LH and PRL secretion in ovariectomized spontaneously hypertensive rats

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Abstract. The pulsatile patterns of LH and PRL secretion, and effects of stress on these patterns were examined in ovariectomized normotensive Wistar, spontaneously hypertensive, and genetically matched normotensive Wistar Kyoto rats. Judged by the overall mean of PRL concentrations, PRL levels in spontaneously hypertensive rats were not different from those in Wistar Kyoto rats, but the pulse amplitudes as well as the overall mean concentrations were significantly greater in both Wistar Kyoto and spontaneously hypertensive rats than in Wistar rats. Increases in PRL release during immobilization stress in spontaneously hypertensive rats occurred earlier than in Wistar rats, but the peak levels were not different between the two animal groups, although significantly lower in Wistar Kyoto rats than in Wistar rats. Overall mean of LH concentrations was higher, but pulse amplitude was smaller in spontaneously hypertensive rats than in Wistar Kyoto rats, and in both groups significantly smaller than in Wistar rats. The decrease in LH release owing to stress was similar in all animal groups. A significant association between LH and PRL peaks was evident in all animals, although the rate of association was lowest in Wistar Kyoto rats. In conclusion, the central nervous system mechanisms for both LH and PRL secretion differ markedly in both spontaneously hypertensive and Wistar Kyoto rats from those in Wistar rats, and no specific difference was found in spontaneously hypertensive rats compared with Wistar Kyoto and Wistar rats as controls.

Although it has been suggested that spontaneously hypertensive (SH) rats have a number of abnormalities in their reproductive endocrine functions, there is disagreement among studies. In comparison with genetically matched normotensive Wistar Kyoto (WKY) or Sprague-Dawley (S-D) control rats, basal PRL levels were elevated in male, (1-3), but not in female rats, either intact (4) or ovariectomized (5). Even so, the release of PRL in response to stress was greater in both male and female rats as compared with WKY and S-D rats (1,2,4), respectively. LH levels were reported to be higher (6) on the one hand, and on the other hand similar (3,4) in intact male and female SH rats.

The objective of the present study, therefore, was to determine the secretion of LH and PRL in SH rats by analysing characteristics of LH and PRL pulses in ovariectomized rats. The response to immobilization stress was investigated in those rats as well.

In the present study, we used ordinary Wistar rats as another control in addition to WKY rats, since the inappropriateness of the WKY rats as a counter model to SH rats has been pointed out (2,7).

Materials and Methods

Animals
Eight-week-old female SH, age-matched WKY normotensive control, and ordinary Wistar rats were purchased from Charles River Japan, Inc. All animals were maintained under conditions of controlled temperature (24-26°C) and light (lights on 5.00-19.00 h). They were ovariectomized at the age of 13-15 weeks.

Systolic blood pressure was measured by an indirect tail cuff method using the rat-tail manometer system (KN-210, Natsume Seisakusho Co, Ltd, Tokyo, Japan) about one week before ovariectomy in conscious SH, WKY and Wistar rats. It was 200 ± 4.5 (N=8), 146 ± 6.8 (N=7), and 126 ± 5.0 (N=10) mmHg, respectively.
Blood sampling

Four weeks after ovariectomy, an intraatrial catheter of silicone tubing (Dow Corning, No. 602-105) was implanted through the jugular vein under ether anesthesia on the day before blood sampling. Control samples, 110 μL, for measuring LH and PRL were taken through the catheter every 6 min for 2 h, from 12.00 to 14.00 h while the animals was moving freely. As soon as the control sampling was finished, the animal was immobilized in its supine position, by fastening its legs to the board with gauze, and samples were taken at 5, 20, 60 and 90 min after the start of immobilization. Altogether sampling was completed in 7 WKY, 8 SH and 10 Wistar rats.

Radioimmunoassay

Serum LH and PRL were measured by double-antibody radioimmunoassay with kits supplied by the NIDDK and are expressed in terms of rat NIH-LH-S1 and NIH-PRL-RP1, respectively. Each hormone was measured in a single assay, in which the minimally detectable amount of LH and PRL were 0.12 and 7.57 μg/l, respectively. The in-trassay coefficients of variation (CV) estimated in 5 replicates of stock serum at the mean LH and PRL concentrations of 14.74 and 27.3 μg/l were 5.9 and 6.9%, respectively.

