Postnatal Undernutrition Accelerates Incidence of Stroke In Stroke-Prone Spontaneously Hypertensive Rats

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SUMMARY  The effect of infantile nutritional levels on the development of hypertension and incidence of stroke was investigated in stroke-prone spontaneously hypertensive rats (SHRSP). Caloric intake was varied during the suckling period by manipulating litter size immediately after birth; however, all animals had free access to food after weaning. Animals reared in large litters of 15 (LL group) weighed significantly less than those in small litters of 5 (SL group) at every age. In the LL group, systolic blood pressure (mean ± SD) increased age-dependently to reach 237 ± 16 mm Hg at 14 weeks of age, and 14 of 15 rats developed stroke from 14 to 19 weeks of age. On the other hand, in the SL group, the blood pressure at 14 weeks of age was 213 ± 6 mm Hg, which was significantly lower than that in LL group, and stroke occurred only in 3 of 10 rats kept up to 22 weeks.

When the drinking water was replaced with a 1% salt solution, the onset of stroke markedly accelerated in both groups; more than 90% of rats developed stroke within 18 days after the salt-loading. However, the time required for the onset of stroke signs was significantly shorter in the LL group (10.5 ± 1.5 days) than in the SL group (15.4 ± 1.7 days). Furthermore, the blood pressure increment for the first week after the salt-loading was significantly greater in the LL group (293 ± 9.5 mm Hg) than in the SL group (14.2 ± 3.0 mm Hg).

These findings indicate that infantile undernutrition may accelerate the development of hypertension and incidence of stroke in SHRSP.

STROKE-PRONE spontaneously hypertensive rats (SHRSP), established by Okamoto et al.1 from spontaneously hypertensive rats (SHR), are used as a model of human hypertension and stroke. SHRSP are more hypertensive and develop cerebrovascular lesions with a much higher frequency than the original strain. The development of the disorders can be modulated by diets; either a high protein diet or high fat diet containing a moderate amount of protein has suppressive effects, but a low protein diet accelerates the disorders.5, 6

It is well known that infantile nutritional levels have profound and permanent effects on animal development. In the present study, we investigated whether infantile nutritional levels might affect the development of hypertension and occurrence of stroke in SHRSP by manipulating litter size immediately after birth in a manner similar to that described by Kennedy4 and Widdowson and McCane.6

Methods

Pregnant SHRSP with known conception times and their offsprings were used. Within 24 hours of the birth, only males from the litters consisting of 8–10 pups were distributed to form a litter size of 5 or 15, because of a much higher incidence and developmental rate of stroke in the males than in the females of the SHRSP.1 Dams were given free access to food (CE-2, Clea Japan) and water, and maintained under controlled conditions of temperature (23–24°C), humidity (50–60%) and light (0800–2000 hours). Pups were weaned at 28 days of age, given free access to food (CE-2) and water, and kept individually after weaning. At 10 weeks of age, half of the animals in each group were loaded with a 1% NaCl solution as drinking water in order to reduce the period required for the development of stroke.4

The systolic blood pressure was measured by the tail pulse pick-up method4 under unanesthetized conditions weekly or biweekly.

Five days after recognition of signs of stroke, such as repetitive lifting and convulsion of paws, paralysis of hindlimbs, or hyperirritability, the rats were killed by decapitation under ether anesthesia. The signs of stroke were generally unequivocal and were identified independently by 2 skilled observers. Cerebral lesions, including cerebral hemorrhage and infarction, were confirmed by autopsy as described previously.4

Brains obtained at autopsy were fixed with 10% neutral formaldehyde solution, and cut into 4 or 5 frontal sections in order to detect macroscopic changes. The tissues were embedded in paraffin to prepare the histological sections that were stained with hematoxylin-eosin for microscopic observation. The histological observations were made blind looking for evidence of hemorrhage or infarction.

Arithmetical means and standard deviation were calculated. The difference between the means from 2 independent samples was analyzed by Student's unpaired t-test.

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Results

Pups reared in large litters of 15 (LL group) weighed less than those in small litters of 5 (SL group). A significant reduction in growth rate in the LL group was observed at 7 days of age and persisted throughout the experimental period (fig. 1). Systolic blood pressure of the LL group increased age-dependently and reached 237 ± 16 mm Hg at 14 weeks of age (fig. 2). The blood pressure of the SL group was significantly higher at 8 weeks of age, but lower at 14 and 16 weeks of age than that of the LL group.

Cumulative occurrence of signs of stroke is shown in figure 3. Five of 15 rats developed signs of stroke at the age of 14 weeks and the frequency gradually increased to reach 93% (14/15) at the age of 19 weeks in the LL group. In the SL group, only 2 of 10 rats had signs of stroke at 16 weeks of age. In rats that developed signs of stroke during the observation period of 22 weeks, mean time for the onset of signs of stroke was calculated to be 15.6 ± 1.6 weeks (n = 14) for the LL group and 18.8 ± 1.2 weeks (n = 2) for the SL group, respectively. Cerebral lesions including cerebral hemorrhage and infarction were observed in all 14 rats with signs of stroke in the LL group, and 3 rats including 2 rats with signs of stroke and one rat without signs of stroke in the SL group. Signs of stroke were coincident with the cerebral lesions except in one animal in the SL group, where clinical signs of stroke were judged to be probably too mild or short-lasting to be detected.

