ANNALS OF ANATOMY

Variations in the cellular proliferation of prolactin cells from late pregnancy to lactation in rats

José Carretero, Manuel Rubio, Enrique Blanco, Deborah J. Burks, José L. Torres, Elena Hernández, Pilar Bodego, José M. Riesco, Juan A. Juanes, and Ricardo Vázquez

Laboratorio de Neuroendocrinología, Instituto de Neurociencias de Castilla y León, Departamento de Anatomía e Histología Humanas, Facultad de Medicina, Universidad de Salamanca, Avda. Alfonso X El Sabio s/n, 37007 Salamanca, Spain

Summary. Lactation is a physiological process associated with hyperactivity of hypophyseal prolactin-producing cells. It is known that the percentage of these cells is increased during lactation, although there are discrepancies in the reports regarding the mechanisms responsible for increasing the number of prolactin cells. In order to analyse whether this increase is a result of previous proliferation, variations in the proliferation rate of prolactin-positive cells were determined from late pregnancy to lactation in adult female rats by means of observation of the immunohistochemical expression of PCNA as a marker of cellular proliferation. During late pregnancy, a very significant increase in the percentage of proliferating prolactin cells was observed in comparison to non-pregnant females in the proestrus phase ($p < 0.01$). Although the percentage of prolactin-positive cells after one week of lactation was higher than in non-lactating or in pregnant females ($p < 0.01$), the proliferation rate was lower than in the other groups studied. In sum, our results suggest that late pregnancy constitutes a preliminary proliferative phase preparatory to the ensuing lactation phase and that endocrine changes in late pregnancy involve the cellular proliferation of hypophyseal prolactin cells in order to prepare the gland for later demands and to prevent proliferative changes from occurring during lactation.

Key words: Prolactin cells – Cellular proliferation – Late pregnancy – Lactation

Introduction

Prolactin is a hypophyseal hormone involved in the regulation of lactation and its release is increased during this physiological process (Meites and Turner 1948; Meites et al. 1972; Cowie et al. 1980). Within the first days and weeks postpartum, basal prolactin levels remain elevated and suckling episodes trigger a rapid release of hypophyseal prolactin (Tyson and Friesen 1973; Noel et al. 1974). This increase in prolactin secretion is accompanied by a suckling-induced increase in hypophyseal prolactin mRNA levels (Lee et al. 1989).

Basal prolactin levels gradually increase throughout the course of pregnancy (Tyson and Friesen 1973; Rigg et al. 1977; Ben-David et al. 1973). This has been attributed to the stimulatory effect of estrogens on hypophyseal lactotrophs. When hypophyseal prolactin synthesis and secretion are stimulated the lactotroph population increases. It has been suggested that this increase could be due to the differentiation of somatotroph to lactotroph cells (Boockfor et al. 1987) or to the proliferation of lactotroph cells (Lloyd et al. 1975; Kalbermann et al. 1979; Perez et al. 1986). A gradual increase in the number of hypophyseal lactotrophs during pregnancy has been reported (Goluboff and Ezrin 1969; Scheithauer et al. 1990). However, the proliferative mechanisms involved in this increase remain obscure.

The aim of the present study was to determine whether cellular proliferation is involved in the increase in the percentage of prolactin cells during late pregnancy and lactation and to determine whether proliferation occurs during both late pregnancy and lactation.

Correspondence to: J. Carretero
E-mail: jcar@usal.es

Materials and methods

Animals. Fifteen female adult Sprage-Dawley rats (200 g b/w) were used and were kept under standard conditions (22 ± 2°C, RH: 50 ± 5%, 8.00 to 20.00 light hours), with water and food (Panlab® maintenance diet) ad libitum. Animals were handled (cleaning, handling, hygiene) according to the guidelines of the European Communities Council Directive (86/609/EEC) and current Spanish legislation for the use and care of laboratory animals (BOE 67/8509-12,1998).

The rats were divided into 3 experimental groups (5 animals per group): (1) untreated rats in the proestrous phase, as determined by vaginal smears, were considered as normal and employed as controls; (2) rats sacrified after 1 week of suckling in their respective litters; (3) animals killed on day 19 of pregnancy.