Statistical analysis

Each animal’s time series of LH and PRL measurements in the control samples was analysed for pulses as follows; a pulse was defined when the CV calculated from all LH or PRL values, comprising ascending as well as descending phases of each LH or PRL pulse, was greater than 1.7 times the intra-assay CV of the corresponding assay (8). Overall mean serum concentrations of LH and PRL in the control samples and the samples obtained during stress, and also pulse frequencies and amplitudes of LH and PRL in the control samples were compared in each group of animals by analysis of variance followed by Duncan's Multiple Range test. Differences among groups were analysed using Student’s t-test.

Results

Characteristics of the LH and PRL pulses

The serum LH and PRL patterns observed during the 2-h control sampling period in representative animals from the 3 animal groups are shown in the upper two panels of Fig. 1, and the means of these groups are shown in the lowest panels of Fig. 1. Parameters which characterize the pulsatile pattern of both hormones in serum, i.e. pulse amplitude, frequency, and the overall mean of both hormones

in each of the 3 animal groups are summarized in Fig. 2.

Serum LH fluctuated in all 3 animal groups in a pulsatile manner, with a mean frequency of 2.4-2.8 pulses/h, and there was no significant difference between groups. However, the pulse amplitude was significantly lower in both WKY and SH rats than in Wistar rats (p<0.05). The amplitude was significantly lower in SH than in WKY rats (p<0.05).

The serum PRL level fluctuated in all 3 groups, indicating that PRL secretion was pulsatile as well. The frequency was 2.3-3.1 pulses/h in all animals, although it was significantly less in WKY and SH rats than in Wistar rats (p<0.05 and 0.05, respectively). The PRL pulse amplitude was significantly higher in both SH and WKY than in Wistar rats (p<0.05). There was no significant difference in any of the parameters for PRL pulses between SH and WKY rats.

Mean serum LH and PRL concentrations

The overall mean serum concentration of LH was significantly higher in SH than in WKY rats (p<0.05). However, the mean LH was significantly lower in WKY rats than in Wistar rats (p<0.05), but not lower than that in SH rats. In comparison with Wistar rats, both WKY and SH rats had significantly higher overall mean PRL concentrations (p<0.05) without any significant difference between the two animal groups.

Concomitant LH and PRL pulses

In all Wistar, WKY and SH rats, the LH and PRL peaks occurred concomitantly, as seen in some representative individual examples (Fig. 1). The percentage of LH peaks concomitant with PRL peaks was determined for each animal using stringent (simultaneous peaks) and lenient criteria (simultaneous peaks ± 6 min), as described by Pohl et al. (9).

When analysed using the lenient criterion, 70-80% of LH peaks were concordant with PRL peaks, against 30-65% using the stringent criterion (Table 1). The Chi-square test of independence (10), which analysed the simultaneous LH and PRL peaks according to the stringent criterion, revealed a significant association between both peaks in ordinary Wistar (p<0.0001), WKY (p<0.005), and SH (p<0.0001) rats. However, the percentage of LH peaks concordant with PRL peaks, as judged by the stringent criterion, was smaller in WKY rats, but not in SH rats, than in Wistar rats by the Chi-square test (p<0.01).

LH and PRL responses to immobilization stress

Effects of immobilization stress, to which the rats were exposed after the 2-h control blood sampling,
Fig. 1.
Serum LH (-----) and PRL (- - -) patterns during the 2-h control blood sampling period in representative animals from the 3 animal groups (upper 2 panels), and the mean values for both hormones at each sampling point (lowest panel). The parameters during the immobilization stress, started at the point indicated by arrows, are also shown. *p<0.05 vs Wistar, **p<0.05 vs WKY.

on the serum concentrations of LH and PRL are shown in Fig. 1. The upper two panels give representative individual examples, the lowest panel gives the mean of LH and PRL concentrations at each sampling time.

Immobilization stress caused a marked decrease in LH secretion in all the Wistar, WKY and SH rats. The mean LH levels were lowest at 60 min after the start of immobilization, and were almost the same in all 3 animal groups regardless of the significant difference in the overall mean LH values during the control sampling period, as described before.

On the other hand, the PRL response to immobilization was very different among animal groups. In all the groups, the mean PRL rose rapidly, attained peak value at 20 min, and recovered the pre-immobilization level at 60-90 min. However, the level at 5 min was significantly greater in SH rats than in WKY rats (p<0.05), indicating higher responsiveness to stress in SH rats than in WKY rats. When compared with the response in Wistar rats, the level at 5 min as well as the peak level at 20 min were significantly lower in WKY rats (p<0.05) for both, but not in SH rats.

