Progesterone Control of the Initiation of Lactose Synthesis in the Rat

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Abstract

The \textit{in vitro} incorporation of \textsuperscript{[14]C}glucose into lactose in mammary tissue, the concentration of lactose in the mammary tissue and the concentration of lactose in the mammary secretion were determined during late pregnancy and lactation in the rat. These changes were related to the decline in blood progesterone during late pregnancy. The incorporation of \textsuperscript{[14]C}glucose into lactose was detected on day 20 of pregnancy; it increased gradually until day 22 and then increased rapidly just prior to term (day 23 of pregnancy) to reach mean ± s.e.m. maximum hourly values of 12.0 ± 1.5 cpm × 10\textsuperscript{4}/g by day 3 of lactation, and then declined to lower values (6.1 ± 0.6 cpm × 10\textsuperscript{4}/g) by day 20 of lactation. The concentration of lactose both in the mammary tissue and in the mammary secretion increased rapidly over the last 24 h of pregnancy and then more gradually after birth to reach mean ± s.e.m. maximum values of 6.85 ± 1.11 mg/g tissue and 43.1 ± 2.1 g/l respectively on day 15 of lactation. The decline in plasma progesterone to low levels between days 21 and 22 of pregnancy preceded the rapid increase in the concentration of lactose in the mammary tissue. A similar relationship was observed between the decline in progesterone and the increase in lactose in mammary tissue in rats Caesarean-sectioned on day 19 of pregnancy, and the administration of progesterone immediately following Caesarean section significantly depressed the accumulation of lactose in the mammary tissue. The results support the involvement of progesterone withdrawal in lactogenesis in the rat and indicate that \textsuperscript{[14]C}glucose incorporation into lactose and the concentration of lactose both in the mammary tissue and in mammary secretion are useful indicators of the synthetic activity of the mammary gland.

Introduction

The temporal relationship between the changes in the concentration of progesterone in the plasma and the accumulation of lactose in the mammary tissue, together with the inhibitory effects of exogenous progesterone on lactogenesis, led Kuhn (1969) to propose that, in the rat, the lactogenic signal was progesterone withdrawal and that this occurred 30 h before parturition. Although the accumulation of lactose in the mammary tissue of the rat is a convenient method of determining the initiation of lactose synthesis, it is not possible to use this criterion in larger species. Therefore, in studies in cows (Hartmann 1973), ewes (Hartmann et al. 1973), women (Kulski et al. 1977, 1978) and sows (Gooneratne et al. 1979), the initiation of lactose synthesis during lactogenesis has been determined by changes in the concentration of lactose in the mammary secretion.

The present study was undertaken to determine the relationship between the various methods used for the assessment of the rate of lactose synthesis in the mammary gland of the rat following progesterone withdrawal at lactogenesis.
Materials and Methods

Chemicals

\([1\alpha,2\beta(n)-3^H]\)Progestosterone (42 Ci/mmol) and \(\beta-[U-\text{I}^4\text{C}]\)glucose (3 mCi/mmol) were obtained from the Radiochemical Centre, Amersham, Bucks., G.B. Progesterone, \(\beta\)-\(\alpha\)-galactosidase (grade II, from Saccharomyces fragilis), peroxidase (type I, from horseradish) and glucose oxidase (type II, from Aspergillus niger) were purchased from Sigma Chemical Company, St Louis, Mo., U.S.A. Oxytocin (Pitocin) was obtained from Parke-Davis and Co., Sydney, and heparin (Pularin) from Evans Medical Speke, Liverpool, G.B. Amberlite IR-120 (\(\text{H}^+\)) was purchased from BDH Chemicals Ltd, Poole, G.B., Dowex 1-X2 (100-200 mesh, chloride form) from BioRad, California, U.S.A., and Sephadex G10 from Pharmacia, Uppsala, Sweden.

