Puberty and Ovulatory Release of Gonadotropins in Spontaneously Hypertensive Rats

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Abstract

The age at vaginal opening, estrous cyclicity, serum levels of luteinizing hormone (LH), follicle stimulating hormone (FSH) and prolactin (PRL) on the day of proestrus, and number of ova and ovarian weight as measured on the day of estrus in spontaneously hypertensive (SH) and genetically matched normotensive Wistar Kyoto (WKY) female rats were compared. In SH rats, there was a significant delay in the vaginal opening, but the regular 4-day estrous cycle followed afterwards. No significant changes were observed in the afternoon increase in serum LH, FSH and PRL on the day of proestrus in SH and WKY rats, although the basal levels of LH and PRL in the morning (11:00h) were lower in SH rats than in WKY rats. The mean number of ova in SH rats was also less than in WKY rats, whereas the ovarian relative weight was similar in both species of rats. It can be said that SH rats undergo certain, but not critical, endocrine and/or neuroendocrine changes related to reproduction.

The spontaneously hypertensive (SH) rat has been shown to exhibit some abnormalities in reproductive endocrine functions. In the comparison with genetically matched normotensive Wistar Kyoto (WKY) rats, male SH rats had higher basal levels of prolactin (PRL), follicle stimulating hormone (FSH) (Amador et al., 1983, Sowers et al., 1978) and luteinizing hormone (LH) (Rodriguez-Padilla et al., 1987). Testicular weight was greater in SH rats than WKY rats, although Plasma levels of testosterone were reported to be either higher (Rodriguez-Padilla et al., 1987) or lower (Amador et al., 1983). Further, the PRL response to TRH was stronger and the LH response to LHRH was weaker in male SH rats than in WKY rats (Sowers et al., 1978).

On the other hand, very few studies have been done on female SH rats. It was only reported that they had a PRL concentration similar to WKY rats (Hodson et al., 1981, Steger et al., 1984), and their LH response to LHRH was weaker, as in the male (Hodson et al., 1981). The litter size was smaller in SH rats (Lawrence & Gray 1982, Scott & Goecke 1984), which was explained by the difference in the number of ova that had been ovulated (Lawrence & Gray 1982) or implanted.
We therefore conducted a series of experiments in order to obtain a more detailed profile of the reproductive function of female SH rats.

Materials and Methods

Animals

Female SH and WKY rats (21 days of age and age-matched) obtained from Charles River Japan, Inc. were maintained under conditions of controlled temperature (24-26°C) and illumination (lights on 05.00-19.00 h). The body weight was checked every 2 days until the day of vaginal opening, for which the examination was performed every day. After the vaginal opening, vaginal smears were checked every morning. SH and WKY rats, which were obtained at 8 weeks of age, were also checked with vaginal smears for the experiment to examine the reproducibility of proestrous LH, FSH and PRL profiles.

Bleeding and radioimmunoassay

Animals were implanted, at 15-18 weeks of age, with a cardiac catheter of silicone tubing (Dow Corning, No. 602-105) through the jugular vein under ether anesthesia in the morning of proestrus. Blood was collected through the catheter at 1-h intervals from 11.00 h to 20.00 h. Serum LH, FSH and PRL concentrations were measured by double-antibody radioimmunoassay with kits supplied by the NIADDK and expressed in terms of rat NIH-LH-S1, NIH-FSH-S1 and NIH-PRL-RP1, respectively. Each hormone was measured in a single assay, in which the minimally detectable amounts of LH, FSH and PRL were 0.48, 86 and 1.14 ng/ml, respectively. The intraassay coefficients of variation (CV) estimated in 5 replicates of stock serum at the mean LH, FSH and PRL concentrations of 12.6, 4200 and 28.2 ng/ml were 5.8, 2.4 and 6.3%, respectively.

Number of ova and ovarian weight

On the day following bleeding, the body weight was recorded, ovaries were dissected and weighed, and the number of ova in the oviduct was counted for each animal.

Statistical analysis

Two-way analysis of variance and the new multiple range test of Duncan were used to test the statistical significance of fluctuations over time in the mean hormone values for each sampling time. Student's t-test was used to test the significance of difference between the values in SH and WKY rats.

Results

Vaginal opening and estrous cycle

There was no significant difference in the body weight increase of SH and WKY rats until the age of 13 weeks, after which SH rats had a lower body weight increase than WKY rats. However, there was a significant (p<0.01) delay in vaginal opening in SH rats; the mean age with SEM for SH rats on the day of vaginal opening was 37.2±0.3 days (n=11) and the age for WKY rats was 33.8±0.7 days (n=12). After the vaginal opening, regular 4-day estrous cycles were observed in both SH and WKY rats.

