EXPERIMENTAL BIOLOGY

Prolactin Receptor in Rat Bile Ducts in Ontogeny

O. M. Petrashchuk, A. N. Smirnov, and O. V. Smirnova

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 122, No. 12, pp. 669-672, December, 1996
Original article submitted November 20, 1995

Expression of prolactin receptors (PR) in the nuclei and plasma membrane of the bile duct cells is demonstrated in 1-45-day-old male and female rats with the use of indirect immunoperoxidase technique. The intensity of PR staining in the bile duct cells is not sex-dependent; it increases from the 1st day till the 45th day and disappears by the 65th day of postnatal ontogeny. The PR-specific staining of hepatocytes also increases during the first 45 days of postnatal life and becomes sex-dependent by the 65th day.

Key Words: *prolactin receptors; immunohistochemistry; rat cholangiocytes and hepatocytes; ontogenesis*

The liver is one of the prolactin targets. Prolactin regulates metabolic processes in the liver and acts as a trophic agent, promoter of carcinogenesis, and stimulator of DNA synthesis and mitoses [3-5]. Previously, we showed that in adult rats only hepatocytes express prolactin receptors (PR), the intensity of expression being higher in females than in males [1,13].

The functions of prolactin are modified during ontogeny [9]. This may be associated with changes in the spectrum of tissues expressing PR. The aim of this study was to analyze the expression of PR in different liver cell types in male and females during the early period of postnatal life.

MATERIALS AND METHODS

Outbred albino male and female rats aged 1, 7, 15, 45, 65, and 90 (puberty) days were used.

Prolactin receptors were identified in the liver by indirect immunoperoxidase technique [1,13]. Murine monoclonal antibodies U6 specific for the extracellular domain of rat PR other than that in the hormone-binding center [10] were used. The antibodies were a generous gift of Dr. P. Kelly (France).

Laboratory of Endocrinology, M. V. Lomonosov Moscow State University

Tissue specimens were fixed in 4% paraform in 0.1 M phosphate buffer (pH 7.4) for 20 h at 4°C, washed, and embedded in paraplast. Sections (3-μ thick) were mounted on glass and consecutively incubated for 10 min at room temperature with 10 mM sodium periodate and 0.01% sodium borohydride. They were then incubated with the monoclonal antibodies in 0.05 M Tris-HCl (pH 7.6) for 18-20 h at 4°C. Control sections were incubated under the same conditions with 0.05 M Tris-HCl or murine IgG (0.1 mg/ml) in the same buffer. Rabbit anti-mouse and donkey anti-rabbit antisera conjugated with peroxidase (N. F. Gamaleya Institute of Epidemiology and Microbiology, Russian Academy of Medical Sciences) were used as "bridge" and developing antibodies, respectively. The antisera were depleted with rat serum (1:2) for 2 h at room temperature, diluted with 0.05 M Tris-HCl 1:10 (rabbit antiserum) and 1:100 (donkey antiserum), and incubated with control and experimental sections for 30 min at room temperature. After each treatment, the sections were washed with the same buffer (5 min, three times). Diaminobenzidine was used as a chromogen. Parallel sections were stained with hematoxylin.

Material from 3-8 rats was analyzed in each group.