Animals

All rats (Rattus norvegicus) were of the Wistar albino strain, aged 3-5 months and weighed between 170 and 250 g. They were provided with food (purchased from either W. H. Milne and Co., Pty Ltd, Perth, or Wesfeeds, Perth) and water \textit{ad libitum} in alternating periods of darkness (2100-0700 hours) and artificial light (0700-2100 hours). The rats were mated overnight and the detection of sperm by vaginal smear on the following morning was taken to indicate day 1 of pregnancy. Rats from this colony normally gave birth early on day 23 and rats which had normal deliveries suckled 10-12 pups. Caesarean-section delivery was carried out by removal of the intact foetus and placenta by manipulation to a small incision in the centre of the uterine horn. Caesarean-sectioned rats were unsuckled. Rats were killed with a blow to the head and blood from the jugular vein collected into heparinized tubes. Samples were centrifuged at 3000 \(g\) for 5 min and the plasma stored at \(-15^\circ\text{C}\) until required.

The inguinal mammary glands from the same rats were immediately excised and weighed. Tissue for the estimation of lactose synthesis was thoroughly washed in Krebs–Henseleit–Ringer bicarbonate buffer (Dawson \textit{et al}. 1969) and blotted dry between filter paper. Mammary gland for the determination of lactose concentration was finely chopped in 5-0 ml of distilled water and frozen at \(-15^\circ\text{C}\) until required. Homogenization was carried out by using a Perspex coaxial homogenizer of the Potter–Elvehjem type. The plunger was motor-driven at 1200 rpm and at least eight excursions of the plunger were made for all preparations. Milk for the determination of lactose content was expressed manually from rats throughout lactation after the separation of the mother and litter for 0.5-1.0 h. The secretion was collected under light ether anaesthesia following the subcutaneous injection of 0.1 i.u. of oxytocin. Prepartum samples were collected 6-15 h before term. Milk at day 0 of lactation was collected before the pups had begun to suckle (i.e. within 2-0 h of birth).

Experimental Design

\textit{Lactogenesis in normal parturient rats}

Rats were killed at daily intervals from day 19 of pregnancy through to day 5 post partum and the capacity of the mammary tissue for \textit{in vitro} synthesis of lactose was determined, together with the concentration of lactose and glucose in the mammary gland. The concentration of progesterone and glucose in the plasma was also measured. Additional rats were milked at 6-15 h before parturition and at day 0, 1, 2, 3, 4, 5, 10, 15 and 20 of lactation for the estimation of milk lactose.

\textit{Lactogenesis in Caesarean-sectioned rats}

Rats were Caesarean-sectioned on day 19 of gestation and killed 1, 2, 3, 4 or 5 days after surgery. The concentration of lactose and glucose in the mammary gland and the concentration of progesterone in the plasma was determined.

\textit{Progesterone inhibition of lactogenesis following Caesarean section}

Rats were Caesarean-sectioned at day 19 of gestation and received progesterone (8 mg/24 h s.c. in peanut oil) either 0, 6 or 18 h after surgery. Control rats were Caesarean-sectioned and received peanut oil. All rats were killed 2 days after Caesarean section and the concentration of lactose and glucose in the mammary gland and the concentration of progesterone in the plasma was determined.
**Progesterone administration during established lactation**

Rats were administered either progesterone (8 mg/24 h or 5 mg/12 h s.c.) in peanut oil or peanut oil (0-1 ml/12 h, control rats) from day 5 to day 8 of lactation and were milked on day 8 for the estimation of lactose.

**Biochemical Analyses**

**Blood progesterone and glucose**

Progesterone was measured by radioimmunoassay as described by Martin et al. (1977). The accuracy was determined in plasma in which the concentration of progesterone was increased by the addition of 4 and 8 μg/l. The estimated increases were equivalent to 107 and 100% of the added progesterone respectively. Intraassay and interassay coefficients of variation were 5·2 and 10·5% respectively. The sensitivity of the standard curve was 0·007 ng per assay tube.

Glucose was estimated using the glucose oxidase method of Bergmeyer and Bernt (1974) following deproteinization of the plasma with 0·0038 M uranyl acetate in 0·9% (w/v) saline.

