Arterial Baroreflex
A Novel Target for Preventing Stroke in Rat Hypertension
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Background and Purpose—Arterial baroreflex is one of the most important mechanisms in the regulation of cardiovascular activities. Arterial baroreflex function can be expressed as baroreflex sensitivity (BRS). The present study was designed to test 2 hypotheses: (1) BRS is a new independent predictor for the incidence of stroke in hypertension, and (2) restoration of BRS can prevent stroke in hypertension.

Methods—First, 82 stroke-prone spontaneously hypertensive rats (SHR-SP) aged 28 to 30 weeks were used. After measuring blood pressure and BRS, the survival time was observed. Second, 12 SHR-SP aged 8 months were used. Blood pressure and BRS were determined separately before and after intragastric administration of ketanserin (0.3 and 3.0 mg/kg). Third, SHR-SP aged 5 months were treated with ketanserin for 12 weeks (0.3 mg and 3.0 mg/kg per day). At the end of the treatment, blood pressure and BRS were determined and the end-organ damage was evaluated. Last, SHR-SP aged 3 months were treated with ketanserin (0.3 and 3.0 mg/kg per day) for life and the survival time was recorded.

Results—Stroke was significantly delayed in rats with high BRS than those with low BRS (time to 50% death was 1.47-fold longer than low BRS group; \( P < 0.01 \)). Ketanserin of 3.0 mg/kg per day decreased blood pressure and enhanced BRS, whereas 0.3 mg/kg per day only enhanced the BRS. Fatal stroke incidences were markedly reduced by treatment with both doses (\( P < 0.0001 \) versus control group).

Conclusions—The present study provides evidence that BRS is an independent predictor for stroke in hypertension. Restoration of BRS may be a new strategy for the prevention of stroke. (Stroke. 2007;38:1916-1923.)

Key Words: arterial baroreflex ■ baroreflex sensitivity ■ hypertension ■ stroke ■ stroke-prone spontaneously hypertensive rats

In many countries, including China, stroke is the third leading cause of death only preceded by heart disease and total cancer.1 According to recent estimates published by the World Health Organization, approximately 15 million people per year fall victim to stroke worldwide, of whom 5 million die and another 5 million are left permanently disabled. Many stroke survivors become dependent and require lifelong assistance. Therefore, prevention is the only possible way to curb the stroke pandemic.2 Blood pressure level is one of the most consistent and powerful predictor of stroke, so blood pressure control is an important way to reduce the morbidity of stroke.3-5 However, blood pressure level is not the unique determinant for stroke. Here we propose another important determinant for stroke: the function of arterial baroreflex (ABR).

ABR is one of the most important mechanisms in the regulation of cardiovascular activities. Since the end of 1980s, the pathological importance of ABR function has attracted the attention of many investigators. Baroreflex function, expressed as baroreflex sensitivity (BRS), was found as an important determinant of cardiac death after acute myocardial infarction.6,7 There is also established evidence of abnormal BRS in animal models of stroke8,9 and patients with chronic cerebrovascular disease.10,11 Indeed, it was found that BRS was impaired after acute stroke.12,13 Poststroke patients with impaired BRS had a poor prognosis. However, to our knowledge, there is no report about BRS on predicting stroke.

The first aim of this study was therefore to investigate whether BRS can be a new predictor for stroke incidence. Ketanserin is an antihypertensive drug with affinity for both 5-HT\(_{2A}\) and \(\alpha\) receptors. Our previous studies demonstrated that ketanserin could significantly decrease blood pressure and improve BRS in conscious spontaneously hypertensive rats. Interestingly, a small dose of ketanserin below the threshold of blood pressure reduction could enhance BRS.14 It might be a good tool for studying the effect of restoration BRS on stroke incidence without blood pressure reduction. The second aim of this study was to investi-
gate whether restoration of BRS can prevent or delay the occurrence of stroke in hypertension. The present work was therefore designed to test both hypotheses.

**Methods**

**Animals**

Stroke-prone spontaneously hypertensive rats (SHR-SP) of either sex were provided by the animal center of our university. They were housed with controlled temperature (23 to 25°C) and lighting (8:00 AM to 8:00 PM light, 8:00 PM to 8:00 AM dark) and with free access to standard food and drinking water. All the animals used in this experiment received humane care in compliance with institutional guidelines for health and care of experimental animals.