Sacrifice of animals and sample processing. The animals were sacrificed by decapitation following anesthesia by Forene inhalation. The hypophyses were carefully dissected out and immediately fixed in a solution of 15% saturated pricric acid in 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4, for 24 h. Then, they were dehydrated in ethanol, cleared with xylene, and embedded in paraffin in order to obtain coronal serial sections of 5 μm thickness. These were placed on slides treated with gelatin-chrome alum and were then used for the inmunohistochemical study.

Immunohistochemistry. To study PCNA-positive cells and to determine the PCNA-Prolactin labelling index, a double labelling immunohistochemical method for PCNA and prolactin was developed. Endogenous peroxidase was blocked with H_2O_2 in methanol and non-specific reactions of the secondary antibody by incubation in normal goat serum (Dako, diluted 1:30). Sections were incubated overnight at 4 °C with mouse anti-PCNA monoclonal antibody (PC 10, Dako, diluted 1:3000 in TBS). Biotinylated goat anti-mouse IgG (Dako, diluted 1:100) and Avidin-Biotinylated horseradish peroxidase complex (ABC kit, Dako, diluted 1:100) were successively applied at room temperature for 40 min and 30 min, respectively. The reaction was developed in freshly prepared 3,3'-DAB (0.025% in TRIS buffer containing 0.03% of H_2O_2). Following PCNA immunolabelling, the peroxidase-antiperoxidase (PAP) reaction was performed for the detection of prolactin, using as primary serum anti-prolactin rabbit serum at a dilution of 1:800, swine anti-rabbit serum (Dako, diluted 1:100), and rabbit-PAP complex (Dako, diluted 1:100). Preabsorption tests with Prolactin and tests substituting the specific serum by normal rabbit serum abolished the reaction. Using ELISA, the specificity of swine anti-rabbit IgG was lower than 1% for rat and mouse IgG and 100% for rabbit IgG. For the washes and dilutions of the sera, TRIS buffer (0.05 M, pH 7.4) containing 0.8% NaCl was used. The reaction was developed in freshly prepared 4-chloro-1-naphthol (1.7×10^{-3} M in 3% absolute ethanol and TRIS-buffer containing 0.3% H_2O_2).

Prolactin- and PCNA-prolactin-immunoreactive cell quantification. Four thousand cells per animal were evaluated using a Leitz Dialux EB-20 microscope at a final magnification of 500×. The cells were randomly selected from different areas of the gland. The following parameters were determined: 1) PCNA-positive cells; 2) Prolactin-positive cells; 3) PCNA- and prolactin-positive cells (always calculated as percentages of the total number of cells analyzed). The percentage of PCNA- and prolactin-immunoreactive cells was also calculated from the total of prolactin-immunoreactive cells.

Statistical analysis. For each parameter evaluated, the values obtained were processed statistically and the differences observed were compared using analysis of variance, accepting p values of < 0.05 as significant for the Scheffé F test. Results are expressed as arithmetic means ± standard error of the mean.

Results

Hypophyseal proliferation. Hypophyseal proliferation, determined as the percentage of cells with PCNA-immunoreactive nuclei, was low in females in proestrous (0.74 ± 0.05). In late pregnancy, a significant increase in cells undergoing proliferation was observed: 9.41 ± 0.79% of glandular cells were PCNA positive ($p < 0.01$ in comparison to nonpregnant females). In suckling females, the percentage of proliferating cells was similar to normal animals (0.55 ± 0.09 vs. 0.74 ± 0.05) and significantly lower ($p < 0.01$) than that observed in pregnant females.

Prolactin-positive cell proliferation. Prolactin-PCNA-positive cells in females in the proestrous phase were 0.44 ± 0.01%. This means that 1.46% of the prolactin cells were proliferating cells. In these animals 30.22 ± 0.28% of the glandular cells were prolactin-positive.

