Pulsatile Secretion of Prolactin and Oxytocin During Nursing in the Lactating Rat

TAKASHI HIGUCHI, KAZUMASA HONDA, TETSUJI FUKUOKA, HIDEO NEGORO, YUTAKA HOSONO* AND ETSURO NISHIDA*

Department of Physiology, Fukui Medical School, Matsuoka, Fukui 910–11 and *Department of Obstetrics and Gynecology, Kanazawa University School of Medicine, Kanazawa 920

Abstract

The secretory profile of prolactin and oxytocin in response to suckling stimuli by litters was studied in unanesthetized and urethane-anesthetized lactating rats. Serum prolactin levels were determined by radioimmunoassay. Oxytocin released at milk-ejection reflex was monitored by the changes in the intramammary pressure and/or the characteristic pup's reaction associated with the milk ejection. Serum prolactin concentrations began to rise earlier than the first milk ejection in unanesthetized rats, but they were never elevated without the appearance of milk ejections in urethane-anesthetized rats. Pulsatile fluctuation in serum prolactin levels at 6-15 min intervals was observed in the nursing period when 10 pups were suckling continually. The intermittent milk-ejection reflex occurred not always but preponderantly (64-91%) when the serum prolactin levels were at the nadir of the fluctuation. Injection of an estimated dose of oxytocin released at each milk ejection (1 mU) did not change the serum prolactin levels. These results indicate that the mechanism for prolactin release may be more susceptible to the effects of anesthesia than that for oxytocin release in response to the suckling stimuli and that the release of both the hormones is pulsatile in nature and be influenced by a common biological clock during the nursing period.

Suckling stimuli by litters are indispensable for maintaining milk production and the milk-ejection reflex in lactating rats. Nerve impulses are conducted to the paraventricular and supraoptic nuclei where they activate oxytocin-neurons projecting to the posterior pituitary gland (Wakerley and Lincoln, 1973, Lincoln and Wakerley, 1975). The same impulses may also inhibit prolactin-release inhibiting factor (PIF) or facilitate prolactin-releasing factor (PRF) release, resulting in elevation of blood prolactin levels (Terkel et al., 1972; Wakerley et al., 1976).

The exact pathway from the nipples to the anterior or posterior pituitary gland is not clear, but it is probable that the pathway conducting the stimuli which induce prolactin and oxytocin release is in common up to a certain level of the central nervous system. Therefore, the releases of prolactin and oxytocin induced by suckling stimuli may show a certain relationship suggesting a common mechanism for control of secretion of both hormones. We report here the existence of pulsatile release of prolactin during nursing and discuss its relationship to the intermittent release of oxytocin.

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The authors would like to dedicate this paper to the late Professor Masazumi Kawakami.
Materials and Methods

Animals

Female Wistar rats, approximately 200 g in body weight, were obtained from Shizuoka Agricultural Cooperative Association for Laboratory Animals (Hamamatsu). They were supplied with pelleted rat food (MF, Oriental Yeast Co. Ltd., Tokyo) and tap water ad libitum. On the night of proestrus they were caged with males and the day of delivery was designated Day 1 of lactation. The litter size was adjusted to 10 to 12 pups within 24 h after birth.

Suckling experiments

The lactating rats were used for the experiment on days 9-12 of lactation. In the experiment using unanesthetized rats, a few days before an experiment the animals were implanted with an intrathoracic cannula of silicone tube (0.64 mm, od) via the jugular vein under ether anesthesia. They were allowed to habituate to the experimental room and to the bleeding procedure in order to minimize the stress effect accompanied with bleeding through the cannulae. On the evening before an experiment, all the pups were removed from the cage. The next day the pups were replaced in the cage and their characteristic reaction associated with the milk-ejection reflex was observed (Lincoln et al., 1973).

In the experiment using anesthetized rats, the mothers were anesthetized with a single dose of urethane (1.1 g/kg body weight) at 0800 h and bleeding was started at 1100-1300 h. A silicone cannula was introduced into the atrium and a stainless tube (0.7 mm, od) was inserted into the teat duct of an inguinal mammary gland for recording intramammary pressure. The other end of the cannula was connected to the pressure transducer (Nihon Kohden, AP-620G). The intramammary pressure was recorded on a pen recorder (Nihon Kohden, WI-641GR). For the estimation of oxytocin released at each milk ejection, synthetic oxytocin (Atonin-O, Teikoku Hormone Mfg. Co., Kawasaki) was injected iv via the intrathoracic cannula at the beginning and the end of the experiment.

Prolactin assay

Blood samples of about 50 µl were taken for 2-3 h through the indwelling cannula at 1- to 6-min intervals according to the frequency of the milk-ejection reflex judged from the pup's stretch behavior and/or rise in intramammary pressure or at fixed 2- or 5-min intervals. About the same volume of physiological saline (0.9% w/v) was replaced at each bleeding. Separated serum was stored at -80°C until assayed for prolactin. Prolactin concentrations in serum were determined with a rat radioimmunoassay kit provided by NIAMDD (USA). Serum levels of prolactin were expressed in terms of NIAMDD rat prolactin RP-2. One way analysis of variance was used for the statistical evaluation of the data.

