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Role of Estrogens in the Regulation of Prolactin Receptors in Liver Cells of Female Rats

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

UDC 612.352.3.014.467:577.175.64.+612.433

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 118, № 9, pp. 264-267, September, 1994
Original article submitted November 27, 1993

Strong expression of prolactin receptors in sinusoidal domains and cytoplasmic granules of hepatocytes, which is independent of cell location in the hepatic lobule and is positively regulated by estrogens, is revealed in pubertal female rats. In estrogen-treated animals, Prolactin receptors are also exposed in the perinuclear space of some hepatocytes surrounding the central veins. Estrogens regulate the intensity of prolactin receptors expression in hepatocytes, but not the number of cells containing these receptors.

Key Words: *immunohistochemistry; prolactin receptors; rat liver; estrogens*

Prolactin produces a broad spectrum of metabolic effects on the liver [2,4]. Prolactin receptors (PR) are present in the liver of humans and various animal species. The receptor protein has been isolated in pure form, and monoclonal antibodies to it have been obtained [2,6,11,12].

With the aid of radioligand techniques it has been demonstrated that estrogens positively regulate the PR content in rat liver [2]. It is unclear however, whether estrogens control the PR level in each liver cell or regulate the number of prolactin-sensitive cells in this organ. Immunohistochemical identification of liver cells containing PR in female rats with different estrogen status was the aim of this study.

Laboratory of Endocrinology, Biology Department, M. V. Lomonosov State University, Moscow. (Presented by I. P. Ashmarin, Member of the Russian Academy of Medical Sciences)

MATERIALS AND METHODS

Experiments were performed on pubertal female albino rats of a mixed population either intact or 25-30 days after ovariectomy. Estradiol-17β (E_2) was administered to ovariectomized females in a dose of 10 μg in 0.4 ml of propylene glycol during a 14-day period. The animals were sacrificed 24 h after the last injection of the hormone.

Prolactin receptors were visualized by the indirect immunoperoxidase technique using murine anti-PR monoclonal antibodies (U6) as the immunoglobulin G fraction isolated from ascitic fluid. Monoclonal antibody U6 is specific for a

0007-4888/94/0009-0962$12.50

receptor site other than the hormone-binding center [11].

The tissue was fixed in 4% paraform in 0.1 M phosphate buffer at pH 7.4 for 20 h at 4°C and embedded in paraplast after washing. Sections (3 μ thick) were mounted on glass and consecutively treated with 10 mM sodium periodine and 0.01% sodium borohydrate (10 min with each solution at room temperature). The sections were incubated with the monoclonal antibodies (0.1 mg/ml of the immunoglobulin fraction 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 antiserum and donkey anti-rabbit antibodies conjugated with peroxidase (N. F. Gamaleya Institute of Epidemiology and Microbiology, Russian Academy of Medical Sciences) were used as a "bridge" and as developing antibodies, respectively. Rabbit anti-mouse antiserum and donkey anti-rabbit antibodies were depleted with rat serum taken in a dilution of 1:2 (2 h at room temperature, diluted with 0.05 M Tris-HCl, pH 7.6, to final dilutions of 1:10 and 1:100, and incubated with control and experimental sections for 30 min at room temperature. Sections were washed after each treatment (5 min, three times). Diaminobenzidine was used as a chromogen. Parallel sections were stained with hematoxylin.

Material obtained from 3-8 animals was analyzed in each group.

RESULTS

In liver tissue specimens of pubertal rats PR-positive immunoreactivity was revealed predominantly in hepatocytes. The PR-specific staining was confined to various subcellular structures of these cells (Fig. 1): sinusoidal domains of plasma membranes, intracellular cytoplasmic granules, and, sometimes, in animals with high estrogen status, to the perinuclear region and/or nuclear membrane of some cells surrounding the central veins. There were no appreciable differences in the level of PR expression in hepatocytes located in different zones of the lobule (Fig. 2). In some animals with a high estrogen level the staining of individual hepatocytes adjacent to the central veins was more intense (Fig. 3 *a, b*).

