Changes in Ambulation and Drinking Behavior Related to Stroke in Stroke-Prone Spontaneously Hypertensive Rats

MASARU MINAMI, M.D.,* HIROKO TOGASHI, PH.D.,* YUICHI KOIKE, M.D.,*
HIDEYA SAITO, M.D.,* NISHIO NAKAMURA, M.D.,† AND HISAKAZU YASUDA, M.D.‡

SUMMARY In order to elucidate the behavioral changes related to stroke, ambulatory activity and water drinking were observed in stroke-prone spontaneously hypertensive rats (SHRSP). Age matched male SHRSP and Wistar Kyoto rats (WKY) were subjected to a 12 hour light and dark alternation cycle. Ambulation and drinking activity counts were determined simultaneously with an Ambulo-Drinkometer. Before stroke, ambulation and drinking activity counts in the dark phase (82%) were higher than those in the light phase (18%). Both parameters were well synchronized with the light and dark alternation cycle. With aging, daily ambulation decreased while daily drinking activity increased in SHRSP and WKY. Daily ambulation and drinking activity in 15 and 30 week old SHRSP were greater than those of WKY. It was demonstrated with an Ambulo-Drinkometer that SHRSP undergo specific behavioral changes before the onset of stroke. For instance, the 40-60 week old SHRSP showed significant individual variation in both ambulation and drinking activity. This desynchronization with the light and dark alternation cycle was followed by stroke. Twenty seven autopsies showed 11 cerebral infarctions, 10 cerebral hemorrhage and 6 cerebral hemorrhage with infarctions to be the causes of death.

STROKES IN HUMANS occur abruptly due to underlying diseases including essential hypertension and cerebral artery sclerosis.1 A retrospective analysis of stroke patients from the literature2-3 reveals that approximately 75% of the patients demonstrated cerebral infarctions and 11% demonstrated cerebral hemorrhages. The lethal course of stroke in stroke-prone spontaneously hypertensive rats (SHRSP) established by Okamoto et al4 coincides well with the lethal course of patients with cerebrovascular lesions.5-7 Yamori et al8 has classified stroke-related behavioral changes in SHRSP from a symptomatical point of view. No reports are available in the literature, however, concerning the behavior of SHRSP during their entire life span. In order to identify prodromes of stroke, the present study was carried out to observe the behavioral pattern changes throughout the SHRSP life span (including the development period of hypertension, the onset of stroke and death). Thus, a chronobiological analysis of the behavioral changes in SHRSP was performed.

Methods
We used SHRSP* and WKY donated by Prof. Dr. Okamoto, Department of Pathology, Kinki University School of Medicine. Age-matched male SHRSP and male WKY were subjected to a 12 hour light (L) and dark (D) alternation cycle (LD). Illumination was provided by fluorescent light (100 Lux). Room temperature was maintained at 22 ± 2°C throughout the experiment. Rats were fed a regular diet (Clea Japan, Inc., CE-2). The automatic Ambulo-Drinkometer (O’Hara & Co., Ltd., Tokyo) was used to determine ambulatory activity and water drinking activity. This apparatus is composed of ten steel cages, which are equipped with micro-switches that are activated by the tilting of the cage floor as the rats move around. A drinking spout is connected to a water tank via an infusion machine made of acrylfiber and stainless steel tubes which delivers 0.05 ml drops. The water falls drop by drop, as the rat drinks. There is a weak electrical current in the upper part of the infusion machine. Short circuits generated by individual drops are amplified to activate an electromagnetic counter. Using this Ambulo-Drinkometer, we could determine exactly the amount and rate of the water drinking. Ambulatory activity and water drinking were recorded every hour. Both Tadokoro et al9 and our group9-10 have described this apparatus in detail previously. A Fujitsu, FM-8 personal computer was used to process our data. Values were expressed as mean ± SD. The data of ambulatory and drinking activities were obtained at hourly intervals and subjected to various statistical analysis: unpaired t-test, analysis of variance and two-tailed t-test. Ambulation and water drinking activity data were subjected to autocorrelation and power spectral analysis. The average period of the rhythms (τ(2))11,12 was determined by power spectral analysis.