Results

Studies with unanesthetized rats

Thirty min after the start of bleeding, 10 fasted pups were replaced in the cage. Mother rats soon retrieved their young and

![Fig. 1. Two examples of the secretory patterns of prolactin and oxytocin release during nursing in unanesthetized rats. Vertical bars indicate the occurrence of milk-ejection reflex judged from pup's characteristic reaction. Horizontal lines represent the period of wakefulness of the mother. Pups were introduced at the time shown by arrow. Blood samples were taken at 5-min intervals.](image-url)
allowed them to suckle. It usually took 5–20 min for all the 10 pups to start suckling. As shown in Fig. 1 serum prolactin levels were low and stable before introduction of the pups to the cage. Five to 10 min after introduction of the pups, serum prolactin levels began to rise. The first milk-ejection reflex occurred after serum prolactin concentrations rose. Mother rats remained somnolent throughout most of the nursing period, but sometimes they left their young behind and moved around in the cage. Serum prolactin levels fluctuated and the milk-ejection reflex occurred intermittently. The milk-ejection reflex appeared to occur when the serum prolactin levels were at nadir except at the time of the initial rise in serum prolactin in response to the suckling stimuli.

Studies with anesthetized rats

In order to make the pups suckle continuously without interference by the mother’s behavior, urethane-anesthetized lactating rats were used. The milk-ejection reflex was monitored with the change in intramammary pressure as well as with the stretch reaction of the pups. As shown in Fig. 2 intramammary pressure increased by about 10 mmHg at each milk-ejection reflex.

The milk-ejection reflex occurred in 11–67 min of contact with their pups in 13 out of 29 rats tested. Eighty min after the onset of nursing the other 16 rats were injected ip with 1–ml hypertonic saline (1.5 M) to facilitate the milk-ejection reflex (Negoro et al., 1983). Four out of the 16 rats never exhibited milk-ejection nor an increase in prolactin release during the nursing period of 4–5 hours. Milk-ejection reflex occurred in the remaining 12 rats following the injection of hypertonic saline, but in 5 of them serum prolactin levels failed to rise.

Blood samples were taken at 1–to 6-min intervals according to the frequency of the milk ejection; blood samples were taken more frequently when the milk ejection occurred with shorter intervals. Serum prolactin concentrations exhibited pulsatile fluctuations in spite of the continuous suckling by the pups (Fig. 3). The intervals between the two peaks of serum prolactin levels were 6–15 min. Milk-ejection reflex did not always occur but 64–91% of the reflexes observed occurred when serum prolactin concentrations were at the nadir. Asynchrony of milk-ejection reflex with serum prolactin fluctuations was usually seen when the intervals between milk ejections were shorter than 5 min.

![Fig. 2. The changes in the intramammary pressure during nursing period. The intramammary pressure was elevated by about 10 mm Hg at each milk-ejection reflex. Reflexes occurred intermittently at 4- to 9-min intervals in this rat.](image-url)
Fig. 3. Two examples of the secretory patterns of prolactin and oxytocin release during nursing in urethane-anesthetized rats. Vertical bars indicate the occurrence of milk-ejection reflex judged from the elevation of intramammary pressure and from pup's characteristic reaction. Blood samples were taken at 1- to 6-min intervals.

Seven other rats were examined to see whether the pulsatile fluctuation in serum prolactin levels existed before suckling started. Serum prolactin concentrations stayed at low levels but seemed to fluctuate before pups began to suckle (Fig. 4).

Effect of oxytocin injection on serum prolactin levels

The results described appear to indicate that oxytocin released at milk ejection might cause prolactin release. To test this hypothesis, anesthetized rats were injected with oxytocin and changes in serum prolactin levels were evaluated. The dose of oxytocin used, 1 mU, was the estimated amount of oxytocin released at spontaneous milk ejection. One-mU oxytocin in 0.1-ml saline caused a rise in intramammary pressure equivalent to that observed at spontaneous milk ejections, but serum prolactin concentrations did not change significantly (p>0.05) up to 30 min after injection as in the control rats administered with 0.1-ml physiological saline (Table 1).
Table 1. Serum prolactin levels after oxytocin injection

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Before</th>
<th>2</th>
<th>5</th>
<th>10</th>
<th>20</th>
<th>30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxytocin</td>
<td>21.3±3.2*</td>
<td>23.1±4.1</td>
<td>20.9±3.7</td>
<td>22.8±2.9</td>
<td>23.0±3.5</td>
<td>22.1±2.6</td>
</tr>
<tr>
<td>Saline</td>
<td>21.8±3.5</td>
<td>22.7±3.8</td>
<td>23.4±4.1</td>
<td>21.5±3.4</td>
<td>23.3±3.6</td>
<td>21.8±3.3</td>
</tr>
</tbody>
</table>