**Blood Pressure and Heart Period Measurements**

Systolic blood pressure (SBP), diastolic blood pressure (DBP), and heart rate were continuously recorded using previously described technique. Briefly, rats were anesthetized with a combination of ketamine and diazepam. A polyethylene catheter was inserted into the lower abdominal aorta through the left femoral artery for blood pressure measurement and another catheter was inserted into the left femoral vein for phenylephrine administration. In experiment 2, a third catheter was placed into the stomach through a midabdominal incision for drug (ketanserin) administration. The catheters were exteriorized through the interscapular skin. After a 2-day recovery period, the animals were placed for blood pressure recording in individual cylindrical cages containing food and water. The aortic catheter was connected to a blood pressure transducer through a rotating swivel that allowed the animals to move freely in the cage. After approximately 4 hours habituation, the blood pressure signal was digitized by a microcomputer. SBP, DBP, and heart rate values were determined online. The mean values of these parameters during a period of time were calculated.

**Determination of Baroreflex Sensitivity**

In the previously mentioned blood pressure recording condition, BRS was measured in the conscious rat by using the previously described method. Briefly, a bolus injection of phenylephrine was used to induce blood pressure elevation. The dose of phenylephrine (5 to 10 $\mu$g/kg) was adjusted to raise SBP approximately $30 \pm 10$ mm Hg. There exists a delay (approximately 1 second) between the elevation of blood pressure (stimulus) and the prolongation of heart rate (response) for ABR. In rats, the heart rate is approximately 5 or 6 per second. So, heart period was plotted against SBP for linear regression analysis for 2 to 8 shifts (calculated by computer); the slope with the largest correlation coefficient ($r$) of heart period/SBP was expressed as BRS (ms/mm Hg). The mean of 2 measurements with proper dose was taken as the final result.

**Stroke Symptom Observation**

To detect the stroke symptoms, the movement of limbs, respiration, diet, fur, and consciousness of all SHR-SP were observed twice daily (at 8 AM and 6 PM).

**Morphological Examination**

In experiments 1 and 4, when the rats died, the brain was removed, and the signs of hemorrhage, edema, or infarction were examined and then photographed. Brains without these distinct signs were immersed in a solution of 4% paraformaldehyde in 0.1 mol/L phosphate buffer (pH 7.4), and then the brain was dissected and fixed in this solution for 24 hours. The specimens were then washed, dehydrated in a graded ethanol series, and embedded in paraffin. Sections cut transversely at 5-$\mu$m thickness separated by 100 $\mu$m from the anterior to the posterior extremity were stained with hematoxylin and eosin for light microscopic study. Other tissues, including the heart and kidney, were also detected grossly.

In experiment 3, the right kidney, thoracic aorta, and heart were dissected quickly after deep anesthesia. Gross detections were performed in the heart (weight and wall thickness), right kidney (appearance, weight, and thickness), and aorta (weight). The ratio of cardiac weight to body weight (mg/g), aortic weight to length (mg/cm), renal weight to body weight, and the ratio of thickness between the renal cortex and medulla were used as indices of cardiac, aortic hypertrophy, and lesion of kidney, respectively.

**Protocol**

**Experiment 1: Effect of Baroreflex Sensitivity and Systolic Blood Pressure in the Prediction of Fatal Stroke in Stroke-Prone Spontaneously Hypertensive Rats**

Ninety SHR-SP at the age of 28 to 30 weeks of either sex were used in this experiment. After approximately 4 hours habituation (from 8:00 AM to 12:00 PM), blood pressure and heart rate were recorded during a period of 4 hours (from 12:00 to 4:00 PM), then BRS was measured using the previously mentioned methods at conscious state (from 4:00 to 5:00 PM, average blood pressure increase is $30 \pm 10$ mm Hg). After blood pressure and BRS measurements, these rats were replaced in the animal house with the previously mentioned conditions. When rats died from fatal stroke, the survival time (from birth to 600 days) was recorded; dead rats without stroke were discarded.

