Disturbance of Circadian Rhythm in Heart Rate, Blood Pressure and Locomotive Activity at the Stroke-Onset in Malignant Stroke-Prone Spontaneously Hypertensive Rats

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ABSTRACT—Malignant stroke-prone spontaneously hypertensive rats (M-SHRSP), separated from SHRSP, develop severe hypertension and spontaneously develop stroke at early ages. Using this model of cerebral vascular stroke, influence of stroke-onset on the autonomic nervous system was investigated. Heart rate (HR), systolic and diastolic blood pressures (SBP and DBP) and locomotive activity were monitored during development of stroke using a telemetry system. Stroke-onset was assessed by neurologic symptoms, changes in body weight, fluid intake and serum NOx level. The rat displayed a nocturnal pattern of circadian rhythms. At stroke-onset, mean HR over 24 h increased by 20 to 30 bpm and rapidly increased at post stroke, approximately 100 bpm higher than that at pre stroke. Circadian variation in HR, which was normally 50 bpm higher during night than during day, attenuated at stroke-onset, and it was blunted or reversed at post stroke. BP variation, which was approximately 7 mmHg higher at night than at day, decreased one or two days before stroke-onset and reversed at post stroke, especially in DBP. Insufficient falls in HR and BP during the day mainly accounted for the disturbed circadian variations. Variation of locomotive activity also decreased. These changes serve as reliable and accurate markers for stroke-onset in evaluation of drugs for the prevention and outcome predictions of stroke.

Keywords: Malignant stroke-prone spontaneously hypertensive rats (M-SHRSP), Stroke, Circadian rhythm, Heart rate, Blood pressure

Stroke-prone spontaneously hypertensive rats (SHRSP), a model of genetic hypertension established by Okamoto and colleagues (1), have a high incidence of spontaneous stroke. Salt-loading accelerates this occurrence. Malignant SHRSP (M-SHRSP) were separated through selective brother-sister breeding between precociously and severely hypertensive SHRSP siblings by Okamoto et al. (2) in 1986. These rats develop extremely severe hypertension at an early age, have a higher incidence of cerebrovascular lesions and a shorter life span (approximately 90 days in males) than do SHRSP. Because M-SHRSP spontaneously develop stroke at comparatively early ages (approximately 80 days) without additional salt-loading, this strain serves as a suitable model of hypertension-induced cerebrovascular stroke.

Nagaoka and colleagues (3) reported that 80% of SHRSP subjected to salt-loading at 8 weeks of age developed stroke. Rats showed neurologic signs, body weight loss and a marked increase in water intake at stroke-onset, and there were brain lesions in these rats at autopsy. Using this temporal definition of stroke, we demonstrated that the serum level of lipid peroxides increased immediately after stroke-onset following a gradual increase preceding the stroke in salt-loaded male SHRSP (4). In addition, there is a significant decrease in glutathione peroxidase (GSH-Px) activity and superoxide dismutase activity in erythrocytes in rats that developed stroke. Murakami et al. (5) later confirmed this finding, reporting that the incidence of stroke was 98% in male SHRSP whose erythrocyte GSH-Px activity dropped below 23 U/ml blood. Recently we found that there was a significant increase of serum NO2− and NO3− (NOx) level at stroke-onset following a steady increase, and a marked and transient rise of serum NOx occurred subsequently at post stroke in male M-SHRSP (6, 7).

To investigate the influence of stroke-onset on the autonomic nervous system, changes in circadian variations of heart rate (HR), blood pressure (BP) and locomotive activ-
ity during the development of stroke were examined using a telemetry system. This study presents the first evidence to show rapid and marked disorder of circadian rhythm at the time of spontaneous stroke-onset.

MATERIALS AND METHODS

Animals and the treatments

M-SHRSP, SHRSP and Wistar-Kyoto (WKY) rats were used. These animals were originally provided by K. Okamoto, Professor Emeritus of Kyoto University, and maintained by brother-sister breeding in our Experimental Animal Laboratory. Rats were kept under specific pathogen free condition with a 12-h cycle of light (light 8:00 – 20:00, dark 20:00 – 8:00). Standard rat chow (CE-2; Clea Japan Inc., Tokyo) and water were available ad libitum. Systolic BP (SBP) was determined using a tail pulse pick-up method with a photoelectric detector (UR-5000; Ueda, Tokyo) after warming the animals for approximately 10 min in a chamber maintained at 37°C. For the stroke experiment, male M-SHRSP were used. They were housed individually in metabolic cages at 8 weeks of age, and daily food and water intake and urinary volume were monitored throughout the experiment. Blood was drawn from the caudal vein for serum NO\textsubscript{x} measurement between 17:00 and 18:00 of the indicated days. Stroke-onset was assessed by the appearance of neurologic symptoms, such as hyperkinetic signs and paralysis of limbs, and the changes in their body weight and fluid intake and metabolic changes such as body weight and fluid intake listed in “Materials and Methods”. Normal serum averages were then calculated.