Pregnancy was seen to be an important proliferation-inducing agent in prolactin-positive cells. In the pregnant animals 4.00 ± 0.51% of all cells were prolactin-PCNA-positive, and hence 14.06% of the prolactin cells were proliferating cells during late pregnancy. Although the

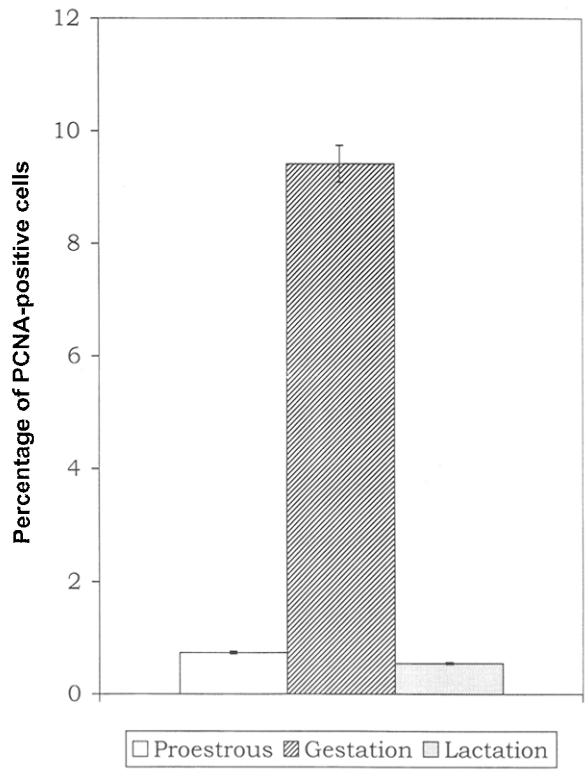

Fig. 1. Plot showing the mean values of the percentage of the percentage of PCNA-positive cells in the different groups studied.

percentage of prolactin-positive cells was lower than in proestrous females, the observed statistical differences were not significant (28.44 ± 0.90 vs. 30.22 ± 0.28).

The percentage of proliferating prolactin-positive cells in suckling females was very low, since 0.22 ± 0.08% were both prolactin- and PCNA-positive cells ($p < 0.05$ in relation to the proestrous animals and $p < 0.01$ in relation to the pregnant females). Thus, during suckling only 0.49% of prolactin-positive cells were undergoing proliferation.

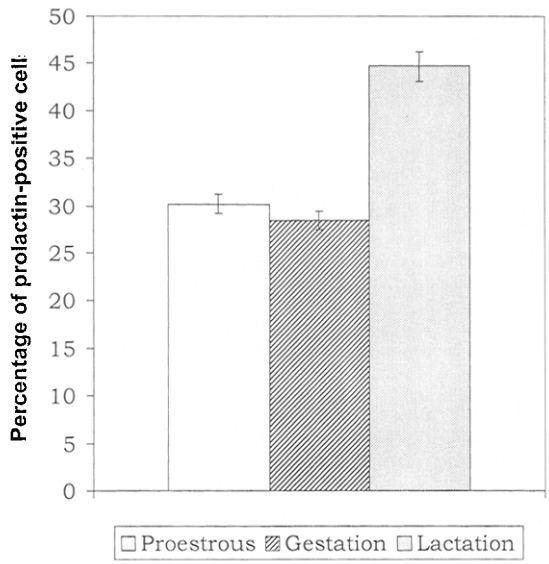

Fig. 2. Plot showing the mean values of the percentage of prolactin-positive cells in the different groups studied.