Results
The Causes of the Death in Both SHRSP and WKY
In this study, 27 male SHRSP were used. As table I shows, from an early age the SHRSP systolic blood pressures were significantly higher than those of WKY. Eleven SHRSP died from cerebral infarction; 10 SHRSP died from cerebral hemorrhage; 6 SHRSP died from cerebral hemorrhage combined with cerebral infarction. These 27 male SHRSP died at the age of 35.55 ± 15.17 weeks old (mean ± SD). Senility was the most frequent cause of death for the control...
TABLE 1  Systolic Blood Pressure (SBP), Heart Rate (HR) in SHRSP and WKY

<table>
<thead>
<tr>
<th></th>
<th>SBP (mm Hg)</th>
<th>HR (bpm)</th>
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<tbody>
<tr>
<td></td>
<td>WKY</td>
<td>SHRSP</td>
</tr>
<tr>
<td>15 W</td>
<td>137.3 ± 14.7</td>
<td>186.8 ± 18.8†</td>
</tr>
<tr>
<td></td>
<td>(11)</td>
<td>(15)</td>
</tr>
<tr>
<td>25 W</td>
<td>139.7 ± 15.1</td>
<td>217.7 ± 34.2†</td>
</tr>
<tr>
<td></td>
<td>(13)</td>
<td>(12)</td>
</tr>
<tr>
<td>45 W</td>
<td>135.8 ± 17.3</td>
<td>213.0 ± 38.4†</td>
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<td>(10)</td>
<td>(6)</td>
</tr>
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</table>

SBP = systolic blood pressure; HR = heart rate; bpm = beats per min; W = week old; WKY = Wistar Kyoto rats; SHRSP = stroke-prone spontaneously hypertensive rats; (n) mean ± sd; †p < 0.05; *p < 0.001 WKY vs SHRSP.

WKY. These WKY died at an age of 82.75 ± 26.01 weeks. In respect to the age at death, a significant difference existed between SHRSP and WKY (t = 6.36, p < 0.001).

Effects of Aging on Ambulatory Activity and Water Drinking Activity in Both SHRSP and WKY

The upper part of figure 1 compares the ambulation of male WKY (dotted line) and male SHRSP (solid line). Values indicate the ambulatory activity during continuous 3 hour periods. The activity counts of ambulation in the dark period were higher than those in the light period. Thus, a pattern typical of nocturnal animals was observed. The lower part of figure 1 compares the water drinking activity of the male WKY (dotted line) and SHRSP (solid line). Values indicate the water drinking activity during continuous 3 hour periods. The activity counts of water drinking in the dark period were also higher than those in the light period. However, no remarkable changes were observed between 15 week old WKY and SHRSP in respect to either of these counts during the 3 hour periods.

Figures 1 and 2 indicate the relationship between aging and behavioral changes in WKY and SHRSP. The upper part of figure 2 depicts the ambulation and the lower part expresses the water drinking activity of WKY and SHRSP. With the lapse of time (15, 30 and 40 weeks), 24 hour ambulatory activity counts tended to decrease in both WKY (left column) and SHRSP (right column). With aging, the drinking activity counts of SHRSP tended to increase as compared with those of WKY. Both the ambulatory and drinking activity of SHRSP were significantly greater than those of WKY at all age levels. At 40 weeks, the SHRSP activity counts of ambulation and water drinking during the light period were much greater than those of WKY during the same period.

Behavioral Changes at the Onset of Stroke in SHRSP

Figure 3 indicates the behavioral changes before death in the SHRSP that died from cerebral infarction.

FIGURE 1. Behavioral changes in relationship to age and circadian rhythm. Values indicate both ambulation (upper part) and drinking activity (lower part) during continuous 3 hour periods. Remarkable difference between the activity counts in the dark period and the activity counts in the light period was noted in respect to ambulation and drinking activity.

FIGURE 2. Histograms of 24 hour ambulatory counts and 24 hour drinking activity counts in WKY and SHRSP. Left columns indicate WKY and right columns indicate SHRSP. Twenty four hour counts of ambulation and drinking activity were divided into two phases: the light and dark periods.
When the same rat was 16–18 weeks old, activity counts of ambulation in the dark period were higher than those in the light period. Synchronization with the light and dark phases was observed and circadian rhythms were noted. As shown in the center of figure 3 (arrow a), the ambulatory activity in the light period increased abruptly followed by a desynchronization with the light and dark periods. Moreover, the SHRSP ambulatory activity was desynchronized with their water drinking activity (arrow b). Figure 4 indicates behavioral changes in the SHRSP whose deaths were caused by cerebral hemorrhage. Desynchronization with the light and dark phases was also observed and the SHRSP ambulatory activity was desynchronized with the water drinking activity.

Dysrhythmicity

The SHRSP whose behavioral changes included desynchronization with the light and dark phases not only underwent behavioral changes in both ambulation and water drinking, but also showed a disturbance in rhythms. Figure 5 indicates the results of power spectral analysis of behavioral changes in SHRSP that died from cerebral hemorrhage (see also figure 4). This same SHRSP, at an age of 20 weeks, had a 24 hour “t” value for both ambulation and drinking activity. However, at the onset of stroke, a much longer periodicity was observed in addition to the 24 hour periodicity in this SHRSP. The other cases of SHRSP that died from stroke showed both desynchronization with the light and dark phases as well as with water drinking activity and ambulation.