**Rate of in vitro incorporation of [14C]glucose into lactose**

The rate incorporation of [14C]glucose into lactose was measured using a modification of the procedures described by Bartley et al. (1966) and Delouis and Denamur (1972). Finely chopped mammary tissue (300 mg) was weighed into a 25-ml vial with 3·0 ml of Krebs–Henseleit–Ringer bicarbonate buffer containing glucose (3·0 mg/ml) and 1·0 μCi d-[U-14C]glucose. The vials were gassed with carbogen, stoppered and placed in a shaking water bath (c. 100 oscillations/min) at 37°C for 2·0 h. The incorporation of [14C]glucose into lactose was found to be linear for at least 3·0 h. The reaction was terminated by immersing the vials in a boiling water-bath for 1·5 min. The tissue was homogenized in its medium, centrifuged at 40 000 g for 1·0 h and a 2·0-ml fraction of the supernatant deproteinized by the addition of 0·4 ml of 30% (w/v) trichloroacetic acid. Precipitated protein was removed by centrifugation at 2000 g for 10 min and the supernatant extracted with an equal volume of ether. The supernatant was then applied to a column of Amberlite IR-120 (H+ form) and Dowex AG 1-X2 (formate form) and the deionized effluent dried at 50°C in vacuo. The constituents of the effluent were redissolved in water and a fraction applied to Whatman No. 1 chromatography paper adjacent to standard glucose and lactose. The chromatogram was developed in a descending system of n-propanol–ethyl acetate–water (7:1:2 by vol.) for 16 h (Baar and Bull 1953). Glucose and lactose standards were localized with an aniline hydrogen phthalate spray (Partridge 1949) and the area of paper corresponding to lactose cut out, eluted with water and a fraction counted in 10 ml of dioxane scintillant (4·0 g PPO, 180 g naphthalene, 1·01 dioxane) in a Nuclear Chicago Isocap 300 liquid scintillation spectrophotometer. Quenching was corrected by the channels-ratio method.

Verification that lactose was the product being measured was shown by paper chromatography in the n-propanol–ethyl acetate–water system before and after incubation with lactase. The area of radioactivity moved from a spot before hydrolysis, which could not be separated chromatographically from authentic lactose, to areas corresponding to glucose and galactose after hydrolysis. The product was also chromatographed on a column of Sephadex G10 (1·3 by 110 cm) after paper chromatography and eluted as a single peak identical to that of lactose.

**Mammary tissue lactose and glucose concentration**

Mammary gland homogenates were centrifuged at 40 000 g for 1·0 h and the supernatant removed from beneath the fat layer. A 1·0-ml fraction was acidified with the addition of 0·1 ml of 28% (w/v) perchloric acid followed by centrifugation and neutralization to approximately pH 7·0 with 18% (w/v) potassium hydroxide. The potassium perchlorate precipitate was removed by centrifugation and the lactose present in 0·1 ml of supernatant was hydrolysed by incubation for 90 min at 37°C with 0·2 ml of 0·1 M potassium phosphate buffer, pH 7·0, containing 0·12 mg of lactase. The determination of glucose before and after hydrolysis of lactose was based on the method of Bergmeyer and Bernt (1974). Standard lactose and glucose samples were included in all assays.

**Milk lactose**

Milk lactose was determined as described by Nicholas et al. (1981).
Results

Lactogenesis in Normal Parturient Rats

Lactose synthesis commenced on day 20 of pregnancy. The rate of synthesis, although remaining low, had doubled by day 22 and increased markedly just prior to term to finally reach maximum levels by day 3 post partum (Fig. 1) and then declined gradually (Table 1).

![Graph showing lactogenesis in normal parturient rats.](image)

**Fig. 1.** Concentration of progesterone (●) in plasma, lactose synthesis ([14C]glucose incorporation into lactose) (▲), lactose (■) and glucose (○) in the mammary gland, and lactose (□) in milk during late pregnancy and early lactation. Day 0 indicates parturition. Each value represents the mean of three to seven observations (the s.e.m. did not exceed 20-2% of the mean of any set of observations).

