BRS with time after stroke or in rats after MCA occlusion; this type of experiment will be necessary to fully understand the influence of cerebral infarction on arterial baroreflex function.

While the relationship between arterial baroreflex function and cardiovascular diseases has been widely studied, the mechanism underlying this relationship remains unclear. Our previous work has shown that inflammatory factors are markedly elevated in the serum of SAD rats. In this work, we observed that the levels of inflammatory factors are also increased significantly in the brains of SAD or low-BRS rats. Inflammation is an important feature of the pathophysiological response to ischemic stroke (Gerritsen et al., 2001) and may also predispose one to the initial development of ischemic stroke (Emsley and Tyrrell, 2002). An increase in the levels of these inflammatory factors and the acute phase response appear to predict a poor outcome after stroke (Vila et al., 1999; Di Napoli et al., 2002). Accordingly, inflammation might be an important part of the mechanism of the regulation of arterial baroreflex dysfunction on the cerebral ischemia in rats.

A detailed explanation of how arterial baroreflex dysfunction increases inflammation is still lacking. It was reported that the parasympathetic nervous system plays an important role in the regulation of the inflammatory response by controlling the release of certain inflammatory factors (Libert, 2003; Borovikova et al., 2000; Wang et al., 2003). The activation of the vagus nerve can suppress inflammation quickly and efficiently. Surgically severing the vagus nerve not only removes this suppression of inflammation, but also sensitizes the animals to lipopolysaccharide. This effect of the vagus nerve was attributed to its nicotinic acetylcholine receptor: one of the five copies of the α7 monomers (Wang et al., 2003). It is well known that an intact arterial baroreflex is required for maintaining the balance of the autonomic nervous systems. This balance is disturbed in rats with BRS dysfunction. Therefore, it is possible that the elevation of the inflammatory factors in SAD or low-BRS rats is due to a removal of the control of the release of inflammatory factors by the vagus nerve. The relationship between inflammation and BRS dysfunction and their effects on cerebral ischemia need to be further investigated.

In conclusion, arterial baroreflex function is an important determinant of acute cerebral ischemia in rats with MCA occlusion. Inflammation might play an important role in the arterial baroreflex dysfunction-induced increase in brain damage in rats with MCA occlusion.

Acknowledgements

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