These results indicate that infantile undernutrition accelerates the development of severe hypertension and incidence of stroke in SHRSP. In order to validate these accelerating effects of infantile undernutrition, we repeated the experiment under the conditions of 1% NaCl-loading.

As shown in figure 4, the salt-loading enhanced the blood pressure increase for the first week to a greater extent in the LL group (29.5 ± 9.3 mm Hg) than in the SL group (14.2 ± 3.0 mm Hg). The blood pressure increase in rats kept under the usual conditions of tap water was small, but significantly higher in the LL group (10.9 ± 3.2 mm Hg) than in the SL group (7.5 ± 4.3 mm Hg).

The development of stroke was dramatically accelerated by the salt-loading (fig. 5). In the LL group, the onset of signs of stroke was recognized in one rat on day 8, and rapidly increased to reach 93% (14/15) on day 13 after the salt-loading. The frequency of signs of stroke in the SL group was similar in pattern to that in the LL group, but delayed for about 5 days. Mean time required for the onset of signs of...
stroke was significantly \( p < 0.001 \) shorter in the LL group \( (10.6 \pm 1.5 \text{ days}) \) than in the SL group \( (15.4 \pm 1.7 \text{ days}) \). In this experiment, cerebral lesions were found in all of the animals that showed signs of stroke.

Discussion

Fostering large litters of 14-20 upon the dam is commonly used in nutritional studies to produce malnutrition in suckling pups. The effect of increasing litter size on body weight is presumably mediated by a reduction in the amount of milk supplied to each pup; thus the pups can thus be considered to be in a state of persistent undernutrition.

In the present study, SHRSP reared in large litters of 15 weigh less, and develop severe hypertension and stroke much earlier than do those in small litters of 5. The result suggests that postnatal undernutrition causes some metabolic changes leading to the development of severe hypertension and onset of stroke in SHRSP.

There have been many reports which found that as a consequence of infantile undernutrition, persistent changes take place in central catecholaminergic systems, protein metabolism, cellularity, body weight gain, and behavior even after a prolonged period of nutritional recovery. A persistent growth retardation in SHRSP with a history of post-

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**Figure 3.** Cumulative incidence of stroke signs in LL (open circle) and SL (solid circle) groups. Cerebral lesions were recognized in 14 rats with stroke signs in LL group, and 2 rats with stroke signs and one rat without stroke signs in SL group. Failure to detect stroke signs in one animal with cerebral lesions seemed to be caused by too weak or short-lasting symptoms.

**Figure 4.** Accelerating effect of salt-loading on blood pressure. Animals were loaded with 1% NaCl at the age of 10 weeks and increase in blood pressure for the first week is shown in the figure. Blood pressure before loading of salt or tap water was 182 ± 3.2 or 185 ± 4.6 mmHg for SL group (solid circle), and 182 ± 4.3 or 183 ± 5.4 mmHg for LL group (open circle), respectively. Increments in blood pressure were significantly different between SL and LL groups in tap water \( p < 0.05 \) and 1% NaCl \( p < 0.001 \), and between tap water and 1% NaCl in SL group \( p < 0.001 \) and LL group \( p < 0.001 \), respectively.

**Figure 5.** Accelerating effect of salt-loading on onset of stroke. Animals were loaded with 1% NaCl at the age of 10 weeks and killed for recognition of cerebral lesions 5 days after the onset of stroke signs. Cerebral lesions were detected in all the animals with stroke signs. Solid circle; SL group, open circle; LL group.
nal undernutrition suggests an occurrence of similar changes in SHRSP to those described above.

Among these changes, either an activation of the central catecholaminergic system or an impairment of protein metabolism, which is induced by postnatal undernutrition, may participate in an acceleration of the development of hypertension and onset of stroke in SHRSP. It has been shown in SHRSP that activation in the central catecholaminergic system may play an important role in initiation of the hypertension, and that a low protein diet markedly accelerates the development of hypertension and onset of stroke. Hypertension is an important risk factor for the development of stroke in the human. In the present study, severe hypertension preceded stroke both under usual conditions (tap water) and after salt-loading. The animals from the LL group had a higher blood pressure than those from the SL group, except at 8 weeks of age. The reason why the SL group had significantly more hypertension than the LL group at the young age appears to be due to earlier maturity in the former group. These findings suggested that postnatal undernutrition accelerated the development of severe hypertension, which resulted in an induction of the onset of stroke. However, the contribution of the other factors could not be excluded in the onset of stroke as suggested by Nagaoka et al. They indicated that the onset of stroke in SHRSP was dependent not only on the severity of hypertension but also on genetic predisposition. Genetic alterations in metabolism of nutrient, especially in the cardiovascular system, may be partly involved in the development of hypertensive vascular lesions in SHRSP, because cerebral blood flow and cerebrovascular reactivity to \( \text{CO}_2 \) inhalation were maintained in the normal ranges in SHRSP given a high protein diet. In the present study, postnatally undernourished SHRSP showed a higher blood pressure in response to salt, which seemed to indicate the presence of an increased vascular reactivity.

The present results suggest that postnatal undernutrition is one of the risk factors for the development of stroke, although the detailed mechanism is unknown.

References
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