Measurement of serum NO\textsubscript{x} levels

Serum NO\textsubscript{2} and NO\textsubscript{3} were analyzed using an automated NO detector – high pressure liquid chromatography system (ENO-10; Eicom, Kyoto) (10, 11). NO\textsubscript{2} and NO\textsubscript{3} were separated on a reverse-phase separation column packed with polystyrene polymer (NO-PAK, 4.6 x 50 mm; Eicom), and NO\textsubscript{3} was reduced to NO\textsubscript{2} in a reduction column packed with copper-plated cadmium filings (NO-RED, Eicom). NO\textsubscript{2} was mixed with a Griess reagent to form a purple azo dye in the reaction coil. The separation and reduction columns and the reaction coil were placed in a column oven that was maintained at 35°C. The absorbance of the color of the product dye at 540 nm was measured using a flow-through spectrophotometer (NOD-10, Eicom). The mobile phase, which was delivered by a pump at the rate of 0.33 ml/min, was 10% methanol containing 0.15 M NaCl/NH\textsubscript{4}Cl and 0.5 g/l Na\textsubscript{2}EDTA. The Griess reagent, 1.25% HCl containing 5 mg/ml sulfanilamide with 0.25 mg/ml N-naphthylenediamine, was delivered at a rate of 0.1 ml/min. Calibration curves were constructed with a standard mixture of sodium nitrite and sodium nitrate, and the amounts in the samples were calculated from the peak areas.

Statistical analyses

Data were expressed as the mean ± S.E.M. (number of rats). Analyses of data were performed by factorial two-way ANOVA and post hoc significance testing with Fisher’s PLSD test of variance in Fig. 1 and Student’s paired t-test in Figs. 3 and 5.

RESULTS

Development of BP

Figure 1 shows SBP of male M-SHRSP, SHRSP and WKY at various ages. SHRSP developed high BP almost linearly up to the age of 11 weeks and reached a plateau at 12 to 15 weeks. The BP of M-SHRSP paralleled that of SHRSP up to 7 weeks of age and then increased sharply for the following 2 to 3 weeks. Thus, the SBP of M-SHRSP was an average of 55 mmHg higher than that of SHPSP (M-SHRSP: 255 vs SHRSP: 200 ± 4 mmHg at 10 weeks of age).

Changes in body weight, water intake and serum NO\textsubscript{x} at onset of stroke

Figure 2a shows typical tracings of serum NO\textsubscript{x} fluctuation during the development of stroke. The dotted line indicates temporal assessment of stroke-onset from neurologic signs and metabolic changes such as body weight and fluid intake listed in “Materials and Methods”. Normal serum...
NO level remained below 20 μM. It began to increase, however, a few days prior to stroke-onset and sharply increased at post stroke. The time of serum NO level coincided well with the time of the occurrence of neurologic signs, a decrease in body weight of more than 3 g in a day, and a sharp increase in water intake. A marked rise in serum NO was observed within a few days after stroke-onset. At autopsy, there were multiple hemorrhages in the right occipital area of the brain (Fig. 2a) in this rat that died at 84 days after birth.

Radio telemetric monitoring of HR, BP and locomotive activity
To detect the influence of stroke-onset and fluctuations in serum NO, on autonomic nervous system activity, rats were implanted with a transmitter in the descending aorta at 7 weeks of age and housed individually in regular cages on telemetric receiver pads. An ambulatory recording of HR, BP and locomotive activity was performed during development of stroke and post stroke while monitoring the metabolic changes and serum NO levels.

Figure 2b shows representative tracings of HR, SBP, DBP and locomotive activity at pre and post stroke in the same rat as in Fig. 2a. There was a rapid and large increase in HR on the day of assessed stroke-onset. A gradual increase in SBP at pre stroke plateaued at stroke-onset. The mean HR over 24 h increased by 20 – 30 bpm at stroke-onset compared with that of previous days, further increasing for a few days, reaching approximately 420 bpm which was 100 bpm higher than the normal HR (refer to Fig. 3a). Such evident changes at stroke-onset were not observed in BP and locomotive activity. When rats were killed soon after the change in HR, clear cerebral lesions were detected in all rats using Evans Blue infusion.

Time course of day and night means of HR, BP and locomotive activity
Figure 3 shows the time course of characteristic changes in day and night mean of HR (panel a), SBP and DBP (panel b), and locomotive activity (panel c) during the development of stroke and post stroke. HR was approximately 50 bpm higher during night than during day before onset of stroke. This circadian variation was attenuated at onset of stroke and blunted at post stroke with a marked increase in HR. A larger magnitude of the increase in HR during day than that of the nocturnal increase accounted for the blunted circadian variation (Fig. 3a). SBP during the night was approximately 7 mmHg higher than that during the day at pre stroke. However, this variation progressively attenuated a few days before stroke-onset, and the variation was absent from one day before to after stroke. The circadian variation in DBP attenuated earlier than in SBP. It was blunted 2 days before stroke-onset and reversed on the day of stroke and after (Fig. 3b). The variation of higher nocturnal locomotive activity remained throughout, although activity became less at post stroke (Fig. 3c).
Changes in profiles of hourly means of HR, BP and locomotive activity

