Changing characteristics of the milk-ejection reflex during pregnancy, lactation and after weaning in the rat

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Summary. The pattern of reflex milk ejection during suckling was investigated in anaesthetized Wistar rats at various stages of pregnancy, lactation and after weaning. Milk-ejection responses were measured using intramammary pressure recordings, and the amount of oxytocin released was estimated from log dose–response lines compiled from the mammary responses to exogenous oxytocin. The number of rats showing intramammary pressure responses to oxytocin increased on Day 22 of pregnancy (the day of parturition) and decreased at 8 days after weaning. The dose–response lines from pregnant animals were shallow, but steepened and shifted to the left during lactation and after weaning. Reflex milk-ejection responses during suckling were detectable in primigravid animals, indicating that birth of the litter and previous suckling experience are unnecessary for the immediate functioning of the reflex. Reflex milk-ejection responses improved during early lactation (such that the frequency and the amount of oxytocin released at each response were maximal at Day 10 of lactation), and subsequently declined in late lactation. Although the frequency of responses in animals 2 and 4 days after weaning was similar to that in late lactating animals, the amount of oxytocin released at each response had risen again to mid-lactation values. In animals undergoing a second pregnancy and lactation the pattern of change in the milk-ejection responses was similar to that of primiparous animals.

Introduction

In the lactating rat, expulsion of milk from the mammary gland is dependent on oxytocin stimulating the contraction of myoepithelial cells (Benson & Cowie, 1956). Oxytocin is produced by cells of the supraoptic and paraventricular nuclei in the hypothalamus and is released from nerve terminals situated in the pars nervosa in response to the sucking stimulus (Lincoln, Hill & Wakerley, 1973). During late gestation and early lactation the myoepithelial cells of the mammary gland enlarge, and spread to form a dense network over the secretory alveoli (Radnor, 1972), and the number of oxytocin receptors associated with the myoepithelium increases dramatically (Soloff, Alexandrova & Fernstrom, 1979; Soloff & Wieder, 1983). Morphological changes also occur in the hypothalamic magnocellular nuclei of parturient and lactating animals, where a greater proportion of oxytocin-staining neurosecretory soma and dendrites are in direct apposition and have ‘double synapses’ (Theodosis, Poulain & Vincent, 1981; Hatton & Tweedle, 1982; Theodosis & Poulain, 1984). This plasticity in the structure of the nuclei may serve to synchronize the activity of oxytocin-secreting neurones, which, coupled with the withdrawal of pituicytes from neurosecretory endings in the pars nervosa, may lead to enhanced hormone release (Tweedle & Hatton, 1982).

To investigate whether those changes in the central and peripheral components of the milk-ejection reflex are associated with altered functional characteristics, the pattern of milk ejection in response to suckling was studied in animals at various stages of pregnancy, lactation and after weaning. Anaesthetized animals were used to allow accurate recording of intramammary pressure and to maintain a constant sucking stimulus during the test.
**Materials and Methods**

*Animals.* Primigravid Wistar rats from the Departmental colony were used at Days 15, 20 and 22 of pregnancy (Groups 1–3), where Day 1 of pregnancy was denoted as the day a vaginal plug was found after mating, and gestation normally lasts 22 days. Other groups consisted of primiparous rats at Days 2, 10, 20 and 28 of lactation (Groups 4–7), where Day 1 of lactation was denoted as the day the litter was born, Day 28 of lactation with a foster litter of 2-day-old young substituted on Day 20 of lactation (Group 8), and at Days 2, 4 and 8 after weaning following a 20-day lactation (Groups 9–11). Additional animals were left for 1 month after a 20-day lactation before being re-mated. These animals were then used at Days 15, 20 and 22 of a second pregnancy (Groups 12–14), and at Days 2 and 10 of a second lactation (Groups 15 and 16). All the lactating animals had litters consisting of 8–10 young.