**Table 1.** Concentration of lactose in milk, mammary gland weight and lactose synthesis ([14C]glucose incorporation into lactose), and lactose, milk and glucose content of mammary gland at 5, 10, 15 and 20 days of lactation in the rat

<table>
<thead>
<tr>
<th>Days of lactation</th>
<th>Lactose concn in milk (g/l)</th>
<th>Weight (g/200 g body wt)</th>
<th>(10^{-4} \times ) Mean hourly lactose synthesis (cpm/g)</th>
<th>Mammary gland Lactose (mg/g tissue)</th>
<th>Milk (ml/gland)</th>
<th>Glucose (mg/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>24·7 ± 1·1</td>
<td>2·23 ± 0·14</td>
<td>8·0 ± 1·3</td>
<td>3·76 ± 0·12</td>
<td>0·267 ± 0·02</td>
<td>0·45 ± 0·02</td>
</tr>
<tr>
<td>10</td>
<td>32·1 ± 2·9</td>
<td>3·57 ± 0·07</td>
<td>7·6 ± 0·4</td>
<td>4·81 ± 0·12</td>
<td>0·417 ± 0·01</td>
<td>0·93 ± 0·13</td>
</tr>
<tr>
<td>15</td>
<td>43·1 ± 2·1</td>
<td>2·64 ± 0·17</td>
<td>—</td>
<td>6·85 ± 1·11</td>
<td>0·326 ± 0·06</td>
<td>0·94 ± 0·09</td>
</tr>
<tr>
<td>20</td>
<td>40·4 ± 4·8</td>
<td>3·28 ± 0·23</td>
<td>6·1 ± 0·6</td>
<td>5·36 ± 1·81</td>
<td>0·340 ± 0·12</td>
<td>1·01 ± 0·16</td>
</tr>
</tbody>
</table>
Lactose accumulated within the mammary gland to measurable levels (0.15 mg/g) by day 22 of gestation. Within the next 12 h the lactose concentration increased to 2.75 mg/g tissue, and by term was 3.75 mg/g tissue. The concentration of glucose in the mammary gland followed a similar pattern to lactose; it remained at approximately 0.1 mg/g tissue from day 18 to 21 of pregnancy, and increased to 0.34 mg/g tissue just before term and to 0.6 mg/g tissue at parturition. Both the lactose and glucose contents of the gland remained relatively constant for the first 5 days post partum (Fig. 1), but increased gradually until day 15 of lactation (Table 1). This sixfold increase in the concentration of glucose in the mammary gland between days 22 and 23 of gestation was not accompanied by any significant change in the concentration of glucose (5.39 ± 0.4 mmol/l) in the plasma.

Prepartum milk proved difficult to collect more than 15 h before term but was freely expressed from rats about the time of parturition. The mean concentration of lactose in milk collected 6–15 h before term was 18.3 g/l and increased to 25 g/l at birth (Fig. 1). The concentration did not change significantly during the first 5 days post partum but subsequently increased gradually to a maximum concentration of 43.1 g/l at day 15 of lactation (Table 1).

The volume of milk present in the tissue was calculated from the concentration of lactose in the milk and the mammary gland, and by assuming that 78% of the lactose was extracellular (Kuhn and White 1975). From 12 h prepartum until day 5 of lactation the milk present in the mammary gland did not change significantly (0.255 ± 0.019 ml/gland, mean ± s.e.m). However, in the latter half of lactation the increase in the weight of the mammary tissue was accompanied by an increase in the concentration of lactose in both the gland and the milk, together with an increase in the volume of retained milk (Table 1).

The peripheral concentration of plasma progesterone was high (40–50 µg/l) on days 19 and 20 of pregnancy (Fig. 1). A decrease in concentration to 30 µg/l occurred by day 21 and was followed by a further decline to 6 µg/l by day 22.

Lactogenesis in Caesarean-sectioned Rats

Lactose (0.15 mg/g) was detected in the mammary tissue 12 h after removal of the foetoplacental unit but did not increase significantly until 48 h after surgery, when a maximum value of 2.6 mg/g was obtained. The concentration of glucose in the gland showed a similar trend to lactose and increased from 0.08 mg/g at 24 h to 0.48 mg/g at 48 h after Caesarean section. In the absence of the suckling stimulus the concentration of both lactose and glucose decreased during the following 3 days as mammary gland involution became apparent. There was a concomitant decrease in the mean wet weight of mammary tissue from 2.2 g/200 g body weight at day 2 to 1.35 g/200 g body weight at day 5 after Caesarean section.