Ambulatory Activity and Water Drinking Behavior in WKY that died from other Diseases

Figure 6 indicates the behavioral changes in a WKY over the age of 100 weeks. This WKY died of senility. The upper part of figure 6 shows low ambulatory activity counts, although a circadian rhythm still existed. The lower part of this figure indicates an increase in drinking activity. This WKY remained ambulation, but ceased drinking two days before death. Both the synchronization with the light and dark phases and the synchronization of ambulation with water drinking were maintained in this WKY.

Discussion

SHRSP is a unique model of stroke developed by Yamori, Okamoto and Nagaoka.4 In this study, rat behavior was recorded with an Ambulo-Drinkometer which was developed by Tadokoro et al.8 This Ambulo-Drinkometer enabled us to take long term simultaneous measurements of the ambulation and water drinking activity of rats without handling them. After the development of hypertension was confirmed, we put the approximately 13–15 week old SHRSP into an Ambulo-Drinkometer. Simultaneous measurements of SHRSP ambulation and water drinking activity were performed up until death. Deaths were caused by cere-
Behavioral changes related to stroke in SHRSP

The behavioral changes related to stroke in SHRSP are characterized by a disruption in normal rhythms. Before stroke, SHRSP showed significantly higher blood pressure and increased plasma norepinephrine concentration as compared with those of age and sex-matched WKY. We have reported previously that SHRSP in which stroke was clearly detected by pathological examination showed higher blood pressure and plasma norepinephrine concentrations than those SHRSP in which stroke was not detected. Similar observations have been reported in human cerebral infarction, cerebral hemorrhage and subarachnoid hemorrhage. It was postulated that the release of some neurotransmitters from an infarcted brain could be responsible for the diffused effects on hemodynamics as well as for the diastolic discharges. Serotonin was shown to be released into the CSF and cerebral venous blood from an infarcted brain. Norepinephrine in cerebral tissues might also contribute to the pathogenesis of hemodynamic disorders that are due to cerebral ischemia. With regard to the relationship between behavioral changes and humoral factors in stroke, further study is necessary.

All 27 SHRSP that were used in this study died from cerebral infarction or cerebral hemorrhage. These SHRSP died at a significantly younger age as compared with the control WKY. Entire life span behavioral analysis is important not only to observe the behavioral changes at the onset of stroke, but also to clarify the prodrome of stroke in SHRSP.