Gonadotropin secretion on the day of proestrus

Both SH and WKY rats showed a marked increase in serum LH in the afternoon of proestrus (Fig. 1). Analysis of variance revealed that the mean LH values fluctuated significantly (p<0.01) and the multiple range test revealed that the concentrations between 14.00 h and 18.00 h were significantly greater than those at 11.00 h and 12.00 h in both SH and WKY rats. The peaks of LH secretion occurred at 15.00 h and 16.00 h, respectively. The only noticeable change was that the level at 11.00 h in SH rats was significantly (p < 0.05) lower than that in WKY rats at corresponding times.

There were observed significant (p<0.01) increases in FSH in both SH and WKY rats in the afternoon of proestrus (Fig. 1), and the mean FSH concentrations in SH and WKY rats were the highest at
a tendency for FSH levels in SH rats to be higher than those in WKY rats was seen.

Significant (p<0.01) fluctuation occurred in PRL concentrations too, and the peak was at 15.00 h in both groups (Fig. 1). It was noticeable that the PRL level at 11.00 h was significantly lower in SH rats than in WKY rats.

Completely similar results were obtained in another set of SH and WKY rats, supporting the reproducibility for the proestrous hormone profile.

**Ovulation**

The number of ova ovulated in SH rats was significantly (p<0.05) smaller than in WKY rats; i.e., the mean number for SH rats was 10.9±0.7 (n=10) and the mean for WKY rats was 14.7±0.4 (n=11). The mean body weight of SH rats was lower (p<0.05) than WKY rats; i.e., 194±6 g vs 229±5 g, but the ovarian relative weight was similar in both groups, i.e., 25±0.5 vs 26±2.0 mg %.

**Discussion**

The present study demonstrates a significant delay in vaginal opening in SH rats compared to genetically matched normotensive WKY rats. There are a number of reports describing abnormalities of catecholamines and neuropeptides in the CNS of SH rats. First of all, age-dependent changes in the level and turnover of epinephrine, norepinephrine and dopamine in the hypothalamus and the brain stem have been described (Yamori et al., 1970, Versteeg et al., 1976, Saavedra et al., 1978, Wijnen et al., 1977, 1980, Koulu et al., 1986). Second, changes in the concentrations of the opiate receptor (Martucci & Hahn, 1979), neuropeptide Y (Maccarrone & Jarrott, 1985), corticotropin releasing factor (CRF) (Hattori et al., 1986), and vasopressin (Crofton et al., 1978) have been
reported. These are all, directly or indirectly, concerned with the regulation of gonadotropin secretion (Ono et al., 1984, Rivier & Vale 1984, Karla 1986), and thus any change will be related with the delay in the first ovulation. Nevertheless, it is possible that handling when observing vaginal opening or checking body weight caused a delay in vaginal opening in SH rats. It has been reported that the response of growth hormone, PRL and corticosterone secretion to a variety of stressful stimuli were much more pronounced in SH rats than in control rats (Iams et al., 1979, McMurry & Wexler 1981), suggesting that in the CNS of SH rats the activities of CRF and opiate are increased. These neuropeptides are also known to inhibit gonadotropin secretion (Ono et al., 1984, Rivier & Vale 1984, Karla 1986).

In SH rats a regular 4-day estrous cycle occurred after vaginal opening and their ovulatory secretions of LH, FSH and PRL in the afternoon of proestrus were comparable in magnitude and timing to those in WKY rats, although an increasing trend was seen in FSH secretion. However, it was noticeable that the basal secretions of LH and PRL, but not of FSH, as indicated by the serum levels at 11.00 h, were significantly lower than those in WKY rats. Although not described in the present paper, this was observed repeatedly in a related study. It is probable that the smaller number of ova in SH rats, as observed in accord with earlier reports (Lawrence & Gray 1982, Scott & Coecke, 1984), was due to this low level of basal LH secretion, since basal secretion of LH has been an important factor in normal follicular maturation (Taya and Sasamoto, 1988). Although a weaker LH response of the pituitary gland to LHRH (Hodson et al., 1981) probably accounts for the low basal LH secretion, an abnormality in the release of LHRH due to a certain abnormality in the CNS, to which the delay in the onset of puberty has been attributed, is also possible. Also, the possibility is not denied that the abnormality in sympathetic nervous activity (Iriuchijima 1973, Takeda & Bunag 1978) is responsible, since adrenergic inputs into the ovary are involved in the regulation of ovarian steroid secretion (Kawakami et al., 1981) and also in the process of ovulation (Espey 1978).

A trend towards an increase in ovulatory FSH secretion in SH rats seems to confirm the observation of the decrease in the number of ova, suggesting that a smaller number of follicles that have matured. There has been a positive correlation between the number of healthy antral follicles and inhibin activity, the latter being inversely related to FSH secretion throughout the estrous cycle (DePaolo et al., 1979, Tsukamoto et al., 1986).

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References


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