The concentration of progesterone in the plasma decreased from 40 µg/l to 10 µg/l within 12 h of Caesarean section at day 19 of pregnancy (Fig. 2).

Progesterone Inhibition of Lactogenesis following Caesarean Section

The increase in the concentration of lactose 48 h after Caesarean section at day 19 of gestation was suppressed by 86, 75, 71 and 48% by the administration of progesterone (8 mg/24 h) at 0, 6, 9 and 18 h respectively, after Caesarean section (Table 2). The concentration of progesterone in the plasma from all progesterone-treated rats
Fig. 2. Concentration of progesterone (●) in plasma, together with mammary gland weight (▲), and the concentration of lactose (■) and glucose (○) in the mammary gland after Caesarean section. Rats were Caesarean-sectioned at day 19 of pregnancy. Each value represents the mean ± s.e.m. for three to five observations, except for 3 days after Caesarean section when the average of two values is shown.

Table 2. Progesterone inhibition of the initiation of lactose synthesis in the mammary gland following Caesarean section at day 19 of gestation

Rats were administered progesterone (8 mg/24 h in peanut oil) at 0, 6, 9 and 18 h after Caesarean section. Control rats (C) received peanut oil only after Caesarean section. Rats were killed 48 h after surgery and the concentration of progesterone in the plasma and the weight, lactose content and glucose content of the mammary gland were determined. Each value represents the mean ± s.e.m., and the values obtained were analysed by a one-way analysis of variance and least significant difference tests. Mean values underlined by the same line are not significantly different at P > 0.05

<table>
<thead>
<tr>
<th>Time (h) of progesterone administration</th>
<th>No. of observations</th>
<th>Plasma progesterone (μg/l)</th>
<th>Mammary gland weight (g/200 g body wt)</th>
<th>Mammary gland lactose (mg/g tissue)</th>
<th>Mammary gland glucose (mg/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7</td>
<td>28 ± 1·0</td>
<td>3·01 ± 0·09</td>
<td>4·59 ± 0·37</td>
<td>0·12 ± 0·02</td>
</tr>
<tr>
<td>0</td>
<td>7</td>
<td>95 ± 7·0</td>
<td>1·83 ± 0·08</td>
<td>0·66 ± 0·21</td>
<td>0·15 ± 0·01</td>
</tr>
<tr>
<td>6</td>
<td>9</td>
<td>119 ± 13·0</td>
<td>1·86 ± 0·11</td>
<td>1·14 ± 0·16</td>
<td>0·18 ± 0·02</td>
</tr>
<tr>
<td>9</td>
<td>7</td>
<td>123 ± 13·0</td>
<td>1·72 ± 0·12</td>
<td>1·32 ± 0·25</td>
<td>0·19 ± 0·02</td>
</tr>
<tr>
<td>18</td>
<td>5</td>
<td>107 ± 6·4</td>
<td>2·43 ± 0·07</td>
<td>2·37 ± 0·12</td>
<td>0·16 ± 0·01</td>
</tr>
</tbody>
</table>

Significance: 0 h 6 h 9 h 18 h C 0 h 6 h 9 h 18 h C 0 h 6 h 9 h 18 h C
was >95 µg/l at the time of death, whereas the concentration of progesterone from control animals was 3 µg/l. In the control rats in this experimental group the concentration of glucose in the mammary gland 48 h after Caesarean section did not increase, although an increase had been observed in the previous experiments (Figs 1 and 2).

**Progesterone Administration during Established Lactation**

The effect of progesterone on lactose concentration in milk during established lactation was investigated by administration of the hormone (8 mg/24 h, 5 mg/12 h, 0.1 ml peanut oil/12 h) from day 5 to day 8 of lactation. The concentration of lactose in milk expressed at day 8 of lactation was 35.1 ± 1.4 (n = 3), 32.2 ± 1.3 (n = 3) and 31.8 ± 1.7 g/l (n = 5, mean ± s.e.m.) respectively. The differences between these means were not significant (P > 0.05).

