

that found in the male fetal circulation<sup>8</sup>. Thus one can speculate that the androgen concentration which exists in the microenvironment of the fetal Leydig cells may exert a local negative influence by the way of an ultra-short loop feedback. Since the fetal testis is able to metabolize testosterone into both estradiol<sup>9</sup> and DHT<sup>10</sup>, it is difficult to predict whether this inhibition is mediated by testosterone itself or via aromatization and/or formation of 5 $\alpha$ -reduced products. The fact that DHT is more effective in reducing testosterone production

than testosterone itself suggests that aromatization is not a prerequisite for androgen action. Whether such an effect results from a direct influence on the fetal Leydig cell is unclear, since more than one type of cell is present in the testicular cell suspension used. This mechanism might offer an explanation for the decrease in circulating testosterone in the face of high concentrations of gonadotropins during late fetal life<sup>11,12</sup> and could take a part in desensitization of the fetal testis as previously suggested<sup>6</sup>.

- 1 Purvis, K., Clausen, O.P.F., and Hanson, V., *Biol. Reprod.* 20 (1979) 304.
- 2 Adashi, E. Y., and Hsueh, A. J. W., *Nature* 293 (1981) 737.
- 3 Darney, K. J. Jr, and Ewing, L., *Endocrinology* 109 (1981) 993.
- 4 Chouraqui, J., Zeis, A., and Weniger, J. P., *C. r. Acad. Sci. Paris* 285 (1977) 1475.
- 5 Naessany, S., Habert, R., and Picon, R., *J. Endocr.* 88 (1981) 359.
- 6 Pointis, G., Rao, B., Latreille, M. T., and Cedard, L., *J. Steroid Biochem.* 20 (1984) 525.
- 7 Schumacher, M., Schäfer, G., Holstein, A. F., and Hilz, H., *FEBS Lett.* 91 (1978) 333.
- 8 Pointis, G., Latreille, M. T., Mignot, T. M., Janssens, Y., and Cedard, L., *J. Steroid Biochem.* 11 (1979) 1609.

- 9 Weniger, J. P., and Zeis, A., *C. r. Acad. Sci. Paris* 296 (1983) 293.
- 10 Weniger, J. P., and Zeis, A., *C. r. Acad. Sci. Paris* 272 (1971) 852.
- 11 Pointis, G., Latreille, M. T., and Cedard, L., *J. Endocr.* 86 (1980) 483.
- 12 Slob, A. K., Ooms, M. P., and Vreeburg, J. T. M., *J. Endocr.* 87 (1980) 81.

0014-4754/84/070756-02\$1.50 + 0.20/0  
© Birkhäuser Verlag Basel, 1984

## Prolactin concentrations in mouse milk during lactation

R. C. Richards and J. M. Beardmore<sup>1</sup>

Department of Medical Cell Biology, University of Liverpool, P.O. Box 147, Liverpool L69 3BX (England), 25 August 1983

**Summary.** Prolactin levels in mouse milk increased from the day of parturition to give a concentration of 230 ng/ml on days 2 and 3 of lactation. Thereafter, levels dropped to 140 ng/ml by day 6 and were maintained at this concentration until weaning.

Milk contains a variety of steroid and polypeptide hormones<sup>2</sup> as well as growth factors<sup>3</sup>. Although we don't know the role (if any) played by these milk hormones in the neonate, many of them have been shown indirectly to cross the mucosa of the gastrointestinal tract and exert a biological effect in the neonate<sup>4,5</sup>. As our knowledge and understanding of milk hormones is still in its infancy, more information is required on the levels of these hormones in milk throughout lactation. As part of our study in mice we have monitored the level of prolactin (PRL) in mouse milk.

**Materials and methods.** Lactating CBA mice were used in this study to provide milk samples. Litter size was standardized to

5–7 pups and mothers were separated from their pups for at least 1 h before being milked at midday. Lactating mice were given i.p. injections of pentobarbitone sodium (60  $\mu$ g/g) and oxytocin ( $0.62 \times 10^{-3}$  units/g) and milk was collected using a suction apparatus<sup>6,7</sup>. Milk samples were immediately defatted and stored at  $-20^{\circ}\text{C}$  for a maximum period of 2 weeks.

