STROKE-PRONE SPONTANEOUSLY HYPERTENSIVE RATS AS A MODEL FOR TOXEMIA OF PREGNANCY AND AGGRAVATING AND PREVENTIVE EFFECTS OF MATERNAL MODIFICATIONS DURING PREGNANCY ON OFFSPRING’S GROWTH

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Genetical differences in changes in blood pressure (BP) were chronologically investigated during pregnancy in stroke-prone spontaneously hypertensive rats (SHRSP), Wister-Kyoto rats (WKY) and Sprague-Dawley (SD) rats. Especially, the early stages were carefully studied. Maternal conditions in SHRSP were modified by the treatments with NaCl and taurine, respectively. BP in SHRSP and WKY rose significantly at the early stage of pregnancy compared to prepregnancy levels (SHRSP; 208±2 mmHg vs 197±5 mmHg, WKY; 133±2 mmHg vs 126±1 mmHg) (p<0.05). In contrast, no such changes were observed in SD rats. Differences in 24-hour urinary epinephrine excretion before and during pregnancy ran parallel with such BP changes among these strains. NaCl-loaded SHRSP died during pregnancy with severe pathohistological changes in their kidneys and severe proteinuria. Taurine treatment had a marked prophylactic effect on these maternal pathological changes during pregnancy, resulting in better growth in offsprings. These results suggest that SHRSP could be one of the suitable animal models for the studies on toxemia of pregnancy and also suggest an important role of hypertensive genetical disposition in the development of toxemia of pregnancy.

MATERNAL hypertension during pregnancy occurs in 7 to 10% of human cases, affecting fetal or offspring’s growth! Preexisting essential hypertension has been speculated to be one of the predisposing factors, based on the epidemiological reports on women who showed pathological blood pressure (BP) rises during pregnancy. These reports prove that these women had much greater genetical disposition to hypertension than those who had normal pregnancy, and the incidence of hypertensive disorders in mothers whose daughters developed preeclamptic symptoms was 28%, in contrast to 13% among mothers whose daughters had normal pregnancy2-3.

As well known, spontaneously hypertensive rats (SHR)4 and stroke-prone SHR

Key words:
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Changes in BP during pregnancy in rats. 

- ****: significant differences from prepregnant values (p < 0.05, 0.01). 
- \*: significant difference from non-pregnant SHRSP (p < 0.05). 
- ☆: significant difference between the 12th and 21st days of pregnancy (p < 0.05).

In the present study, changes in BP were followed during pregnancy in SHRSP, Wistar-Kyoto (WKY) and Sprague-Dawley (SD) rats. Genetical differences in chronological changes in BP were investigated.

The early stages were particularly focussed on. Further, aggravating or preventive modifications of the effects of pregnancy on maternal conditions and their offspring's growth in SHRSP were investigated by treatment with NaCl or taurine.

**MATERIALS AND METHODS**

**Experiment 1**

Four-month-old pregnant SHRSP (n=5), age-matched non-pregnant SHRSP (n=5), pregnant SD rats (n=5) and age-matched non-pregnant SD rats (n=5) were used. In addition, age-matched pregnant WKY (n=4) were also used as the normotensive control from which SHRSP had been derived. These animals were placed in stain-

Changes in urinary epinephrine excretion during pregnancy. 

- \*: significant differences from non-pregnant rats (p < 0.05, 0.01, 0.001). 
- \*:\*:\*:\*: significant difference from prepregnancy (p < 0.001).
Fig. 3. Changes in BP during pregnancy in NaCl-loaded SHRSP, untreated SHRSP and untreated WKY. ⋆ ⋆: significant differences from prepregnant values (p<0.05, 0.01).

less steel cages at 20±2°C with a 12-hour photoperiod and were fed on a standard laboratory diet (SP diet, Funahashi farm, Chiba, Japan) with tap water for drinking.

After mating, vaginal smears were checked every morning for the detection of spermatozoa. Days of pregnancy were accounted 24 hours after the recognition of spermatozoa in the vaginal smear.

After checking daily gain in body weight (BW) and water and food intake, 24-h urine was collected in a plastic metabolic cage connected with a bottle containing 0.5 ml 6N hydrochloric acid for the determination of 24-h urinary catecholamine and creatinine excretions. Urinary catecholamine was assayed by high performance liquid chromatography with electrochemical detection (HPLC-ECD), as described by Kissinger. Urinary creatinine levels were determined by the addition of an alkaline picrate solution to the diluted urine, followed by the colorimetrical measurement of the resultant creatinine picrate at 515 nm.