References

9. Togashi H, Minami M, Bando Y, et al: Effects of clonidine and rhythms. Further study is necessary to elucidate the relationship between onset of stroke and the stroke related behavioral changes in SHRSP. SHRSP should be sacrificed just after the onset of disruption of normal behavior to determine whether these behavioral changes occur before stroke or only after the onset of stroke. In this study, desynchronization between the light and dark alternation cycle and desynchronization of ambulation and water drinking behavior occurred before death. Moreover, these stroke-related behavioral changes were observed in other cases of SHRSP. In another series of experiments which was done in our laboratory to study the stroke-related humoral factors, male SHRSP showed significantly higher blood pressure and increased plasma norepinephrine concentration as compared with those of age and sex-matched WKY. Ambulatory activity of SHRSP and WKY tended to decrease with age (fig. 2). Conversely, the activity counts during the light period increased significantly as compared with the activity counts during the dark period. After this behavioral abnormality occurred, SHRSP became desynchronized with the light and dark alternation cycle and new rhythms were observed in addition to the circadian rhythm (fig. 5). Thus, the typical behavioral pattern of low activity counts during the light period and high activity counts during the dark period was disrupted in SHRSP. The ambulation of young SHRSP was synchronized with their water drinking behavior. After the occurrence of desynchronization with the light and dark alternation cycle, desynchronization between ambulation and water drinking was also observed. The behavioral abnormality of these SHRSP did not recover to the initial regular behavioral patterns. Post-mortem examination revealed cerebral hemorrhage or cerebral infarction at approximately 40 weeks old. Rats were subjected to a 12 hour light and dark alternation cycle. Before stroke, SHRSP displayed a typical nocturnal behavior pattern with low ambulation and water drinking counts during the light period and high counts during the dark period. No significant difference was demonstrated between SHRSP and WKY in respect to ambulation and water drinking behavior at an age of 15 weeks. At 30 weeks, the ambulation and water drinking of SHRSP were significantly greater than those in age and sex matched WKY. Ambulatory activity of SHRSP and WKY tended to decrease with age (fig. 2). Conversely, the activity counts during the light period increased significantly as compared with the activity counts during the dark period. After this behavioral abnormality occurred, SHRSP became desynchronized with the light and dark alternation cycle and new rhythms were observed in addition to the circadian rhythm (fig. 5). Thus, the typical behavioral pattern of low activity counts during the light period and high activity counts during the dark period was disrupted in SHRSP. The ambulation of young SHRSP was synchronized with their water drinking behavior. After the occurrence of desynchronization with the light and dark alternation cycle, desynchronization between ambulation and water drinking was also observed. The behavioral abnormality of these SHRSP did not recover to the initial regular behavioral patterns. Post-mortem examination revealed cerebral hemorrhage or cerebral infarction to be the causes of death. No remarkable difference existed between cerebral hemorrhage (fig. 4) and cerebral infarction (fig. 3) in respect to behavioral changes. On the other hand, the WKY that died from senility did not show a desynchronization with the light and dark phases (fig. 6). Power spectral analysis of behavioral rhythm (T10) in both young WKY and young SHRSP revealed a 24 hour periodicity (circadian rhythm). Just before death, SHRSP had rhythms with periodicities greater than 24 hours as well as circadian rhythms. The significance and the mechanisms of these newly discovered rhythms (fig. 5) need to be evaluated. It is speculated that the suprachiasmatic nuclei (SCN) are neural time keepers for the maintenance of the circadian rhythms of sleeping, drinking, general activity, plasma corticosterone level and N-acetyl-transferase activity. Several investigators suggest that SCN are the critical cell group in respect to the circadian rhythms. Further study is necessary to elucidate the relationship between onset of stroke and the stroke related behavioral changes in SHRSP. SHRSP should be sacrificed just after the onset of disruption of normal behavior to determine whether these behavioral changes occur before stroke or only after the onset of stroke. In this study, desynchronization between the light and dark alternation cycle and desynchronization of ambulation and water drinking behavior occurred before death. Moreover, these stroke-related behavioral changes were observed in other cases of SHRSP. In another series of experiments which was done in our laboratory to study the stroke-related humoral factors, male SHRSP showed significantly higher blood pressure and increased plasma norepinephrine concentration as compared with those of age and sex-matched WKY. We have reported previously that SHRSP in which stroke was clearly detected by pathological examination showed higher blood pressure and plasma norepinephrine concentrations than those SHRSP in which stroke was not detected. Similar observations have been reported in human cerebral infarction, cerebral hemorrhage and subarachnoid hemorrhage. It was postulated that the release of some neurotransmitters from an infarcted brain could be responsible for the diffused effects on hemodynamics as well as for the diastolic discharges. Serotonin was shown to be released into the CSF and cerebral venous blood from an infarcted brain. Norepinephrine in cerebral tissues might also contribute to the pathogenesis of hemodynamic disorders that are due to cerebral ischemia. With regard to the relationship between behavioral changes and humoral factors in stroke, further study is necessary.
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Nationwide Co-Operative Study of Intracranial Aneurysm Surgery in Japan

AKIRA NISHIMOTO, M.D., KIYOTAKA UETA, M.D., HIDEAKI ONBE, M.D.,
KATSUTOshi KITAMURA, M.D., TERUO OMAE, M.D., FUMIO GOTO, M.D., GENJI OHNEDA, M.D.,
HIROO CHIGASKA, M.D., MITSUO TSURU, M.D., JIRO SUZUKI, M.D., Tokuo WADA, M.D., KEJI SANO, M.D.,
TORU MANNEN, M.D., MASUMI YOSHIOKA, M.D., OSAMU NAKAI, M.D., NAOKI KAGYEMAYA, M.D.,
TAKAYOshI NOMURA, M.D., HAJIME HANDA, M.D., and KENZO TANAKA, M.D.

SUMMARY A cooperative study was made of 4750 intracranial aneurysm cases collected from 133 neurosurgical clinics in Japan by letter inquiry for the period of 2 years from January 1974 to December 1975. Among them, 4124 cases (87%) had a single aneurysm, and 626 cases (13%) had multiple ones. Direct radical surgery was done in 78% of all cases, carotid ligation in 2% and non-surgical treatment in 17%. Direct surgery resulted in a mortality rate of 15% for ruptured aneurysm cases and 7% for nonruptured cases. Radical surgery within 24 hours after rupture had a mortality of 51%, while those within 1 week and 2 weeks were 39% and 30% respectively; grade I or II patients, however, showed much better surgical results even in early operations. The neurosurgical clinics included in this study were spread throughout most of Japan. Micro-surgical technic was already in use of aneurysm surgery at the time of this study in Japan.
Changes in ambulation and drinking behavior related to stroke in stroke-prone spontaneously hypertensive rats.
M Minami, H Togashi, Y Koike, H Saito, N Nakamura and H Yasuda

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