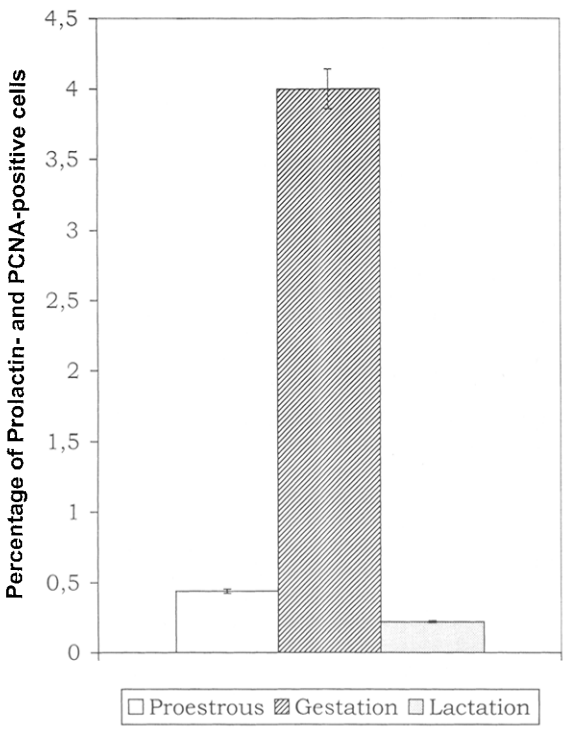

Fig. 3. Plot showing the mean values of the percentage of PCNA- and prolactin-positive cells in the different groups studied.

Comparatively, the percentage of prolactin-immunoreactive cells was higher in suckling females than in the animals in proestrus (44.67 ± 1.38 vs. 30.22 ± 0.28, $p < 0.01$) or late pregnancy (44.67 ± 1.38 vs. 28.44 ± 0.90, $p < 0.01$).

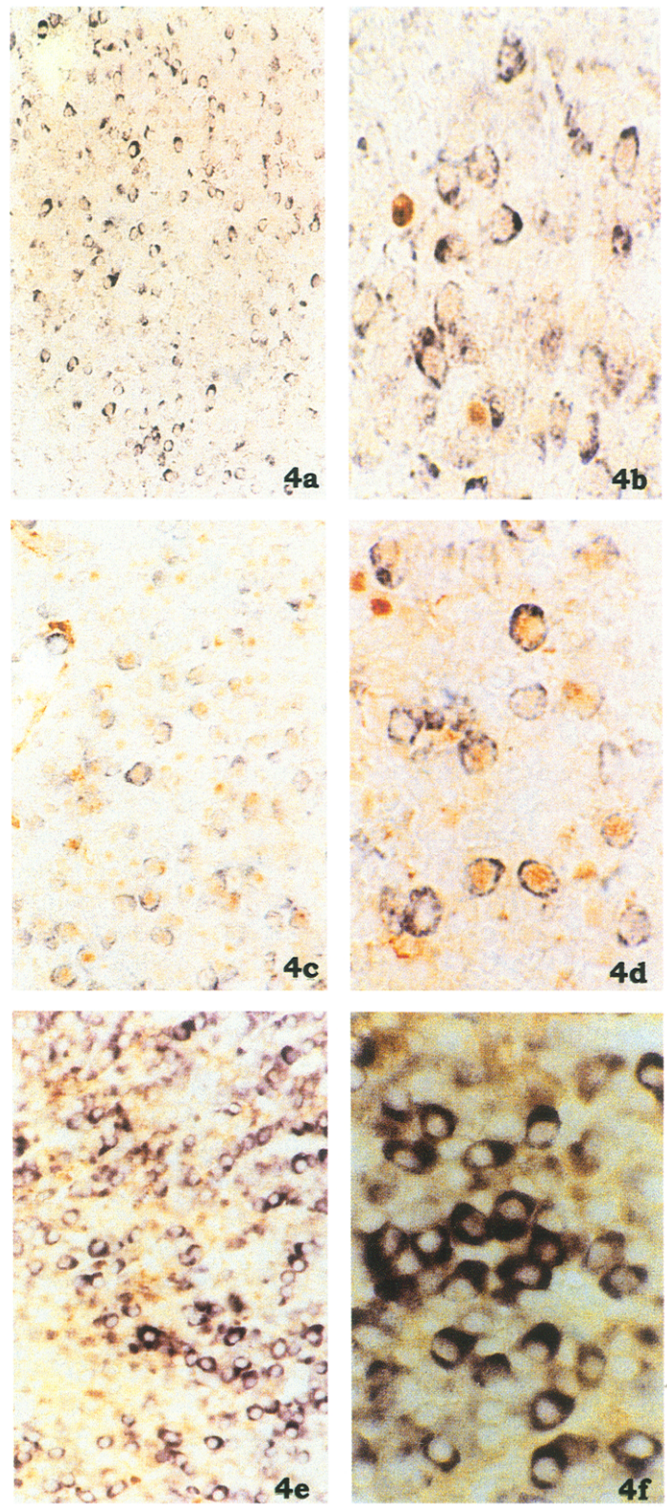

Fig. 4. Micrographs showing the immunostain for PCNA (brown) and/or prolactin (dark blue) in proestrus female rats (4a: ×100, 4b: ×300), pregnant female rats (4c: ×100, 4d: ×300) and lactating female rats (4e: ×100, 4f: ×300).