Urine collection and BP measurements (tail-pulse pickup method) were performed before mating, on the 7th (just after implantation), 12th (complete placental formation), 18th (placental functional maturation) and 21st (just before delivery) days of pregnancy.

Experiment II

Four-month-old female SHRSP (n=15) from 3 litters fed on the SP diet were divided into 3 groups. Animals in A (n=5), B (n=5) and C (n=5) groups were given 1% NaCl, 3% taurine and tap water as controls, respectively. Further experimental procedures during pregnancy were the same as Experiment I.

BW of their offsprings were measured every two days until 45 days of age. They were sacrificed under ether anesthesia and their femur lengths were measured as an additional growth index. The date 24-h after birth was defined as the first day of age for the uniformed accountment. All data were statistically analized by the Student’s t-test, and each value revealed mean±SE.

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RESULTS

Experiment I

BP was significantly elevated at the early stage of pregnancy in SHRSP (207 ± 2 mmHg) (p < 0.05) and markedly fell just before delivery (177 ± 4) (p < 0.01) as compared to BP measurements taken before pregnancy (197 ± 5). (Fig. 1-above). BP increments during gestation in SHRSP (16 ± 3 mmHg) were significantly greater than those in WKY (7 ± 3) (p < 0.05). BP in WKY also rose significantly at the early stage of pregnancy (133 ± 2) compared to prepregnancy levels (126 ± 1) (p < 0.05). In contrast, there were no significant BP changes during pregnancy in SD rats (Fig. 1-below).

Twenty-four-hour urinary epinephrine excretions in SHRSP were significantly greater than those in non-pregnant SHRSP during pregnancy (p < 0.05). The highest level was recorded on the 7th day of pregnancy (77.0 ± 4.1 ng/mg). These measurements were significantly greater than those recorded before pregnancy (48.2 ± 3.9) (p < 0.05) (Fig. 2-left). It was also revealed that the adrenal glands of pregnant SHRSP were more activated, showing heavier adrenal glands (33.0 ± 2.0 mg/100g) than those before pregnancy (24.5 ± 2.3) (p < 0.05).

In contrast, 24-h urinary epinephrine excretions in pregnant SD rats were significantly greater only on the 12th day of pregnancy (53.8 ± 6.1 ng/mg) as compared to those in age-matched non-pregnant SD rats (25.1 ± 4.6) (p < 0.01). These levels, however, were not significantly higher than those before pregnancy (Fig. 2-right).

Experiment II

BP was markedly elevated at the early stage of pregnancy in 1% NaCl-loaded pregnant SHRSP (262 ± 4 mmHg) compared to BP before pregnancy (235 ± 4). These rats showed greater BP increments (27 ± 7 mmHg) than both pregnant SHRSP (16 ± 3) and pregnant WKY (7 ± 3) without NaCl loading (p < 0.05) (Fig. 3). All NaCl-loaded SHRSP showed a marked proteinuria (184.0 ± 4.9 mg/day) and eclampsia-like seizures and died of severe cerebral edema.
This was probably due to metabolic disturbances caused by renal failure during pregnancy. These rats revealed severe pathohistological changes in their kidneys. These changes included the widespread and diffuse damage of both glomeruli and tubules partly replaced by proliferative connective tissue and remarkable thickening of arterial walls (Fig. 4-below).

On the other hand, BP in taurine-treated SHRSP did not significantly rise at the early stage of pregnancy (202±5 mmHg) compared to prepregnancy levels (199±5). This contrasted to significant BP increases (208±2) from control prepregnancy levels (195±3) (p<0.05) found in SHRSP (Fig. 5). The taurine-treated SHRSP showed significantly less proteinuria (31.0±5.7) and only slight pathohistological changes in their kidneys (Fig. 4-above).

Twenty-four-hour urinary epinephrine excretions in taurine-treated SHRSP (38.5±4.5 ng/mg) were less than those in control SHRSP at the early stage of pregnancy (49.6±5.7) (p<0.05) (Fig. 6).