0007-4888/96/0012-1228$15.00

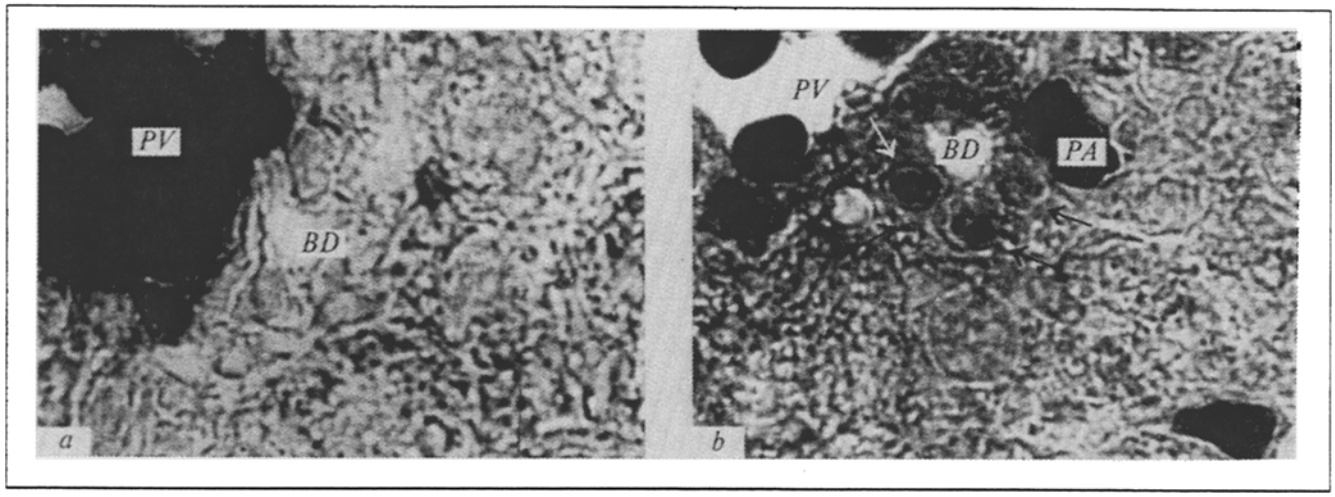

Fig. 1. Immunoperoxidase identification of subcellular prolactin receptors (PR) in the bile duct cells of a 45-day-old male rat. Control section (*a*), section incubated with U6 monoclonal antibodies (*b*): PR-positive staining of the nuclei (black arrows) and plasma membrane (white arrow). ×1250. Here and in Figs. 2 and 3: *BD:* bile duct, *PA:* portal artery; *PV:* portal vein.

RESULTS

The bile duct cells and hepatocytes of male and female rats stained positively for PR from day 1 till day 45 of postnatal life.

In the bile duct cells of males and females, the PR-positive staining was confined predominantly to the nuclei and sometimes was seen in the plasma membrane (Fig. 1). Its intensity increased from the 1st till the 45th day (Fig. 2) and then decreased. On

