Inhibition of Oxidative Stress in Rostral Ventrolateral Medulla Improves Impaired Baroreflex Sensitivity in Stroke-Prone Spontaneously Hypertensive Rats

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Summary

Reactive oxygen species (ROS) in rostral ventrolateral medulla (RVLM) of brainstem contribute to sympathoexcitation and are critically involved in the pathogenesis of hypertension. Baroreflex sensitivity (BRS) is a valuable prognostic parameter of the autonomic nervous system, and is impaired in hypertension. The aim of the present study was to determine whether or not a chronic reduction of ROS in the RVLM improves impaired BRS in hypertensive rats. We transfected adenovirus vectors encoding either manganese superoxide dismutase (AdMnSOD) or ß-galactosidase (Ad-LacZ) into the RVLM of stroke-prone spontaneously hypertensive rats (SHRSP). We measured BRS using the spontaneous sequence method. BRS was significantly lower in SHRSPs than in Wistar-Kyoto rats. In the AdMnSOD-transfected SHRSP, blood pressure, heart rate, and sympathetic nervous system activation were significantly decreased from day 5 after the gene transfer. BRS in the AdMnSOD-transfected SHRSP was significantly increased from day 4 after the gene transfer with the reduction of ROS in the RVLM. Furthermore, in the AdMnSOD-transfected SHRSP, intravenous infusion of atropine dramatically decreased BRS. In contrast, in the AdLacZ-transfected SHRSP, atropine did not decrease BRS. These results suggest that chronic reduction of ROS in the local RVLM improves the impaired BRS in SHRSP through inhibition of the sympathetic component. (Int Heart J 2012; 53: 193-198)

Key words: Baroreflex sensitivity, Reactive oxygen species, Brain, Sympathetic nervous system, Hypertension

A change in arterial blood pressure (BP), such as that resulting from postural change, is detected by baroreceptors, and the afferent nerves provide the information to the central nervous system. 1,2 The arterial baroreflex regulated by the central nervous system acts to oppose the increase in BP by inhibiting sympathetic activity, causing vasodilation and slowing heart rate (HR) in the short-term. 1,3,4 Previous reports have suggested that baroreflex sensitivity (BRS) is impaired in cardiovascular diseases such as hypertension, 5,6 heart failure, 7,8 and myocardial infarction. 9 It has been suggested that BP variability, which is a cardiovascular risk factor, contributes to end-organ damage. 10-12 The assessment of BRS has been considered to be the established tool for the evaluation of autonomic control of the cardiovascular system. 9,13 Classically, BRS has been evaluated by the drug-induced Oxford method. 14 However, pharmacological assessment of BRS is difficult to determine repeatedly in humans, but can be done in acute experiments with anesthetized animals. Recently, the spontaneous sequence method has proved to be a reliable and noninvasive method for assessing BRS, and the assessment of BRS can now be performed repeatedly in humans and animals in a conscious state. 13,15

The rostral ventrolateral medulla (RVLM) is the vasomotor center that determines basal sympathetic nervous system (SNS) activation, and is responsible for maintaining sympathetic out flow. 1,2 The RVLM receives input from multiple areas of the brain and determines the SNS activation to maintain the stability of BP and HR. Previously, we reported that nitric oxide in the RVLM caused sympathoinhibition and improved impaired BRS in hypertensive rats. 15 Furthermore, we have demonstrated that the reduction of reactive oxygen species (ROS) induced by manganese superoxide dismutase (MnSOD) gene transfer into the bilateral RVLM causes sympathoinhibition in hypertensive rats. These results suggest that ROS in the RVLM causes sympathoexcitation in hypertensive rats. 16 Therefore, we believe that ROS in the RVLM might impair BRS in hypertensive rats. However, it has not been demonstrated whether the chronic reduction of ROS in the RVLM could improve the impaired BRS in hypertensive rats. The aim of the present study was to determine whether or not the chronic reduction of ROS via overexpression of MnSOD in the local RVLM of conscious hypertensive rats improves the impaired BRS.
METHODS

This study was reviewed and approved by the Committee on the Ethics of Animal Experiments of Kyushu University Graduate School of Medical Sciences and was conducted according to the Guidelines for Animal Experiments of Kyushu University.