**Experiment 2: Effects of a Single Dose of Ketanserin on Blood Pressure and Baroreflex Sensitivity in Stroke-Prone Spontaneously Hypertensive Rats**

The experiment was performed in 8-month-old male SHR-SP. They were randomly divided into two groups ($n=6$ in each group) and received ketanserin of 0.3 and 3.0 mg/kg (intragastric), respectively. After approximately 4 hours habituation (from 8:00 AM to 12:00 PM), blood pressure was recorded during a period of 60 minutes (from 12:00 to 1:00 PM) and BRS was measured using the previously mentioned methods. These values were taken as basal control values. A single dose of ketanserin was given through an intragastric catheter. Approximately 30 minutes after drug administration, blood pressure was recorded for another 60 minutes and BRS was determined again.

**Experiment 3: Effects of Long-Term Treatment of Ketanserin on Blood Pressure, Baroreflex Sensitivity, and End-Organ Damage in Stroke-Prone Spontaneously Hypertensive Rats**

The experiment was performed in 5-month-old male SHR-SP. They were randomized into 3 groups: control rats ($n=9$) and 2 groups treated with ketanserin (low-dose group, 0.3 mg/kg per day, $n=10$; high-dose group, 3.0 mg/kg per day, $n=10$). The drugs were mixed into food and administered consecutively for 12 weeks. After hemodynamic measurement, the animals were killed and the right kidney, thoracic aorta, and heart were dissected quickly. Morphological examination was performed using the previously mentioned methods.

**Experiment 4: Effects of Lifelong Treatment of Ketanserin on Stroke Incidence in Stroke-Prone Spontaneously Hypertensive Rats**

The experiment was performed in 3-month-old male SHR-SP. They were randomly divided into 3 groups: control rats ($n=31$) and two groups treated with ketanserin (low-dose group, 0.3 mg/kg per day, $n=24$; high-dose group, 3.0 mg/kg per day, $n=23$). The drugs were mixed into food and administered consecutively. The survival time was observed (from birth to 600 days, dead rats without stroke had been discarded).

**Statistical Analysis**

Investigators were blind to the procedures during blood pressure and heart rate recording, BRS determination, weighing, and morphological examination. Statistical analysis data are expressed as the mean±SD. The comparisons between pre- and postdrug were made by paired $t$ tests and between 2 groups by unpaired $t$ tests. In experiments 1 and 4, Kaplan–Meier analysis was used to estimate
Results

Experiment 1: Effect of Baroreflex Sensitivity and Systolic Blood Pressure in the Prediction of Stroke in Stroke-Prone Spontaneously Hypertensive Rats

Among 90 rats studied, 82 animals that died from stroke were confirmed by displaying neurological symptoms of stroke and/or brain pathological examination (Figure 1). The survival rate is shown in Figure 2. According to the mean BRS value (0.3 ms/mm Hg), 82 SHR-SP were divided into 2 groups: group BRS–low with BRS less than 0.3 ms/mm Hg (n=34) and group BRS–high with BRS more than 0.3 ms/mm Hg (n=48). There was no difference in blood pressure level between these 2 groups. The survival time expressed by Kaplan–Meier survival curves is shown in the Figure 2A. There existed an obvious difference between the 2 curves (log-rank testing $\chi^2=7.601, P=0.0058$). When half of the animals died, it was 406 days in group BRS–high, whereas it was 276 days in group BRS–low from birth. The survival time was approximately 1.47-fold longer in group BRS–low than group BRS–high. When the rats were divided into 2 groups according to the mean heart rate, the curves were not different between the high heart rate group and low heart rate group (log rank testing $\chi^2=0.095, P=0.757$, figure not shown).

Univariate Regression Analysis

The associations among BRS, SBP, and survival time are presented in Figures 2B and 2F. The survival time is significantly and positively correlated with BRS ($r=0.441$, $P<0.0001$) and significantly and negatively correlated with SBP ($r=-0.392, P<0.001$), whereas not significantly correlated with heart rate ($r=-0.013, P=0.905$).

Stepwise Multivariate Regression Analysis

The independent effects and relative importance of SBP, DBP, heart rate, and BRS on the survival time are assessed by stepwise multivariate regression analysis. The regression analysis shows that only SBP and BRS are significant at the 0.05 level (BRS: $P<0.0005$; SBP: $P<0.005$), which indicates they may have independent effects on survival time. The standard partial regression coefficients of BRS were 0.195, and the value of SBP is 0.083. These values mean that the independent BRS and SBP explain 19.5% and 8.3% of the variation in the survival time in SHR-SP, respectively.