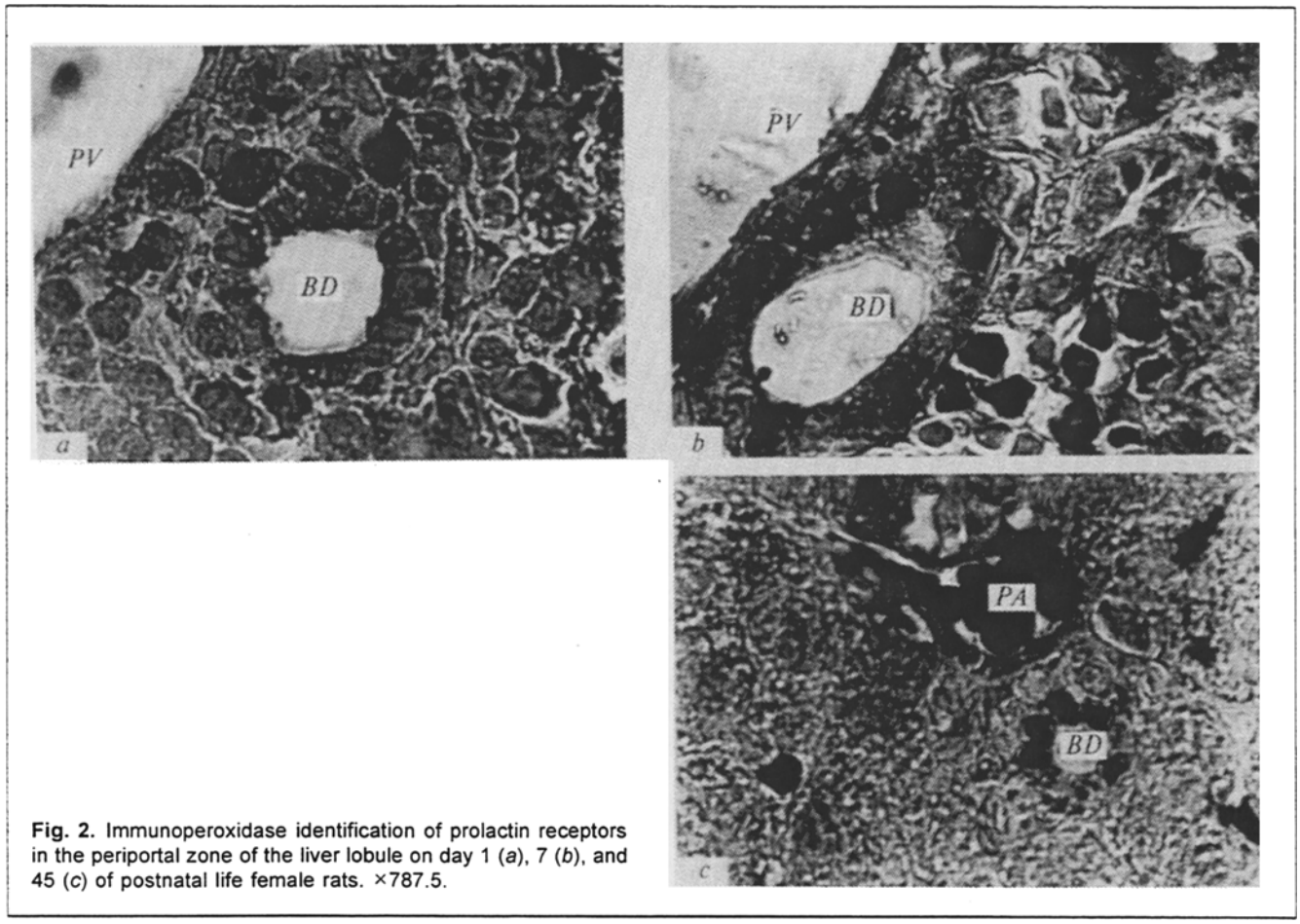

Fig. 2. Immunoperoxidase identification of prolactin receptors in the periportal zone of the liver lobule on day 1 (*a*), 7 (*b*), and 45 (*c*) of postnatal life female rats. ×787.5.

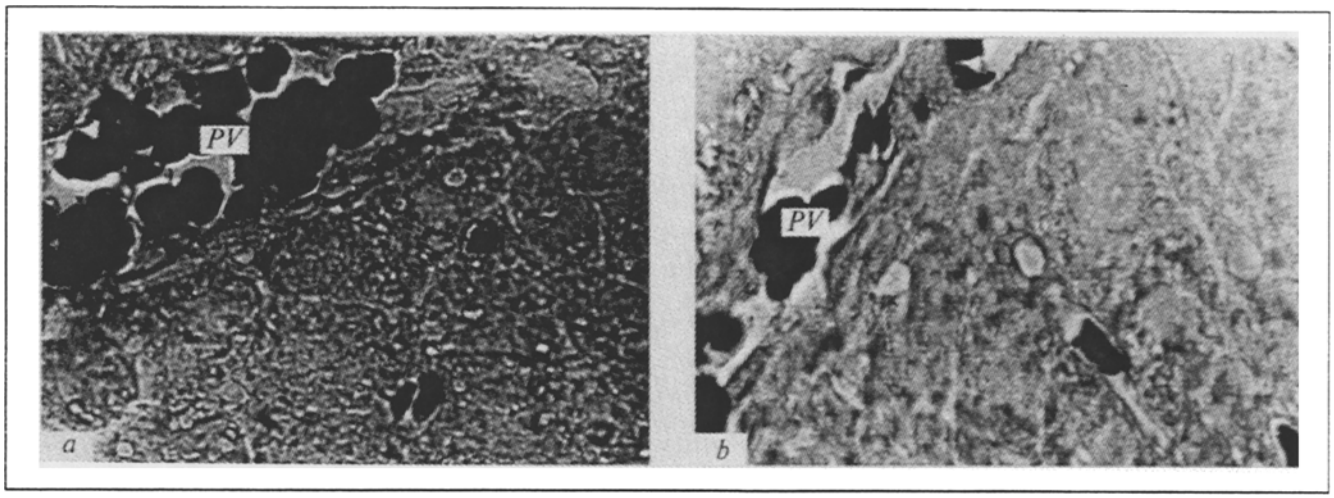

Fig. 3. Immunoperoxidase identification of prolactin receptors in the periportal zone of the liver lobule on day 65 of postnatal life female (*a*) and male (*b*) rats. ×787.5.

day 65 of postnatal ontogeny, the staining was revealed neither in males nor in females (Fig. 3). There were no sex-specific differences in the intensity of PR-specific staining of the bile duct cells at all stages of ontogeny.

In hepatocytes of males and females, the intensity of PR-positive staining also increased from the 1st till the 45th day of life. In males, it decreased after 45 days, on day 65 its intensity being similar to that in adult males. In 45-day-old females, the intensity of the staining was stable and high (Fig. 3).