Discussion
Although it has been reported that intact male SH rats have elevated basal levels of PRL in serum in comparison with WKY rats (1-3), the results of the present study demonstrate that the PRL levels in the ovariectomized SH rats, judged by the overall mean of PRL concentrations, are not different from those in WKY rats. This is in agreement with the report by Steger et al. (5). However, when comparing all 3 animal groups, not only the overall mean of PRL concentrations but also the amplitude of the
pulses are significantly greater in WKY and SH rats than in ordinary Wistar rats, indicating that both types of animals are hyperprolactinemic when ovariectomized, lacking ovarian hormones. We have observed that PRL levels in serum are significantly lower in SH rats than in WKY rats in the morning of proestrus; i.e. 20.04 ± 4.35 μg/l (N=13) for SH rats and 41.23 ± 4.29 μg/l (N=11) for WKY rats (11), suggesting that sensitivity to estrogen in the neuroendocrine system controlling PRL release differs between the two animal groups. In addition, it is also possible that the sensitivity to testosterone concerning PRL regulation (12) is different between male WKY and SH rats, which accounts for the difference in PRL levels between the two animals (1-3). The significant difference in PRL levels in intact female SH rats in comparison with intact S-D rats as controls, as reported by Hodson et al. (4), could not be explained since they used an inappropriate control.

It has been reported that SH rats, both male and female, showed a greater response to stressful stimuli than other rats; the release of PRL in SH rats in diestrus was exaggerated in response to ether stress compared with that in S-D female rats in diestrus (4), and in response to immobilization stress compared with that in male WKY rats (1). The results of the present study that the ovariectomized SH rats showed a greater response to immobilization stress than WKY rats, as judged by the level at 5 min after the onset of stress, support these reports. However, the present study demonstrates that in both WKY and SH rats, the PRL response to immobilization stress, as judged by peak levels at 20 min, was markedly small compared with that in ordinary Wistar rats, without any significant difference between the two animals. This indicates that the neuroendocrine system controlling PRL release is somewhat different in both WKY and SH rats from that in ordinary Wistar rats.

The present study shows that in ovariectomized SH rats, LH levels, as judged by overall mean values, are higher and the amplitude of the pulses are lower than those in WKY rats. However, it must be noted that in WKY rats, both LH levels and pulse amplitudes were lower than those in Wistar rats, suggesting also that the neuroendocrine system controlling LH release in WKY rats is different from that in ordinary Wistar rats. Thus, it is possible that controversial results are drawn owing to the control animal used in the experiments. However, differential effects of ovarian hormones on LH secretion between SH and WKY rats were again suggested, because it was observed in our laboratory that LH levels in the morning of proestrus in SH rats were significantly lower than in WKY rats: 0.66 ± 0.12 μg/l (N=13) for SH rats and 1.30 ± 0.24 μg/l (N=11) for WKY rats (11). Even

### Table 1.
Concordance of LH and PRL peaks.

<table>
<thead>
<tr>
<th></th>
<th>No. of rats</th>
<th>Criterion of simultaneous peaks</th>
<th>Criterion of simultaneous peaks ± 6 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>WKY</td>
<td>7</td>
<td>31.0+6.1%</td>
<td>72.9+7.8%</td>
</tr>
<tr>
<td>SH</td>
<td>8</td>
<td>51.9+7.7</td>
<td>77.6+2.6</td>
</tr>
<tr>
<td>Wistar</td>
<td>10</td>
<td>64.4+7.7</td>
<td>83.7+4.8</td>
</tr>
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*: Significant difference (p<0.01) was evaluated with χ² test of comparison of rates.
so the response of LH release in ovariectomized rats to immobilization stress, namely a decrease in the release (13,14), apparently was not different between the 3 animal groups.

The present results show that PRL release in the ovariectomized rats is pulsatile and that many of the PRL peaks are concomitant with the LH peaks (10), confirming the observation by Pohl et al. (9), although our ordinary Wistar rats showed higher rates of concomitant pulses than reported (31% by the stringent criterion and 62% by the lenient criterion). The rate in WKY rats, which is the lowest among the 3 animal groups, is similar to theirs. Studying the mechanism for such a temporal association between LH and PRL pulses, Pohl et al. (9) suggested that neurons secreting PRL-releasing factors other than GnRH, which is known to release PRL (15,16), are involved, being loosely coupled to the GnRH pulse generator in the hypothalamus. The WKY rats may have some disturbances in this mechanism, although the details are unclear.

The present results indicated some crucial disturbances in the neuroendocrine system controlling the secretion of LH and PRL in WKY rats. This supports previous reports proposing that WKY rats as well as SH rats have several abnormalities, including the neuroendocrine functions, compared with the normotensive S-D rats (2,7). Therefore, it seems plausible to argue that both Wistar and WKY rats need to be used as controls in experiments studying functions of SH rats.

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References