*Experimental design.* Lactating rats were separated from all but one of their young for 2–3 h before the experiment. Rats were anaesthetized with urethane (0-9 g/kg i.p.) and xylazine (7 mg/kg i.m.) (Isherwood & Cross, 1980), occasionally supplemented with methohexitone i.v. if necessary. A saphenous vein was then cannulated, and an inguinal mammary gland was cannulated for intramammary pressure recording as described by Lincoln *et al.* (1973). To obtain reliable recordings of intramammary pressure a small volume (0.1–0.2 ml) of cow’s milk was injected into the cannulated gland of pregnant animals until consistent mammary responses were obtained. At 3 h after induction of anaesthesia the log dose–response of the mammary gland to exogenous oxytocin was determined, using doses of between 0-2 and 2 mU oxytocin injected rapidly as a single bolus. These log dose–response lines provided the basis for the subsequent estimate of oxytocin release at each reflex milk-ejection response. A 2-h suckling test was then performed on animals which had shown mammary contractions to exogenous oxytocin, using a foster litter of 8 hungry 2-day-old young. These 2-day-old young were separated from their mother for 3 h before the suckling test. Young pups were used to keep the suckling stimulus constant, since young pups tend to suck more vigorously than do older pups after deprivation, and do not limit their milk intake (Cramer & Blass, 1983). Oxytocin (1 mU i.v.) was injected periodically throughout the suckling test to check the sensitivity of the gland. The frequency of response was calculated for each animal from the 5 intervals after the first milk ejection. This was to avoid inclusion of erratic milk ejection intervals which sometimes occur as a result of periods of cortical desynchronization (Lincoln *et al.*, 1980).

**Results**

*Responses to exogenous oxytocin.*

The number of rats that showed a measurable rise in intramammary pressure in response to 1 mU oxytocin increased throughout pregnancy, from 3/8 in Group 1 to 7/8 in Group 3 (Table 1). All the animals responded to oxytocin during lactation and shortly after weaning, but 8 days after weaning (Group 11) oxytocin caused a measurable contraction of the gland in only 3/7 animals. The mean log dose–response line of the glands to oxytocin for some of the groups are shown in Fig. 1. The slopes of the log dose–response lines for the pregnant animals were shallow, in that a given dose of oxytocin caused a small rise in intramammary pressure. The log dose–response lines from lactating animals were similar in slope and position to those from animals 4 days after weaning, and were steeper and had shifted to the left compared with those from pregnant animals. The mean pressure changes of the mammary gland to 1 mU oxytocin for all the groups are shown in Fig. 2. When compared with the response at Day 10 of lactation (Group 5), significant differences in the amplitude of the response were found in pregnant animals (*P* <0.01 for Groups 1, 2, 3 and 13, *P* <0.05 for Group 14), in Group 8 (*P* <0.05) and Group 11 (*P* <0.01). The results from animals in a second pregnancy or lactation were similar to those from primiparous animals in that the
Table 1. Comparison of reflex milk ejection during pregnancy, lactation and after weaning