During the first 45 days of postnatal life, hepatocytes and bile duct cells of male and female rats express PR. Presumably, these 45 days are a specific period in the differentiation and function of these cells. Hepatocytes and bile duct cells are known to have common precursors; however, it is unclear whether these precursors are oval cells or immature hepatocytes [12,14]. In rats, the development of bile ducts and differentiation of hepatocytes occur during the first month of postnatal life. In addition, both cell types intensely proliferate during the formation of liver lobules and loss of hematopoietic cells [14,15]. Bearing in mind that prolactin induces proliferation and differentiation of liver cells [3-5], it can be suggested that during the first 45 days of life prolactin maintains proliferative activity and differentiating potential of both hepatocytes and bile duct cells at a high level. In adult animals, the proliferative index of liver cells is very low, and the metabolic processes are different in hepatocytes and bile duct cells [2,6]. It can be suggested that prolactin becomes one of the main metabolic regulators during puberty. The liver metabolism is sex-dependent. In adult animals, the expression of PR is controlled by sex steroids [1,2,13], which leads to a sex-dependent differentiation of metabolic reactions regulated by prolactin.

The present study shows that subcellular distribution of PR in the bile duct cells differs from that in hepatocytes [1,13]: immunoreactive PR are present in the nuclei of the bile duct cells. Prolactin receptors have been recently identified in the nuclei and nuclear membranes of various cell types [8,11]. It was hypothesized that endocytosis is not only the mechanism by which PR are eliminated but also the initial state of PR transport into the cell. Signal transduction via membrane-bound and nuclear PR may differ considerably [11]. The possibility cannot be excluded that different compartmentalizations of PR in different cell types result from variations of the expression of PR differing by the length of the intracellular domain and activating different second messenger systems [7].

Thus, our results indicate that at the early stages of postnatal ontogeny two types of liver cells in male and female rats are sensitive to prolactin. The bile duct cells lose PR during puberty, while hepatocytes express these receptors under the control of sex-related factors.

This study was financially supported by the Russian Foundation for Basic Research (project codes: 93-04-20127 and 95-04-11115-a).

REFERENCES

1. O. M. Petrashchuk, O. V. Smirnova, and A. N. Smirnov, *Byull. Eksp. Biol. Med.*, **118**, No. 9, 264-267 (1994).
2. V. B. Rosen, G. D. Mataradze, O. V. Smirnova, and A. N. Smirnov, *Sex-Dependent Differentiation of Liver Functions* [in Russian], Moscow (1991).
3. A. Buckley, C. Putman, D. Montgomery, and D. Russell, *Biochem. Biophys. Res. Commun.*, **138**, 1138-1145 (1986).
4. A. Buckley, C. Putman, and D. Russell, *Life Sci.*, **37**, 2569-2576 (1985).
5. P. Crowe, A. Buckley, N. Zorn, and H. Rui, *Mol. Cell. Endocrinol.*, **79**, 29-35 (1991).

6. J. Greengard, M. Federman, and W. Knox, *J. Cell Biol.*, **52**, 261-272 (1972).
7. L. Lesueur, M. Edery, S. Ali, *et al.*, *Proc. Natl. Acad. Sci. USA*, **88**, 824-828 (1991).
8. P. Lobie, J. Garcia-Aragon, and M. Waters, *J. Endocrinol.*, **139**, 371-382 (1993).
9. Nicol, *Fed. Proc.*, **39**, 2563-2566 (1980).
10. H. Okamura, J. Zachwieja, S. Raguet, and P. Kelly, *Endocrinology*, **124**, 2499-2508 (1989).
11. J.-P. Rao, D. Buckley, M. Olson, and A. Buckley, *J. Cell. Physiol.*, **163**, 266-276 (1995).
12. S. Sell, *Cancer Res.*, **50**, 3811-3815 (1990).
13. O. V. Smirnova, O. M. Petraschuk, and P. A. Kelly, *Mol. Cell. Endocrinol.*, **105**, 77-81 (1994).
14. P. VanEyken, R. Sciot, and V. Desmet, *Lab. Invest.*, **59**, 52-59 (1988).
15. N. Varceau, M.-J. Bljuin, L. Germain, and M. Noel, *In Vitro*, **25**, 336-341 (1989).