Experiment 2: Effects of a Single Dose of Ketanserin on Blood Pressure, Heart Rate, and Baroreflex Sensitivity in Stroke-Prone Spontaneously Hypertensive Rats

The effects of ketanserin on blood pressure in conscious SHR-SP are summarized in Figure 3. The large dose (3.0 mg/kg) of ketanserin decreased SBP by 34 mm Hg and the small dose (0.3 mg/kg) did not lower SBP. The decrease in DBP was similar to SBP but much slighter. Neither dose of ketanserin affected heart rate. The BRS is less than 0.3 ms/mm Hg in most SHR-SP in the conscious state. Ketanserin significantly enhanced BRS in both dose groups (Figures 3 and 4). The enhancement of BRS by ketanserin was not dose-dependent and had a similar effect on BRS (small dose, $0.41\pm0.12$ versus $0.19\pm0.11$ ms/mm Hg; large dose, $0.40\pm0.13$ versus $0.17\pm0.08$ ms/mm Hg).

Experiment 3: Effects of Long-Term Treatment With Ketanserin on Blood Pressure, Heart Rate, Baroreflex Sensitivity, and End-Organ Damage in Stroke-Prone Spontaneously Hypertensive Rats

Compared with the control group, long-term treatment with ketanserin did not affect the body weight in SHR-SP. The effects of ketanserin on blood pressure, heart rate, and BRS in conscious SHR-SP were similar to the results of experiment 2. The large dose (3.0 mg/kg per day) of ketanserin decreased SBP by 29 mm Hg and the small dose (0.3 mg/kg per day) did not lower SBP (Figure 5A). Two doses did not affect heart rate. Compared with the control group, small and large doses
significantly enhanced BRS (0.37±0.16, 0.36±0.13 versus 0.16±0.09 ms/mm Hg).

Compared with control group, a small dose of ketanserin (0.3 mg/kg) did not significantly affect cardiac weights, whereas the large dose (3.0 mg/kg) markedly reduced the cardiac hypertrophy. The ratio of kidney weight to body weight as well as the thickness ratio of renal cortex to medulla was significantly increased in rats treated with the small dose. The aortic weight to length ratio was markedly decreased in rats treated with a small or large dose.

Figure 2. BRS and SBP predict stroke death in SHR-SP. First, 82 rats were divided into 2 groups according to BRS: group BRS-H: BRS more than 0.3 ms/mm Hg (n=34); group BRS-L: BRS less than 0.3 ms/mm Hg (n=48). A, Kaplan–Meier survival curve by subgroup of BRS in SHR-SP. B, Representative scatter plots showed the significant and positive relationships between survival time and BRS. C, The blood pressure has no difference between the 2 groups. D, The mean heart rate of group BRS-L (open bar, 157±27.2 ms) is significantly lower than the group BRS-H (black bar, 168±30.1 ms). Second, rats were divided into 2 groups according to SBP: group SBP-H: SBP more than 220 mm Hg (n=41); group SBP-L: SBP less than 220 mm Hg (n=41). E, Kaplan–Meier survival curve by subgroup of SBP in SHR-SP. F, Representative scatterplots showed the significant and negative relationships between survival time and SBP. G, The BRS has no difference between the 2 groups. H, The mean heart rate of group SBP-H (black bar, 163±27.1 ms) is higher than group SBP-L (open bar, 151±20.2 ms).*P<0.05, **P<0.01 versus group SBP-L.
Experiment 4: Effects of Lifelong Treatment of Ketanserin on Stroke Incidence in Stroke-Prone Spontaneously Hypertensive Rats

The survival time expressed by Kaplan–Meier survival curves is shown in Figure 6. There existed an obvious difference between the control group and the 2 doses of ketanserin groups (log rank testing $\chi^2=59.13$, $P<0.0001$). When half of the animals died, it was 319 days in the control group, whereas it was 406 days in the small dose (0.3 mg/kg) group and 426 days in the large dose (3.0 mg/kg) group.
Compared with the control group, survival time was approximately 1.27-fold longer in the small dose group and approximately 1.34-fold longer in the large dose group.