Animals: Male stroke-prone spontaneously hypertensive rats (SHRSP) and age-matched Wistar-Kyoto (WKY) rats (12 to 14 weeks old) fed standard feed were used. The rats were purchased from SLC Japan (Hamamatsu, Japan).

Radio-telemetry monitoring of BP and HR: A UA-10 telemetry system (Data Science International) was used to measure BP and HR, as described previously. The rats were allowed to recover from implantation of the telemetry system for 7-8 days before the start of the protocol. HR was derived from the BP interval using a PowerLab (AD Instruments Inc.) data acquisition system.

In vivo gene transfer of MnSOD into the RVLM: Adenovirus vectors encoding either bacterial β-galactosidase gene (AdLacZ) or MnSOD gene (AdMnSOD) were transfected into the bilateral RVLM (SHRSP transfection with AdMnSOD [AdMnSOD-transfected SHRSP], SHRSPs transfection with AdLacZ [AdLacZ-transfected SHRSPs], and WKY rats transfected with AdMnSOD [AdMnSOD-transfected WKY rats]). The method of transfection was described previously. AdMnSOD and AdLacZ were constructed in the Gene Transfer Core Laboratory at the University of Iowa, Iowa City. Procedures for microinjection of the vectors into the RVLM have been described previously.

Analysis of gene overexpression for MnSOD: To confirm the local overexpression of MnSOD in the RVLM, Western blotting analysis for MnSOD in protein in the RVLM or cortex tissues of AdMnSOD-transfected SHRSP was performed at day 0, 7, and 21 after the gene transfer. In the Western blotting analysis for MnSOD protein, mouse IgG monoclonal antibody to MnSOD (1:1000, Transduction Laboratories) was used as described previously.

Measurement of ROS in the RVLM: To confirm ROS activity, thiobarbituric acid-reactive substance (TBARS) levels in the RVLM tissue were measured at day 7 after the gene transfer as described previously.

Measurement of urinary norepinephrine excretion: Twenty-four hour urinary norepinephrine excretion was measured before and at day 7 after the gene transfer as described previously as a noninvasive indicator of SNS activation.

Measurement of conscious BRS by spontaneous sequence method: BRS was measured using a spontaneous sequence method. The subjects were given a 10-minute rest period to allow for stabilization of BP or HR. To analyze approximately 5 minutes of hemodynamic recordings from the telemetry system, we selected all sequences of three or more successive heart beats in which there was a concordant increase (Up sequence) or decrease (Down sequence) in arterial systolic BP and peak-to-peak systolic BP interval change. Linear regression analysis was applied to each sequence, and an average regression slope was calculated for the sequences. This slope represents the cardiac BRS. The threshold values for including beat-to-beat systolic BP and its interval changes in a sequence were set at 1 mmHg and 2 milliseconds, respectively.

Effects of autonomic blockade on BRS: Autonomic nervous system blockade was performed to determine the sympathetic and parasympathetic components. Intravenous injection via a tail vein was performed under light pentobarbital anesthesia. Metoprolol bitartrate (dominant to parasympathetic component) (a selective β1-receptor blocker, 0.2 mg/kg) or atropine methyl bromide (dominant to sympathetic component) (0.02 mg/kg) was injected intravenously at day 7 after the gene transfer, and then the changes in HR and BRS in each group were measured.

Statistical analysis: All data are expressed as the mean ± SEM. ANOVA was used to compare the TBARS level, and changes in mean blood pressure, heart rate, and BRS. An unpaired t-test was used to compare the expression of MnSOD determined by Western blotting analysis and urinary norepinephrine excretion. Differences were considered to be statistically significant at a P-value of <0.05.

RESULTS

Analysis of gene expression of MnSOD: Western blot analysis demonstrating the expression of MnSOD protein in the RVLM (A) or cortex (B) in AdMnSOD-transfected SHRSP before (day 0) and on day 7 and day 21 after the gene transfer. MnSOD indicates manganese superoxide dismutase. *P < 0.01, n = 5 for each group.