A high intensity of PR-positive hepatocyte staining was characteristic of intact pubertal rats (Fig. 3, *a*). Ovariectomy of adult rats (1 month after the operation) led to a uniform lowering of PR expression in all hepatocytes of a lobule, mainly due to a decrease in the cytoplasm immunoreactivity (Fig. 3, *b*). In these animals, positive staining of the perinuclear area of hepatocytes was virtually absent. Administration of E_2 to ovariectomized rats resulted in a rise of PR in all hepatocytes of a lobule. The intensity of PR expression increased both in the cytoplasm and in the sinusoidal domains of the hepatocyte membrane (Fig. 3, *c*). In estrogen-treated animals, much more intense intracellular staining and the presence of PR-positive immunoreactivity in the perinuclear area were revealed in some hepatocytes surrounding the central veins (Figs. 1 and 3, c).

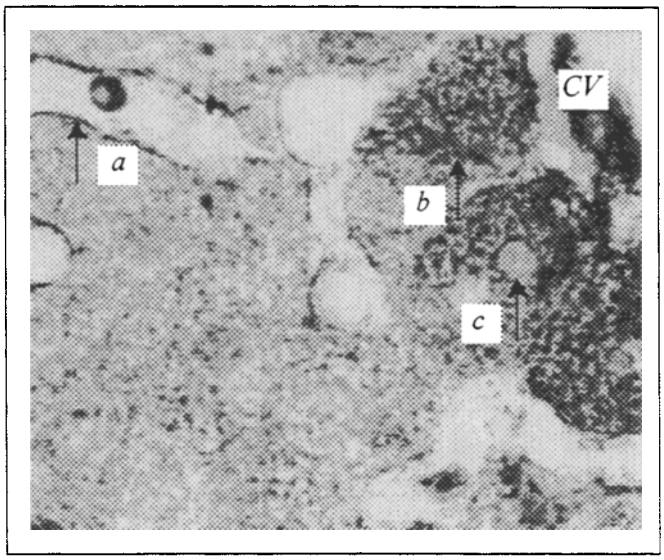

Fig. 1. Immunoperoxidase identification of subcellular localization of PR in liver cells of an ovariectomized rat given 10 μg E_2 during a 14-day period. Arrows indicate PR-positive staining. *a*) sinusoidal domain of plasma membrane; *b*) cytoplasm; *c*) perinuclear area and/or nuclear membrane of hepatocytes. ×1250. Here and in Figs. 2 and 3: *CV* stands for central vein.

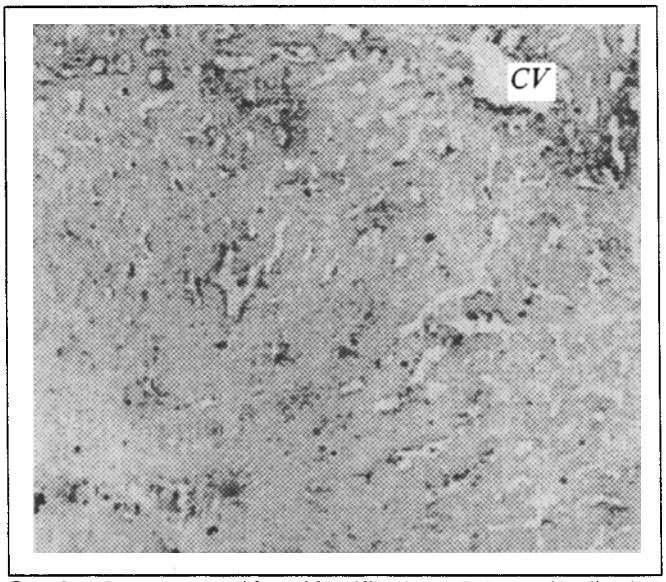

Fig. 2. Immunoperoxidase identification of tissue distribution of PR in the liver of an intact pubertal rat. ×200.