Discussion

Blood pressure level is an important determinant for stroke. The risk of stroke is increased by approximately 25% with each 10 mm Hg increase in SBP.3,4,22,23 In the present work, when the rats were divided into 2 groups according to SBP level, the stroke was significantly delayed in the relatively lower SBP group compared with the higher SBP group. Using the parameter measuring the time when 50% of the rats died, it was found that the survival time was 1.43-fold longer in the SBP–low group than in the SBP–high group. The univariate regression analysis also confirmed that the survival time of SHR-SP was significantly associated with blood pressure level. However, heart rate had no effect on the prediction of stroke.

Most interestingly, our results intensively demonstrate that BRS is a new predictor for stroke in hypertension. The survival time was 1.47-fold longer in the BRS–high group than in the BRS–low group. The univariate and step multivariate regression analysis also confirmed the significant association between survival time and BRS. These results indicate that BRS is a very important and independent novel predictor for stroke in hypertension. The value of standard partial regression coefficients of BRS is higher than SBP. It means that BRS is more important than SBP in the determination of survival time of SHR-SP. However, it should be noted that the baseline of blood pressure level is very high in these animal models, which may influence this analysis.
Many researchers had confirmed that baroreflex dysfunction was associated with many diseases.\textsuperscript{7,13,24,25} La Rovere and colleagues assessed BRS within 30 days of acute myocardial infarction. During a follow-up period of 2 years, they found the mortality rate is 50\% in patients with a BRS less than 3.0 ms/mm Hg, whereas it was 3\% in patients with a BRS more than 3.0 ms/mm Hg.\textsuperscript{26} Robinson and colleagues reported that poststroke patients with impaired BRS values (\(\geq 5.0\) ms/mm Hg) had a significantly poorer prognosis than patients without impaired BRS (\(>5.0\) ms/mm Hg).\textsuperscript{13} Recently, a series of studies concerning the baroreflex dysfunction and cardiovascular diseases in rats was performed in our department. It was found that baroreflex dysfunction was related to the severity of atherosclerosis induced by a high-cholesterol diet, the survival times in coronary artery ligature-induced myocardial infarction, in aconitine-induced ventricular arrhythmia, and in endotoxin-induced shock.\textsuperscript{27–29} Concerning the present work, to our knowledge, it is the first time it has been demonstrated that BRS is a very important predictor for the most important complication in hypertension: stroke.

Because baroreflex dysfunction contributes importantly to the poor prognosis of many cardiovascular diseases, it is reasonable to propose that restoration of impaired BRS might be as a new strategy for cardiovascular diseases. However, little information on this aspect is available until now. In the present work, ketanserin was used to enhance BRS in SHR-SP and the hypothesis that restoring BRS could prevent stroke in hypertension was tested.

Our previous work\textsuperscript{14} showed that ketanserin significantly decreased blood pressure and improved BRS in the conscious SHR. The effect of ketanserin on BRS was not secondary to a decrease in blood pressure attributable to the following facts: a small dose (0.3 mg/kg) of ketanserin did not decrease blood pressure but enhanced BRS. This finding was repeatedly confirmed in our experiments. It was found that the large dose (3.0 mg/kg) of ketanserin markedly decreased SBP, whereas the small dose (0.3 mg/kg) did not. Ketanserin significantly enhanced BRS in both 2-dose groups. The enhancement of BRS by ketanserin was not dose-dependent or blood pressure-dependent. It possesses a direct action on BRS. Recently, we reported that the action site of ketanserin enhancing baroreflex function is within the rostral ventrolateral medulla in anesthetized rats and mediated by 5-HT\textsubscript{2A} receptors.\textsuperscript{30}

The most exciting finding of the present work is that, without blood pressure reduction, restoration of BRS by the small dose of ketanserin significantly prevented stroke in SHR-SP. Indeed, the lifelong treatment with the small dose of ketanserin significantly delayed the occurrence of stroke. The effects produced by the small dose nearly approached those produced by the large dose of ketanserin. Because the small dose of ketanserin did not significantly lower blood pressure level, its effects on stroke may be mainly attributable to the restoration of impaired BRS in SHR-SP. Nevertheless, we cannot exclude the possibility that other unknown beneficial effects besides the restoration of BRS are also involved in the protection of ketanserin against stroke.

Finally, it should be noted that it is not clear why restoration of BRS leads to the extension of survival time. Further investigations are necessary to elucidate the possible mechanisms.

In conclusion, the present study provides evidence that BRS is a new and important predictor for stroke incident in hypertension. Restoration of ABR function is a new target for the prevention of stroke.

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Disclosures

None.

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