TBARS level in RVLM tissue from nontransfected SHRSP, AdMnSOD-transfected SHRSP, and AdLacZ-transfected SHRSP. The RVLM tissues from AdMnSOD-transfected and AdLacZ-transfected SHRSP were obtained at day 7 after the gene transfer. *P < 0.01 versus nontransfected SHRSP, n = 5 for each group.
ysis showed that the expression of MnSOD protein was significantly increased in tissue from the RVLM of AdMnSOD-transfected SHRSP at day 7 after the gene transfer (Figure 1). The expression of MnSOD protein in RVLM was decreased at day 21 after the gene transfer (Figure 1). These changes in expression were not seen in cortex tissue.

TBARS levels in RVLM tissue: TBARS levels were significantly lower in AdMnSOD-transfected SHRSP than in nontransfected SHRSP or AdLacZ-transfected SHRSP (Figure 2).

Mean BP, HR, and urinary norepinephrine excretion: Figure 3 shows the time courses in mean BP (A) and HR (B) in AdMnSOD-transfected and AdLacZ-transfected SHRSP. Mean BP and HR decreased significantly between day 5 to 9 or 11 after the gene transfer in AdMnSOD-transfected SHRSP, but were not seen in AdLacZ-transfected SHRSP. Twenty-four hour urinary norepinephrine excretion was significantly higher in AdMnSOD-transfected WKY rats before the gene transfer (Figure 4). At day 7 after the gene transfer, urinary norepinephrine excretion was significantly decreased in AdMnSOD-transfected SHRSP (Figure 4). In contrast, these changes were not observed in AdLacZ-transfected SHRSP (Figure 4).

Effect of overexpression of MnSOD in RVLM of SHRSPs on BRS: BRS was significantly lower in AdMnSOD-transfected SHRSP than in AdMnSOD-transfected WKY rats before the gene transfer (Figure 5). In contrast, BRS was significantly increased in AdMnSOD-transfected SHRSP to a greater extent than in AdLacZ-transfected SHRSP (Figure 5). BRS was slightly increased at day 3 after the gene transfer in AdMnSOD-transfected SHRSP, and was significantly higher at day 4 after the gene transfer in AdMnSOD-transfected SHRSP than in AdLacZ-transfected SHRSP (Figure 5). The peak value was at day 7 after the gene transfer, and the peak value of BRS was significantly higher in AdMnSOD-transfected SHRSP than in AdMnSOD-transfected WKY rats (Figure 5).

Effect of autonomic blockade on HR and BRS: Blockade of
the parasympathetic component via systemic infusion of atro-
pine significantly increased HR in AdMnSOD-transfected
SHRSP to a greater extent than in non-transfected SHRSP
(Figure 6A). Blockade of the sympathetic component via sys-
temic infusion of metoprolol significantly decreased HR in
non-transfected SHRSP to a greater extent than in AdMnSOD-
transfected SHRSP (Figure 6B). In AdMnSOD-transfected
SHRSP, atropine significantly decreased BRS to a greater ex-
tent than in metoprolol (Figure 7A). However, in AdLacZ-
transfected SHRSP; neither atropine nor metoprolol changed
BRS (Figure 7B).

**Discussion**

The new findings of the present study are that the chronic
reduction of ROS induced by overexpression of MnSOD in the
RVLM improves impaired BRS in SHRSP through inhibition of
the sympathetic component, and that the time course of the
change in BRS caused by overexpression of MnSOD in the
RVLM of SHRSP is similar to that of the SNS activation.
These results indicate that ROS in the RVLM of SHRSP im-
pairs BRS with SNS activation.

We have already demonstrated that ROS in the RVLM
contributes to SNS activation,16 and that NO in the RVLM de-
creases the SNS activation and improves the impaired BRS in
SHRSP.5 In the present study, we demonstrated that ROS in
the RVLM of SHRSP impaired BRS due to activation of the
sympathetic component, and that the impaired BRS was im-
proved at almost the same time as the sympathoinhibition.
Other reports suggested that intracerebroventricular infusion of
exogenous angiotensin II impaired BRS with increased ROS
in the RVLM, and that the reduction of ROS in the brain by
exercise training or central infusion of simvastatin contributed
to the improvement of impaired BRS in cardiovascular dis-
ases.24-26 These previous reports support the results obtained in
the present study. Furthermore, other reports have indicated
that electric stimulation of baroreceptors decreased BP, HR
and SNS activation,27 and that BRS was improved during
baroreceptor stimulation. Interestingly, oral administration of
atorvastatin reduced oxidative stress in the RVLM and improved
the impaired BRS in SHRSP without sympathoinhibition.17

We believe that the reduction of ROS in the RVLM improves
the impaired BRS in close correlation with sympathoinhibition
in SHRSP.