The highly sensitive homologous radioimmunoassay for mouse PRL used in this study has already been described<sup>1,8</sup>. Iodination of PRL was by a modification of the chloramine-T method<sup>8</sup> and separation of free iodine from labelled PRL was accomplished on a Biogel P60 column (BioRad Laboratories, Bromley, Kent). The iodinated PRL had a mean sp. act. of 105  $\mu\text{Ci}/\mu\text{g}$  and was stored at  $-20^{\circ}\text{C}$  for up to 1 month. Prior to use in the radioimmunoassay the  $^{125}\text{I}$  PRL was further chro-

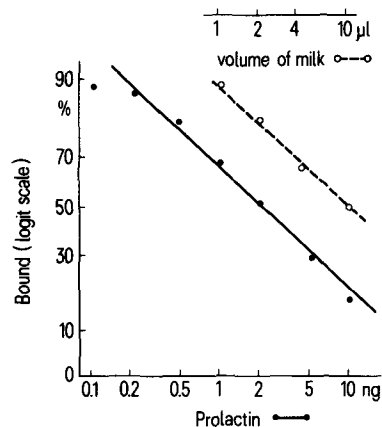


Figure 1. Dilution dose response curves for mouse prolactin standards and samples of mouse milk. Ordinate: logit scale of %  $^{125}\text{I}$ -prolactin bound. Abscissa: log<sub>10</sub> standard concentration or volume of milk sample.

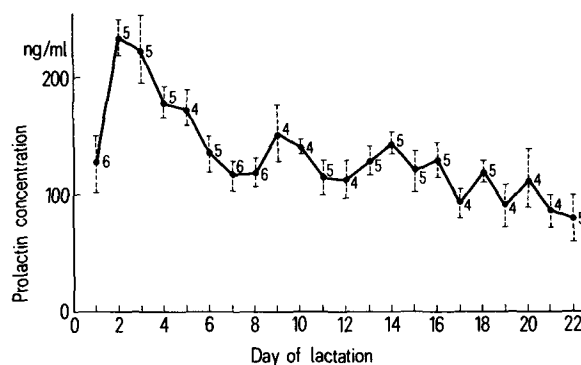


Figure 2. Prolactin concentrations in mouse milk throughout lactation. Variation bars represent SEM and n-values are included for each determination.

matographed on a Sephadex G100 column (2.3 × 13 cm) and the middle peak pooled as unaggregated PRL.

Milk samples were assayed at 25–200-fold dilutions, as more concentrated samples either did not yield a dilution curve parallel to that of the standards or reduced the total bound value when assayed in the presence of excess antibody.

**Results and discussion.** Inter- (6.9%) and intra-assay (7.7%) variations, when calculated from a series of milk samples were slightly higher than those reported for serum<sup>8</sup>. There was no incubation damage by milk samples diluted 25-fold or greater and the dose response curve for milk (10–100-fold dilution) was parallel to the purified PRL standards (fig. 1).

Prolactin concentrations in mouse milk increased from the day of parturition to give an early peak of 230 ng/ml on days 2 and 3 of lactation (fig. 2). Levels then decreased to 140 ng/ml by day 6 and were maintained at approximately this level until weaning.

This pattern of milk PRL concentrations differs from that reported for bovine and human milk<sup>9,10</sup>, where PRL levels are high in colostrum and decrease dramatically down to relatively low levels in mature milk. However, it is similar to the reported pattern of EGF levels in mouse milk<sup>3</sup>.

The observed peak of PRL on days 2 and 3 may be directly due to variations in the concentration of circulating PRL<sup>11</sup> and/or the number of PRL receptors expressed on the mammary epithelial cells<sup>12</sup>, assuming that these receptors are involved in the receptor-mediated transcellular transport of PRL.

The prolactin concentrations reported for mouse milk are higher than those found in human<sup>10</sup>, goat, sheep or cow's milk<sup>9</sup>, but are lower than the values recorded for bovine colostrum<sup>9</sup>. Whether milk PRL plays a role in the neonate is not known. However, plasma levels of PRL are very low in suckling rats<sup>15</sup> and it has been shown that PRL can cross the mucosa of the neonatal gut<sup>13,14</sup>.

- 1 Acknowledgments. Mouse PRL and its antiserum was the generous gift of Y.N. Sinha (Scripps Clinic and Research Foundation, La Jolla, California). J.M.B. was in receipt of a Liverpool University Studentship.
- 2 Koldovsky, O., *Life Sci.* 26 (1980) 1833.
- 3 Beardmore, J.M., and Richards, R.C., *J. Endocr.* 96 (1983) 287.
- 4 Baram, T., Koch, Y., Hazum, E., and Fridkin, M., *Science* 198 (1977) 300.
- 5 Kelly, W.A., *Nature* 186 (1960) 97.
- 6 Haberman, B.H., *Lab. Anim. Sci.* 24 (1974) 935.
- 7 Nagasawa, H., *Lab. Anim. Sci.* 29 (1979) 633.
- 8 Sinha, Y.N., Selby, F.W., Lewis, U.J., and Vanderlaan, W.P., *Endocrinology* 91 (1972) 1045.
- 9 Malven, P.V., *J. Anim. Sci.* 46 (1977) 609.