<table>
<thead>
<tr>
<th>Group†</th>
<th>No. of rats responding to oxytocin</th>
<th>% rats responding which milk ejected</th>
<th>Interval to 1st milk-ejection response (min)</th>
<th>Frequency of milk-ejection responses (per min)‡</th>
<th>Estimated oxytocin release at each milk-ejection response (mU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>12</td>
<td>8</td>
<td>9.9 ± 2.1</td>
<td>0.11 ± 0.03‡</td>
<td>0.62 ± 0.07</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>7</td>
<td>9.2 ± 0.9</td>
<td>0.18 ± 0.06*</td>
<td>0.69 ± 0.09</td>
</tr>
<tr>
<td>4</td>
<td>9</td>
<td>9</td>
<td>8.9 ± 1.9</td>
<td>0.21 ± 0.02*</td>
<td>0.66 ± 0.08</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>6</td>
<td>7.2 ± 1.9</td>
<td>0.46 ± 0.10</td>
<td>0.95 ± 0.05</td>
</tr>
<tr>
<td>6</td>
<td>7</td>
<td>7</td>
<td>17.9 ± 4.9</td>
<td>0.25 ± 0.04*</td>
<td>0.50 ± 0.11</td>
</tr>
<tr>
<td>7</td>
<td>7</td>
<td>7</td>
<td>30.5 ± 15.9</td>
<td>0.19 ± 0.06*</td>
<td>0.58 ± 0.07</td>
</tr>
<tr>
<td>8</td>
<td>8</td>
<td>8</td>
<td>9.9 ± 3.2</td>
<td>0.14 ± 0.02*</td>
<td>0.73 ± 0.11</td>
</tr>
<tr>
<td>9</td>
<td>10</td>
<td>10</td>
<td>9.7 ± 3.4</td>
<td>0.19 ± 0.03*</td>
<td>1.20 ± 0.15</td>
</tr>
<tr>
<td>10</td>
<td>9</td>
<td>9</td>
<td>9.0 ± 2.8</td>
<td>0.19 ± 0.04*</td>
<td>1.10 ± 0.06</td>
</tr>
<tr>
<td>11</td>
<td>7</td>
<td>3</td>
<td>9.2 ± 0.5</td>
<td>0.12 ± 0.01‡</td>
<td>0.71 ± 0.09</td>
</tr>
<tr>
<td>12</td>
<td>6</td>
<td>1</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>13</td>
<td>7</td>
<td>4</td>
<td>25</td>
<td>(0.13)</td>
<td>(0.65)</td>
</tr>
<tr>
<td>14</td>
<td>6</td>
<td>4</td>
<td>8.4 ± 3.6</td>
<td>0.16 ± 0.02*</td>
<td>0.72 ± 0.07</td>
</tr>
<tr>
<td>15</td>
<td>7</td>
<td>7</td>
<td>8.8 ± 1.2</td>
<td>0.47 ± 0.02‡</td>
<td>0.91 ± 0.03</td>
</tr>
<tr>
<td>16</td>
<td>7</td>
<td>7</td>
<td>8.5 ± 2.6</td>
<td>—</td>
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</tr>
</tbody>
</table>

Values are mean ± s.e.m.
† Group 1, Day 15 pregnant; 2, Day 20 pregnant; 3, Day 22 pregnant; 4, Day 2 lactation; 5, Day 10 lactation; 6, Day 20 lactation; 7, Day 28 lactation; 8, Day 28 lactation with young foster litter substituted on Day 20 lactation; 9, Day 2 after weaning following 20 day lactation; 10, Day 4 after weaning following 20 day lactation; 11, Day 8 after weaning following 20 day lactation; 12, Day 15 of 2nd pregnancy; 13, Day 20 of 2nd pregnancy; 14, Day 22 of 2nd pregnancy; 15, Day 2 of 2nd lactation; 16, Day 10 of 2nd lactation.
‡ Frequencies are derived from the 5 intervals after the first milk-ejection response.
* P < 0.05, ** P < 0.01 (ANOVA, Newman–Keuls test) (only significant differences versus Day 10 of lactation (Group 5) are shown).

Fig. 1. Dose–response relations of the rat mammary gland to oxytocin (mean ± s.e.m.) during pregnancy, lactation and after weaning. Group 2, Day 20 of pregnancy; Group 3, Day 22 of pregnancy; Group 4, Day 2 of lactation; Group 5, Day 10 of lactation; Group 10, Day 4 after weaning, after 20-day lactation; Group 16, Day 10 of a second lactation.
**Fig. 2.** Amplitudes of mammary responses (mean ± s.e.m.) to 1 mU oxytocin throughout pregnancy, lactation and after weaning. Numbers in parentheses denote number of animals in the group. Group 1, Day 15 of pregnancy; 2, Day 20 of pregnancy; 3, Day 22 of pregnancy; 4, Day 2 of lactation; 5, Day 10 of lactation; 6, Day 20 of lactation; 7, Day 28 of lactation; 8, Day 28 of lactation with young foster litter substituted on Day 20 of lactation; 9, Day 2 after weaning following a 20-day lactation; 10, Day 4 after weaning following a 20-day lactation; 11, Day 8 after weaning following a 20-day lactation; 12, Day 15 of 2nd pregnancy; 13, Day 20 of 2nd pregnancy; 14, Day 22 of 2nd pregnancy; 15, Day 2 of 2nd lactation; 16, Day 10 of 2nd lactation. *P < 0.05, **P < 0.01, ANOVA, Newman–Keuls test (only significant values versus Group 5 (Day 10 of lactation, shaded) are indicated).