In the present study, the reduction of ROS in the RVLM
via overexpression of MnSOD contributed to inhibition of the
sympathetic component. These results are compatible with
those of our previous study in which the production of NO in
the RVLM via overexpression of endothelial NO synthase im-
proved the BRS with inhibition of the sympathetic compo-
nent.5,23 Previous reports have demonstrated that a predomi-
nant sympathetic component caused impaired BRS.5,6,24 In
the RVLM, the increase in ROS and decrease in NO resulted in
SNS in SHRSP.18,23 We believe that a reduction in ROS and/or
production of NO in the RVLM improved the impaired BRS,
probably due to the sympathoinhibition in SHRSP.

Impairment of BRS is considered to be the marker of the
risk of mortality or a cardiovascular event in hypertension,29
heart failure,30 and ischemic heart diseases.31 The ATRAMI
study demonstrated the prognostic importance of parasympa-
thetic activity as a strong predictor of postmyocardial infarc-
tion cardiac mortality.32 However, cardiovascular events or sud-
den death occur most frequently toward the end of the night,
which coincides with a period when sympathetic activity, BP,
and HR change suddenly.33 Previous reports also have sug-
gested that several antihypertensive agents improved the im-
paired BRS in hypertension.34 Because several antihyper-
tensive drugs, such as calcium channel blockers, have an effect
on refractory SNS activation,35 antihypertensive drugs with
sympathoinhibition are preferable for the treatment of hyper-
tension.32,36 Moreover, because the improvement of BRS con-
tributes to long-term mortality in cardiovascular diseases,37
antihypertensive agents that improve BRS and possess sym-
pathoinhibition are preferable. In the present study and our
previous studies, we have demonstrated that the reduction of
ROS in the RVLM causes sympathoinhibition and improve-
ment of BRS in SHRSP.16,17,23 It is possible that antihyper-
tensive agents with sympathoinhibition and that improve BRS via
reduction of oxidative stress in the RVLM might be the most
preferable for the treatment of hypertension, because such
agents should improve long-term mortality in cardiovascular
diseases.

Several questions remain unsolved in the present study.
First, the reason why the BRS of AdMnSOD-transfected SHR-
SP exceeded that of WKY rats could not be clearly elucidated.
Second, we used only SHRSP as a hypertensive rat model in
the present study. Oxidative stress in the brain was suggested
to contribute to SNS activation in other hypertensive mod-
els,27-40 and BRS was impaired in other hypertensive mod-
els.41-42 Thus, it is possible that the results obtained in the
present study might be common in hypertensive models. Fur-
ther studies are needed to determine these issues. Finally, we
did not clarify the relationship between BRS and SNS activa-
tion in the present study. Recently, we reported that MnSOD-
regulated ROS in the RVLM enhanced glutamatergic excito-
try inputs and attenuated gamma amino butyric acid (GABA)-
mediated inhibitory inputs to the RVLM.43 Glutamate and
GABA in the RVLM are key mediators of baroreflex control.
1,2,5,18 We speculate that these mechanisms could contribute
to the improvement of baroreceptor function and sympathoin-
hibition via overexpression of MnSOD in the RVLM in hyper-
tensive rats.
Conclusions: The present results suggest that a reduction in ROS via overexpression of MnSOD in the RVLM improves the impaired BRS in SHRSP through the inhibition of the sympathetic component. These results indicate that ROS in the RVLM of SHRSP impairs BRS with sympathectomy, and that ROS in the RVLM should be the target of treatment for cardiovascular diseases.

References

1. Dampney RA. Functional organization of central pathways regulating the cardiovascular system. Physiol Rev 1994; 74: 323-64. (Review)
2. Guyenet PG. The sympathetic control of blood pressure. Nat Rev Neurosci 2006; 7: 335-46. (Review)