*: Serum prolactin concentrations (ng/ml serum), mean±SEM in 10 animals

Discussion

Suckling stimuli brought about prolactin release in both unanesthetized and urethane-anesthetized rats as reported earlier (Terkel et al., 1972; Wakerley et al., 1978). It has been reported that prolactin levels do not become significantly raised until the intermittent milk ejections are under way (Burnet & Wakerley, 1976) and that the release of prolactin is never observed without the release of oxytocin in the urethane-anesthetized rats (Wakerley et al., 1978). We confirmed that the milk-ejection reflex could occur even without an apparent rise in serum prolactin levels in urethane-anesthetized rats. However, in unanesthetized rats serum prolactin concentrations started to rise earlier than the first milk-ejection reflex. The mechanism for prolactin release may be more susceptible to the effects of anesthesia than that responsible for oxytocin release in response to the suckling stimuli.

Serum prolactin levels fluctuated rhythmically during the nursing period when the pups were continually suckling, indicating a pulsatile release in prolactin. Before nursing, the existence of a pulsatile release of prolactin was indicated (Fig. 4) but the pulse amplitude was too small to be determined from this study. Pulsation in prolactin secretion has been reported to be observed only when the prolactin secretion was liberated from the inhibitory control by such as pentobarbital (Belchetz et al., 1978; Wehrenberg & Ferin, 1982) or tranquilizer (Shin & Chi, 1979). Suckling stimuli provided by the pups can induce a synchronized electroencephalographic pattern similar to that seen in a light sleep period (Lincoln et al., 1980). Therefore, suckling stimuli might unmask suppressed pulsatile release of prolactin as anesthetics or tranquilizers did. Another interpretation that small pulsatile fluctuation of serum prolactin levels was magnified enough to be easily recognized by suckling stimuli is possible.

The milk-ejection reflex occurred preponderantly when serum prolactin levels were in the trough of pulsatile fluctuations. Oxytocin released at milk ejection may not be responsible for the change in serum prolactin levels, because exogenously injected oxytocin failed to facilitate prolactin release at a dose comparable to that released at each milk ejection in nursing rats. The results are in accord with those in male rats (Vaugham et al., 1978; Shin, 1982). The relationship observed between oxytocin and prolactin secretion may not be due to the direct action of oxytocin released into the general circulation. It is more likely that the release of both the hormones may be regulated by a common oscillator.

Episodic secretion of prolactin has been reported to be mediated by noradrenergic neurons (Negro-Villar et al., 1979). They reported that episodic secretion of prolactin was suppressed after the inhibition of noradrenaline synthesis by the administration of diethyldithiocarbamate (DDC) and that stimulation of α-adrenergic receptors induced a rise in blood prolactin levels in DDC-treated rats. Moreover, the prolactin secretory pulse is synchronized with the discharge of LH in rhesus monkey (Belchetz et al., 1978; Wehrenberg & Ferin, 1982). The LH secretory pulse also is caused by
\(\alpha\)-receptor-mediated adrenergic input (Bhattacharya et al., 1972; Gnodde & Schuiling, 1976; Weick, 1978). Therefore, this oscillatory apparatus participating in the pulsatile secretion of LH and prolactin may consist of or be driven by adrenergic neurons. In terms of oxytocin secretion, central adrenergic receptors have been reported to be involved in the reflex release of oxytocin (Tribollet et al., 1978). The adrenergic pathway projects to the paraventricular and supraoptic nuclei from the dorsal part of the midbrain (Fuxe & Hökfelt, 1967) and microiontophoresis of noradrenaline inhibited electrical activity of the paraventricular neurons of which axons reached the posterior pituitary gland (Moss et al., 1972). These results together with the present ones suggest that the oscillatory mechanism associated with noradrenergic neurons may also control the timing of the milk-ejection reflex; when adrenergic pathways are activated, serum prolactin and probably LH levels may increase but milk ejection may be inhibited, however, when the adrenergic influence is attenuated, the milk-ejection reflex can occur and prolactin secretion may be suppressed.

In addition to noradrenergic neurons, dopaminergic neurons may participate in the mechanism of the intermittent secretion of prolactin and oxytocin as well. Dopamine is one of the possible PIFs and/or facilitates PIF release from the hypothalamus (MacLeod, 1976). Dopamine has also been reported to stimulate oxytocin release (Bridges et al., 1976) and to be necessary for periodic release of oxytocin when the milk ejection reflex occurs (Moos and Richard, 1982). Therefore, our finding that oxytocin release occurred preponderantly at the nadir of serum prolactin levels indicates that dopaminergic neurons may be activated at the time of the milk ejection reflex, resulting in temporal inhibition of prolactin secretion.

References