Amplitudes of responses to 1 mU oxytocin were significantly different in Groups 13 (P < 0.01) and 14 (P < 0.05) from Group 16. There were no significant differences in amplitude of responses between primiparous and multiparous animals at any stage.

**Reflex milk ejection**

The milk-ejection reflex was functional in all groups tested (Table 1; Fig. 3), and approximately the same proportion (70–100%) of animals which responded to oxytocin subsequently milk ejected in each group, apart from Groups 4 and 13. The reflex appeared to be potentiated in mid-lactating animals, both in terms of frequency of responses and estimated amount of oxytocin released at each reflex milk ejection. Thus for all the groups except 16, the frequency of the milk-ejection responses was significantly lower than in Group 5 (Table 1).

The mean amount of oxytocin released at each reflex milk ejection also increased between pregnancy and mid-lactation (Table 1; Fig. 3); animals in Group 5 gave the largest responses, while
those in Groups 6, 7 and 8 gave responses of a similar amplitude to Group 4 animals (Table 1). The responses of Group 8 were not significantly different in amplitude from those of Group 6 and 7. The size of the responses improved shortly after weaning such that the amounts of oxytocin released by Groups 9 and 10 were larger than those by Groups 6 and 7 ($P < 0.01$) and Group 8 ($P < 0.01$, $P < 0.05$), but were not significantly different from those of Group 5 (Fig. 3; Table 1). Multiparous animals showed a similar improvement throughout pregnancy and lactation in both the frequency of responses and the amount of oxytocin released, with the largest and most frequent responses in Group 16 (Table 1). There were no significant differences in either the frequency or amount of oxytocin released at each response between primiparous and multiparous animals at any stage.

The reflex milk ejections from the pregnant animals were not accompanied by the characteristic ‘milk treading’ and ‘stretch’ responses of the young (Vorherr, Kleeman & Lehman, 1967; Lincoln et al., 1973; Drewett, Statham & Wakerley, 1974) (Fig. 3).

![Fig. 3. Examples of intramammary pressure records from animals at Day 22 of pregnancy (a), Day 10 of lactation (b), and Day 4 after weaning following a 20-day lactation (c). Triangles denote pup stretch reactions. The milk-ejection responses from the pregnant animal were not accompanied by pup stretch reactions.](image_url)

**Discussion**

The fact that reflex milk ejection was seen in response to suckling in primigravid rats in late pregnancy indicates that birth of the litter and previous suckling experience are not prerequisites for the immediate functioning of the reflex. Furthermore, of the pregnant rats that responded to exogenous oxytocin, the proportion of animals which then showed reflex milk-ejection responses was similar to those in the lactating groups, suggesting that in these animals responsiveness to
Oxytocin and reflex milk ejection could not be dissociated. To investigate this further, animals at an earlier stage of pregnancy (Day 15, Group 1) were included in the study. Unfortunately the suckling test could not be performed on these animals as the young could not attach to the nipples, so without an adequate afferent stimulus this question could not be answered.

Reflex milk-ejection responses in pregnant animals were not accompanied by the characteristic behavioural responses of the young (Vorherr et al., 1967; Lincoln et al., 1973; Drewett et al., 1974). Milk production starts on Day 20 of pregnancy, but increases markedly on the day of parturition (Nicholas & Hartman, 1981). Although the absence of stretch behavioural responses by the young during the suckling tests on pregnant animals could be attributed to the young receiving little milk (Drewett et al., 1974), the absence of milk-tread behavioural responses indicated that the young could not detect the myoepithelial contractions in pregnant animals. Figure 2 demonstrates the small pressure change of the mammary gland to 1 mU oxytocin in pregnant animals compared with those on Day 10 of lactation. The myoepithelium is undeveloped in pregnant animals (Radnor, 1972) with few oxytocin receptors (Soloff et al., 1979; Soloff & Wieder, 1983), which could account for the difference in the size of contractions compared with lactating animals. In addition, it is possible that the high plasma concentrations of hormones such as relaxin (Sherwood, Crnekevic, Gordon & Rutherford, 1980), oestradiol, progesterone, cortisol and oxytocin (Yoshinaga, Hawkins & Stocker, 1969; Yoshida, Suzuki, Hattori & Noda, 1981) present in pregnant animals may inhibit the functioning of myoepithelium (Deiss, 1971; Summerlee, O'Byrne, Paisley, Breeze & Porter, 1984).