Further, taurine-treated mothers' offsprings, which had lower birth weight (below their mean birth weight), showed significantly greater growth compared to those in offsprings of untreated mothers (Fig. 7), although there were no differences in their birth weights. Femurs of the taurine-treated mothers' offsprings were significantly longer than those of untreated mothers' offsprings.

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Fig. 8. Correlation between birth weight and BW, and between birth weight and femur lengths at 45 days of age in male offsprings.

(Table).

In control mothers' male offsprings, there were positive linear correlations between birth weights and BW, and birth weights and each femur length at 45 days of age (p<0.001) (Fig. 8). A similar correlation was also found in females (p<0.001) (Fig. 9). In contrast, there were no such correlations in offsprings of taurine-treated SHRSP at 45 days of age.

DISCUSSION

It has been previously reported that maternal BP in SHR elevates in the early and intermediate stages of pregnancy and decreases during the last week of pregnancy. In relation to such a decrease in BP, there was the report that the magnitude of the decrease in BP correlated with the number of live pups born although precise mechanisms have not been completely clarified. The decline in BP before delivery might be linked with the increased circulating levels of progesterone or increased production of PGE2 and PGI2. In the present study, a similar BP decrease was recognized. However, catecholamine
levels did not seem to be directly related to such a reduction of BP occurring just before delivery in SHRSP as well as SHR, as suggested through the chronological analyses of urinary catecholamine excretion.

On the contrary, SHRSP and WKY showed significant rises in BP at the early stage of pregnancy compared to pre-pregnancy levels, in contrast to no changes in SD rats.

Urinary excretions of epinephrine in SHRSP during pregnancy were significantly higher than those in age-matched non-pregnant SHRSP (p<0.05). There were no differences between pregnant SD rats and age-matched non-pregnant SD rats. An investigator has suggested that urinary excretion of norepinephrine is an indicator of average daily sympathetic nervous activity, while it is partly accelerated by presynaptic

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stimulation of epinephrine as an indicator of adrenal medullary activity. Results of this study might be due to the differences in a sympathetic genetical disposition between SHRSP and WKY. These differences, probably caused by the pituitary-adrenal system, manifested itself through a greater hypertrophic response of the adrenal medulla in SHRSP than that in WKY during pregnancy. The maternal adrenal glands of SHRSP were significantly heavier than those in WKY.

NaCl-loaded SHRSP died during pregnancy and showed severe pathohistological changes of the kidney. These results clearly suggest that maternal NaCl loading in SHRSP is one of the aggravating factors for both maternal conditions and their fetal or offspring’s growth partly through exaggerated sympathetic activation.

Taurine, one of the sulphur aminoacids, might have a lot of important roles in humans. It was reported that fetuses and neonates have only low taurine-synthesis activity, and taurine is to be given to fetuses and neonates through transplacental route and maternal milk.

Taurine has also been suggested to have a suppressive effect on activities in the sympathetic nervous system. In the present study, urinary epinephrine levels in taurine-treated SHRSP were lower than those in controls at the early stage of pregnancy. However, a significant difference in BP changes was not recognized. Taurine had marked attenuating effects on such maternal sympathetic activity during pregnancy in SHRSP.

Further, it was reported that taurine has an important biological role in developmental growth and that around 60-70% of the whole amount of taurine present in rat pups is derived from their mother’s taurine pool at birth.

In the present study, taurine-treated mothers’ offsprings with a low birth weight showed significantly greater growth compared to those of control mothers 2 weeks after birth and thereafter, although there were no significant differences in birth weight between both groups. These results might be due to the maternal taurine administration during pregnancy.

Our results suggest that SHRSP might be one of the suitable animal models from which to study toxemia of pregnancy in humans, and also suggest an important role of hypersympathetic genetical disposition in the development of such disorders.

Our data indicate that a maternal sympathetic suppression by taurine at the early stage of pregnancy results in better growth of offsprings after birth. In contrast, NaCl loading caused an opposite effect. Still, the precise mechanisms of the beneficial effects of taurine have yet to be discovered.

In addition, these facts may also explain one of the speculations on the initial pathogenesis of toxemia of pregnancy, i.e., reduced placental blood flow due to acute atherosclerosis of the uterine spiral arteries which might occur with allograft rejection, suggesting an immunologic basis for the initial vascular lesion in uteroplacental vessels.

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