**Discussion**

Previous studies on the initiation of lactose synthesis in the late-pregnant rat have been limited to the determination of lactose concentration of the mammary gland (Shinde et al. 1965; Wrenn et al. 1965; Kuhn and Lowenstein 1967; Kuhn 1968, 1969; Yokoyama et al. 1969). In contrast, the present investigation compares the concentration of lactose in the mammary tissue with the concentration of lactose in the mammary secretion and with the in vitro incorporation of [14C]glucose into lactose. It is apparent from all three methods of assessment that lactose synthesis and secretion increased abruptly just prior to parturition (Figs 1 and 2). Therefore all three methods provide a reliable indication of the initiation of lactose synthesis in the mammary gland.

A two-stage induction of lactose synthesis has been reported in the cow (Hartmann 1973), sheep (Hartmann et al. 1973), goat (Fleet et al. 1975) and woman (Kulski et al. 1977). In these species, limited secretion of milk constituents occurred during late pregnancy while progesterone remained elevated, and then the onset of copious milk secretion was temporally related to a rapid decline in progesterone. Thus, the relationship between progesterone withdrawal and the initiation of lactose synthesis in the rat (Fig. 1) is consistent with the relationship which has been observed for a number of other species. The above species represent extremes in the variation in the blood levels and tissue locations of progesterone synthesis during pregnancy (Bedford et al. 1972), and therefore the similarity between them in the initiation of lactose synthesis suggests that progesterone withdrawal may be the trigger for lactogenesis in most mammals.

The increase in the concentration of glucose in the mammary gland which accompanied lactogenesis (Figs 1 and 2) is not supported by the findings of either Kuhn and Lowenstein (1967) or Murphy et al. (1973). This study, together with the findings of Sutter-Dub et al. (1974), shows that the plasma concentration of glucose and insulin remain relatively constant during the latter stages of pregnancy. Consequently, the observed changes in gland glucose cannot be attributed to a change in either plasma glucose or insulin. Subsequent studies have shown that the increase in glucose concentration in the mammary tissue appeared to be a consequence of the animal diet since the increase was not observed (see Table 2) after the introduction
of an alternative commercial diet (W. H. Milne and Co.) to the rats. The reason for the observed increase in mammary glucose is at present unclear. However, the former commercial diet (Wesfeeds) was found to be low in energy.

The detection of lactose in the mammary gland 12 h after Caesarean section at day 19 of gestation is considerably earlier than that previously reported (Shinde et al. 1965; Kuhn 1969). However, this finding suggests that the onset of lactose synthesis, which followed the decline in the plasma concentration of progesterone after Caesarean section, is rapid and comparable with that observed in normal pregnant rats (Fig. 1).

The administration of progesterone immediately after Caesarean section suppressed the appearance of lactose in the mammary gland by 86% (Table 2). However, the administration of progesterone at 18 h after Caesarean section, i.e. at least 6 h after the decline in the concentration of progesterone in the plasma (Fig. 2), inhibited the increase in lactose content by only 48%. The inverse relationship between the timing of administration of progesterone at 0, 6, 9 or 18 h after Caesarean section and the increase in the concentration of lactose in the mammary gland 48 h after the operation (Table 2) suggests that the initial synthesis attained before injection, proceeds in the presence of exogenously elevated progesterone levels. This finding contrasts the observations of Davis et al. (1972), who demonstrated a complete inhibition of the appearance of casein in the mammary gland of pregnant rats if the administration of exogenous progesterone was delayed 8 h after progesterone withdrawal by ovariec­tomy. Taken collectively, these results support the findings of Kuhn (1969) that the initiation of lactation in Caesarean-sectioned rats differs temporally to that in rats following ovariec­tomy.

Progesterone was ineffective in suppressing the concentration of lactose in milk during established lactation. Similarly, Hartmann et al. (1973) have shown that ewes receiving progesterone immediately after Caesarean section had a significantly decreased milk yield and lactose concentration whereas the administration of progesterone to ewes with established lactation did not lead to any significant change in either milk yield or concentration of lactose in the milk. In this connection Haslam and Shyamala (1979, 1980) recently reported the absence of specific receptors for progesterone in mammary tissue from mice in mid-lactation. Thus, the inability of lactating mammary glands to respond to progesterone by inhibiting lactose synthesis may be a consequence of the disappearance of the receptor during the initial stages of lactation.

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References


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