The log dose–response lines of the gland to oxytocin steepen and shift to the left between pregnancy and lactation. In the first 4–8 days after weaning the myoepithelial cells do not regress concurrently with the secretory cells (Radnor, 1972). The continuing presence of the myoepithelial cells maintains the structure of the gland, the number of oxytocin receptors (Soloff & Wieder, 1983) and consequently the response to oxytocin (De Nuccio & Grosvenor, 1967). It was not surprising, therefore, that the slope and position of the dose–response line from animals 4 days after weaning (Group 10) is similar to those from lactating animals. It could be argued that the position of the log dose–response line from any animal depends on the amount of milk present in the gland, since volume has been shown to affect the size of mammary contractions (De Nuccio & Grosvenor, 1971). However, since only small amounts of milk were present in the gland of lactating animals due to the short period of separation from the young before the experiment (see experimental design), and small volumes of milk were injected into the gland of pregnant animals, it seems unlikely that the position of the lines can be totally attributed to the influence of milk volume. The size of the pressure change in the mammary gland appears to depend on various additional factors, such as the development of the myoepithelium and the number of functional oxytocin receptors.

The morphological changes in the hypothalamic supraoptic nucleus were observed at the end (Day 21) of pregnancy (Theodosis & Poulain, 1984), and the withdrawal of pituicytes from neurosecretory endings occur immediately post partum (Tweedle & Hatton, 1982). The fact that the milk-ejection reflex continued to improve until mid-lactation suggests that there is not a clear-cut temporal relationship between the morphological changes in the central components of the milk-ejection reflex and the high frequency of responses and increased hormone release seen at mid-lactation (Table 1). It seems likely, therefore, that other factors may also be involved in influencing the milk-ejection reflex, such as the hormonal environment (Clarke, Wood, Merrick & Lincoln, 1979; Wright, Pill & Clarke, 1983; Summerlee et al., 1984; Petraglia et al., 1985), or possibly structural changes elsewhere in the components of the reflex and activity in the pathway.

As lactation progresses and the young become less dependent on milk, plasma prolactin concentration decreases (Amenomori, Chen & Meites, 1970), milk production declines (Flint, Clegg & Knight, 1984) and the morphological changes in the supraoptic nucleus start to regress (Theodosis & Poulain, 1984). Perhaps not surprisingly the frequency of responses and the amount of oxytocin released at each response declined in late lactation. In animals 2 and 4 days after weaning the frequency was low, similar to that in late-lactating animals, but the amount of oxytocin released
had increased to mid-lactation values (Table 1). The periods after weaning could have replenished pituitary stores of oxytocin which are known to be depleted in lactation (Cross, 1951; Heller, 1959; Nicholson & Pickering, 1977) such that more hormone was readily available for release. Alternatively, it is possible that the separation from the young altered nipple sensitivity and so could have affected the reflex, since nipple sensitivity is known to change throughout lactation in the human (Robinson & Short, 1977).

To investigate the effects of prolonged lactation on the reflex, Group 8 was included in the study. Milk production continues to decrease in late-lactating animals even when suckled by young foster litters (Flint et al., 1984) and plasma prolactin concentration decreases in late lactation (Amenomori et al., 1970). Neither the frequency of responses in Group 8 nor the amount of oxytocin released were significantly different from those in Groups 6 or 7. Possibly the foster litters were placed with the mothers too late (Day 20 of lactation) to affect the characteristics of the reflex, since Day-20 lactation animals (Group 6) already showed less frequent and smaller responses than did those in Group 5 (Table 1).

The multiparous animals showed a similar pattern of changing characteristics of the reflex throughout pregnancy and lactation as the primiparous animals (Table 1), and were not significantly different from primiparous animals at any stage. It can be concluded, therefore, that the interval between the first and second lactation periods was sufficient to prevent any increase in the efficiency of the second lactation compared with the first. However, in the 1-month period, the changes in the central and peripheral components of the reflex would have regressed, and the hormonal state of the animals would have become similar to virgin animals (Radnor, 1972; Yoshida et al., 1981; Theodosis & Poulain, 1984).

References


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