Discussion

It is conventionally accepted that the hypophyseal cellular proliferation rate is very low in adult animals (Pomerat 1941; Hunt 1943; Städtler et al. 1970; Stepién et al. 1978). Oishi et al. (1993) reported important variations related to the different phases of estrual cycle and lactotroph cells were found to show the highest proliferation rate of all types of hypophyseal cells. In agreement with the findings reported by Oishi and co-workers, our results demonstrate a low cellular proliferation in female rats during the proestrous phase.

The cellular proliferation of lactotroph cells has mainly been analysed following treatment with estradiol and dopamine antagonists or in tumor cells (Lloyd et al. 1975; Kalbermann et al. 1979; Pérez et al. 1986; Stefaneanu et al. 1992; Oishi et al. 1993; Carretero et al. 1995).

Except during postnatal development (Carbajo-Pérez y Watanabe 1990), the proliferation of lactotroph cells decreases progressively in relation to age (Takahashi et al. 1984). However, the proliferation of lactotroph cells is very important for the maintenance of total hypophyseal proliferation because 60.6% of proliferating cells in female rats are lactotroph cells (Oishi et al. 1993).

Although variations in the number of prolactin cells along pregnancy and lactation have been reported (Goluboff y Ezrin 1969; Amat and Muñoz-Barragán 1974; Scheithauer et al. 1990), very few studies using proliferation markers have been published (Kalbermann et al. 1979).

Kalbermann et al. (1979) reported a decrease in BrdU incorporation in the hypophysis at the end of pregnancy, which they explained as being a result of hyperprolactinemia. The present study – the first to use PCNA as a proliferation marker – demonstrates a significant increase in proliferating lactotroph cells during late pregnancy.

The discrepancies with the findings of Kalbermann could be due to the fact that PCNA is 5-fold more sensitive for the detection of hypophysial proliferating cells than BrdU (Oishi et al. 1993).

However, our results are consistent with the massive hyperplasia of lactotroph cells described in women by Scheithauer et al. (1990) and with the increase in mitosis in lactotrophs of gestating women reported by Stefaneanu et al. (1992).

In view of the elevated prolactin activity during lactation – probably due to the stimulating effect of oxytocin, TRH and VIP, a decrease in dopamine tonic inhibition and suckling stimulation (Matsuzaki et al. 1997) – it is clear that during lactation lactotroph cells must be numerous and hyperactive, as seen here. Nevertheless, this increase in the number of cells does not agree with the finding in our study of low proliferative activity during lactation. Therefore, the need for prolactin to be produced during post-partum lactation requires a previous preparative phase, which is achieved by high proliferative activity during late gestation, as demonstrated in this study.

In sum, our results demonstrate that the increase in lactotroph cells observed during lactation is a consequence of cellular proliferation in late pregnancy and, without rejecting the possibility of the appearance of mammosomatotroph cells, suggest the existence of a preparatory proliferative phase of lactotroph cells prior to the demands for prolactin during lactation.

Acknowledgements. Supported by the FIS 99/1187 program and the SA58/98 Social European Funds and JCyL program.

References

Amat P, Muñoz-Barragán L (1974) Mitosis en la adenohipófisis. Ann Anat 23: 15–27

Ben-David M, Rodbard D, Bates RW, Bridson E, Chrambach A (1973) Human prolactin in plasma, amniotic fluid and pituitary: identity and characterization by criteria of electrophoresis and isoelectric focusing in polyacrylamide gel. J Clin Endocrinol Metab 36: 951–964

Boockfor FR, Hoeffler JP, Frawley SL (1987) Estradiol induces a shift in cultured cells that release prolactin or growth hormone. Am J Physiol 250: 103–106

Carbajo-Pérez E, Watanabe YG (1990) Cellular proliferation in the anterior pituitary of the rat during the postnatal period. Cell Tissue Res 261: 333–338