- 10 Healy, D.L., Rattigan, S., Hartmann, P.E., Herington, A.C., and Burger, H.G., *Am. J. Physiol.* 238 (1980) E83.
- 11 McMurtry, J.P., and Malven, P.V., *Endocrinology* 95 (1974) 559.
- 12 Hayden, T.J., Bonney, R.C., and Forsyth, I.A., *J. Endocr.* 80 (1979) 259.
- 13 Whitworth, N.S., and Grosvenor, C.E., *J. Endocr.* 79 (1978) 191.
- 14 Mulloy, A.L., Keen, S.J., and Malven, P.V., *Biol. Neonate* 36 (1979) 148.
- 15 Dussault, J.H., Walker, P., and Dubois, J.D., *Can. J. Physiol.* 55 (1977) 84.

0014-4754/84/070757-02\$1.50 + 0.20/0  
© Birkhäuser Verlag Basel, 1984

## Melatonin in the plasma of growing sheep subjected to short and skeleton long photoperiods

B.R. Brinklow, J.M. Forbes and R.G. Rodway

*Department of Animal Physiology and Nutrition, University of Leeds, Leeds LS29JT (England), 25 August 1983*

**Summary.** The levels of melatonin in plasma were measured at hourly intervals for 24 h in 8 sheep, 4 under 8L:16D (short day) and 4 under 7L:10D:1L:6D (skeleton long day) after 38 days of exposure. Mean concentrations did not differ significantly between treatments (52 pg/ml under short days; 91 pg/ml for skeleton long days), but the levels were more stable during 24 h in the SD treatment. Under skeleton long days there were 3 peaks during the 10D scotophase, with low levels during the 6D scotophase and the 7L photophase.

A daily rhythm of the pineal hormone melatonin has been demonstrated in rodents<sup>1</sup> and sheep<sup>2–4</sup> with highest blood levels during the scotophase of the daily cycle. However, the amount of melatonin secreted does not reflect proportionately the duration of darkness and sheep kept under long photoperiods show higher night-time plasma concentrations than do sheep kept under short photoperiods<sup>4</sup>. In sheep under natural, changing photoperiods the situation is complicated by the extremely pulsatile nature of melatonin secretion<sup>3,5</sup>. Sheep are more active during the day than during the night, the reverse of the situation in the rat, even though the diurnal rhythms of melatonin are similar in the 2 species.

The melatonin rhythm will continue in sheep kept in constant darkness, but under constant illumination melatonin secretion is suppressed<sup>2</sup>. Rollag et al.<sup>6</sup> showed that at the onset of darkness melatonin concentrations in plasma rose to their maximal level within 10 min and fell to basal levels within 10 min of the lights being switched on again. However, the changes at dusk and dawn are not always so rapid as this<sup>7</sup>.

In rats, brief exposure to light during the night causes a rapid decline in the activity of pineal N-acetyl transferase, the first

specific enzyme in the pathway to melatonin, and a decline in pineal and plasma melatonin concentrations. As little as 1 min of exposure to light is sufficient to cause a marked inhibition for at least 5 h even though the rats were maintained in darkness after exposure to the flash of light<sup>8</sup>. The administration of melatonin has been shown to mimic the effects of photoperiod on the reproductive processes of hamsters<sup>1</sup> and sheep<sup>9</sup>.

The present study describes the patterns of melatonin secretion in sheep during short (8 h light:16 h dark – 8L:16D) and skeleton long (7L:10D:1L:6D) photoperiods which both have 8 h of light per day but have been shown to have different effects on both reproductive paradigms<sup>10</sup> and prolactin secretion<sup>11,12</sup>.

**Materials and methods. Animals.** 8 Suffolk ♂ × Greyface ♀ crossbred lambs (4 males castrated neonatally and 4 females) born during March were weaned to a concentrate diet on 3 May. On 10 May they were transferred from natural photoperiodic conditions to individual pens in 2 exactly similar rooms of a lightproofed building (2 males and 2 females to each room) and subjected to a photoperiodic regime of 12L:12D for 12 days. At the end of this time the lighting in one room was changed to 8L:16D and in the other to