Profiles of hourly means of HR, SBP and DBP, and locomotive activity were compared on the day of stroke-onset (0 day) and 5 days before (+5 days) and after (+5 days) (Fig. 4). Figure 4a shows that HR during day at stroke-onset was considerably elevated compared with that of day +5, and a further greater increase with absent circadian variation was observed on day +5. SBP was elevated at stroke-onset with concomitant absence of characteristic morning fall and evening rise. The daily variation appeared to be slightly recovered 5 days at post stroke, although SBP remained still high. DBP steadily increased at onset of stroke and post stroke with disordered circadian variation. Locomotive activity during the night was progressively reduced with developing of stroke, while the activity during the day remained similar.

Fig. 3. Time courses of day- and night-mean of heart rate, blood pressure and locomotive activity at pre and post stroke. Day 0 on the abscissa indicates the day of stroke. Each point and vertical bar indicate the mean ± S.E.M. (n=6–9). ○: day (light 8:00 – 20:00), ●: night (dark 20:00 – 8:00). Significance: *P<0.05, **P<0.01, ***P<0.001 night vs day by Student’s paired t-test.

Fig. 4. Profiles of hourly means of heart rate, blood pressure and locomotive activity in male M-SHRSP at the onset of stroke, and before- and after-stroke. Individual 10-s measurements of heart rate, SBP and DBP were sampled at a 5-min interval, and means during 60-min period were then calculated. Each point and vertical bar indicate the mean ± S.E.M. (n=9). ●: the day of stroke, ○: 5 days before stroke, ▽: 5 days after stroke.
Disturbed Circadian Rhythm at Stroke-Onset

Changes in day-night differences in HR, SBP and DBP, and locomotive activity were observed during the development of stroke and post-stroke. Day-night differences were calculated by subtracting the day value from the night value of each rat. Day-night differences in HR were significantly attenuated at stroke-onset compared with that of 5 days, almost absent on days 1 to 3, and reversed (lower during night than during day) on days 4 to 5. The 6- to 7-mmHg day-night differences in both SBP and DBP on 5 days, gradually attenuated at pre-stroke days and reversed at stroke-onset. DBP remained reversed during the observation, but SBP remained reversed only on day 0 and the following day 1. Locomotive activity variation was attenuated day 0 and afterwards.

DISCUSSION

In humans, there is a circadian pattern of changes in BP and HR, with morning values being higher than in the evening, decreasing further during the night. A subgroup of hypertensive patients loses this circadian variation with reduction of nocturnal BP decline (non-dippers) (12, 13), and they are at an increased risk of hypertensive target organ damage (14, 15). Recent studies suggest that stroke in humans can produce changes in autonomic mechanisms that may contribute to sudden death (16). A reduced nocturnal BP fall has also been reported following stroke (17–19).

Rats display nocturnal patterns of circadian variation in BP, HR and locomotive activity that are higher during the night and lower during the day. Normotensive WKY and SHRSP show similar profiles of circadian variations in HR and BP, while rats with secondary hypertension, which were made by transgenic implantation of mouse salivary gland renin gene, displayed reversed variation in BP, being higher during the day (20). However, their HR remained undisturbed. This coincided with the finding in patients with secondary hypertension who showed abnormal circadian rhythms characterized by failure to reduce BP at night (12, 13). Cechetto and coworkers reported that occlusion of the left middle cerebral artery (MCAo) in normotensive rats produces some changes in autonomic and cardiac functions (21) that resemble those in humans after cerebral infarction. Perez-Trepichio and coworkers reported that cerebral infarction in rats (left MCAo) produced a transient elevation of mean BP and HR (22).

These studies in humans and rats suggest that autonomic activity is disturbed due to a subtype of hypertension and stroke. The primary purpose of our present study with use of spontaneous stroke model M-SHRSP is to detect the characteristic changes of autonomic activities at the time of stroke-onset. Such an attempt would be extremely difficult in humans and experimental MCAo rats.

In our experiment, a sudden alteration of HR preceded by progressive attenuation of circadian variation in BP was observed at the time of stroke-onset. This change in autonomic activity occurred in all rats which developed stroke regardless of the anatomic location of a cerebral lesion. This may suggest that humoral factors related to stroke such as nitric oxide, reactive oxygen species, and a potential...
neurotoxic peroxynitrite, a reaction product of NO and superoxide (23), contribute to the disturbance of autonomic activity (6, 7).

Various chemicals, components of foods and environmental factors are evaluated for the efficacy of prevention of stroke using spontaneously hypertensive rats. In most of the cases, however, the survival rate of SHRSP was used to evaluate the effect of stroke prevention because of the difficulty in detecting the time of stroke-onset. An acute and marked change in autonomic activity serves as a more reliable and useful marker of stroke than changes in body weight, fluid intake and neurologic symptoms in evaluating efficacy of various drugs for prevention and outcome prediction of stroke.

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