Carretero J, Rubio M, Navarro N, Nieto P, Vázquez RJ, Sánchez F, Vázquez R (1995). In vitro modifications in the proliferation rate of prolactin cells are accompanied by nuclear morphometric variations. Histol Histopathol 10: 135–139

Cowie AT, Forsyth IA, Hart IC (1980) Hormonal control of lactation. Springer, Berlin

Goluboff LH, Ezrin C (1969) Effect of pregnancy on the somatotroph and the prolactin cell of the human adenohypophysis. J Clin Endocrinol Metab 29: 1533–1541

Hunt TE (1943) Mitotic activity in the anterior hypophysis of female rats of different age groups and at different periods of the day. Endocrinology 32: 334–339

Kalbermann LE, Szijian I, Burdman JA (1979) Cell proliferation in the rat pituitary gland: A mechanism of control in prolactin cell. Experientia 35: 689–690

Lee LR, Haisenleder DJ, Marshall JC (1989) The role of the suckling stimulus in regulating pituitary prolactin mRNA in the rat. Mol Cell Endocrinol 64: 243–249

Lloyd HM, Meares JD, Jacobi J (1975) Effects of oestrogen and bromocryptine on in vivo secretion and mitosis in prolactin cells. Nature 255: 497–498

Matsuzaki T, Irahara M, Aono T (1997) Physiology and action of prolactin. Nippon Rinsho 55: 2871–2875

Meites J, Lu KH, Wuttke W, Welsh CW, Kagasawa H, Ovadri SK (1972) Recent studies on function and control of prolactin secretion in rats. Rec Prog Horm Res 28: 471–526

Meites J, Turner CW (1948) Studies concerning the induction and maintenance of lactation. I. The mechanism controlling the initiation of lactation and parturition. Mo Agr Exp Sta Res Bull 415: 1–65

Noel GL, Suh HK, Frantz AG (1974) Prolactin release during nursing and breast stimulation in postpartum and nonpostpartum subjects. J Clin Endocrinol Metab 38: 413–423

Oishi Y, Okuda M, Takahashi H, Fujii T, Morii S (1993) Cellular proliferation in the anterior pituitary gland of normal adult rats: influences of sex, estrous cycle, and circadian change. Anat Rec 235: 111–120

Pérez RL, Machiavelli GA, Romano MI, Burdman JA (1986) Prolactin release, oestrogens and proliferation of prolactin-secreting cells in the anterior pituitary gland of adult male rats. J Endocrinol 108: 399–403

Pomerat GR (1941) Mitotic activity in the pituitary of the white rat following castration. Am J Anat 69: 89–121

Rigg LA, Lein A, Yen SCC (1977) Pattern of increase on circulating prolactin levels during human gestation. Am J Obstet Gynecol 129: 454–456

Scheithauer BW, Sano T, Kovacs K, Young WF, Ryan N, Randall RV (1990) The pituitary gland in pregnancy: A clinicopathologic and immunohistochemical study on 69 cases. Mayo Clin Proc 65: 462–474

Städler F, Stocker E, Dhom G, Tietze HU (1970) Autoradiographic studies on nuclear DNA and RNA synthesis in the adenohypophysis of castrated rats. Acta Endocrinol 64: 324–338

Stefaneanu L, Kovacs K, Lloyd RV, Scheithauer BW, Young WF Jr, Sano T, Jin L (1992) Pituitary lacotrophs and somatotrophs in pregnancy: a correlative in situ hybridization and immunocytochemical study. Virchows Archiv B Cell Pathol 62: 291–296

Stepién H, Wolaniuk A, Pawlikowski M (1978) Effects of pimozide and bromocryptine on anterior pituitary cell populations. J Neural Transm 42: 239–244

Takahashi S, Okazaki K, Kawashima S (1984) Mitotic activity of prolactin cells in the pituitary glands of male and female rats of different ages. Cell Tissue Res 235: 497–502

Tyson JE, Friesen HG (1973) Factors influencing the secretion of human prolactin and growth hormone in menstrual and gestational women. Am J Obstet Gynecol 116: 377–387

Accepted September 25, 2002