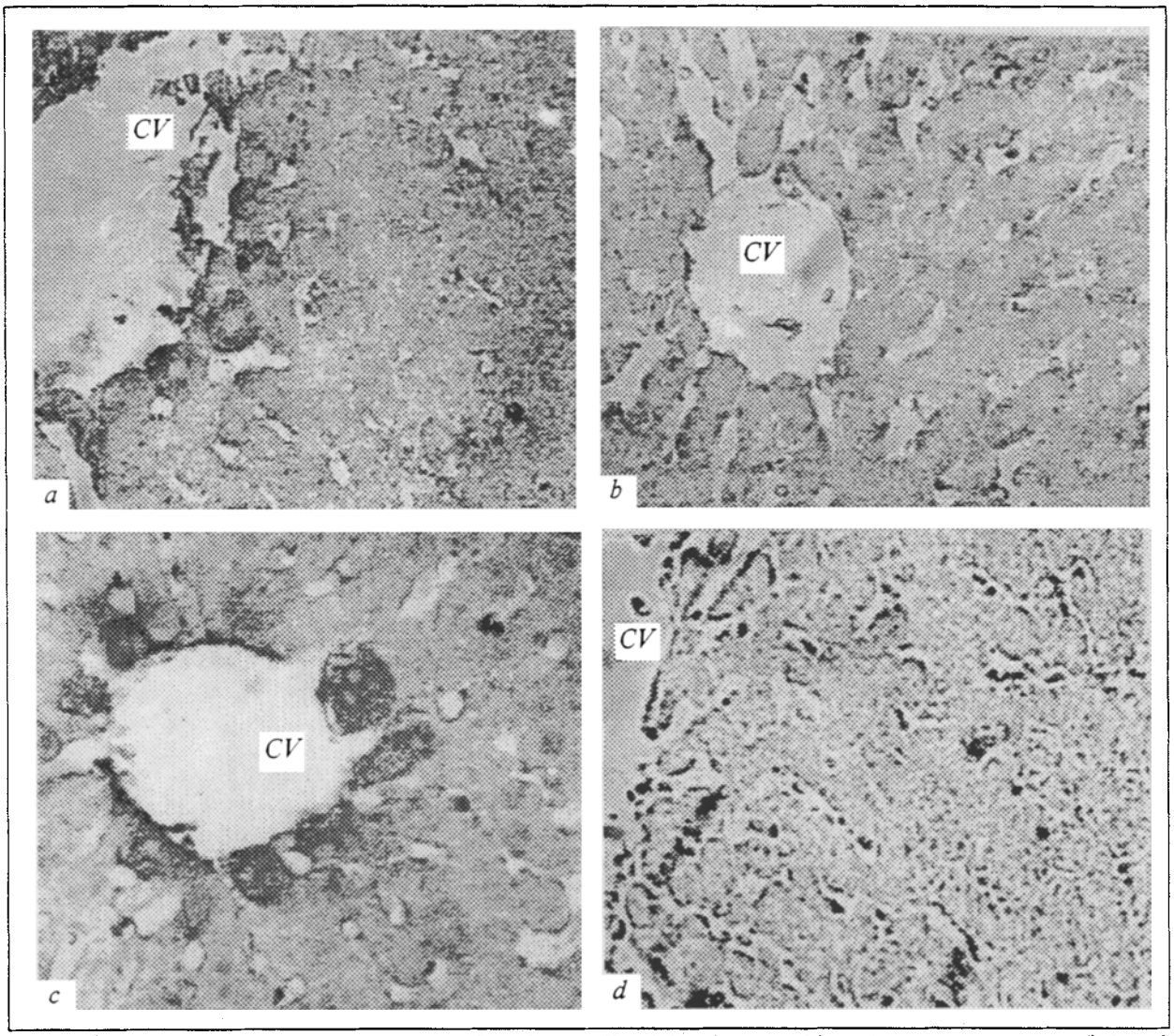

Fig. 3. Immunoperoxidase identification of PR in liver cells of female rats with various estrogen status. Pubertal females: *a*) intact; *b*) ovariectomized; *c*) ovariectomized, 10 μg E_2 during a 14-day period; *d*) intact females, control section. ×500.

Thus, in adult female rats with a well-developed specific structure of the liver tissue the sinusoidal domain hepatocyte plasma membrane is one site of PR localization. It is known that this domain on the hepatocyte surface is in charge of the exchange of metabolites between blood and liver. Presumably, localization of PR at this membrane site creates optimal conditions for receiving the hormonal signal conveyed by the blood. At the same time, using immunohistological methods we found that considerable amounts of PR are concentrated in the cell. This is consistent with the data on a high PR content in the microsomal fraction of hepatocytes [2,5,6]. In animals with a high content of E_2 (intact and E_2-treated ovariectomized rats) the ratio between exposed and internalized PR is markedly shifted toward the latter, which confirms the results obtained by other methods [5,6,12]. The presence of a large pool of intracellular receptors is a distinctive feature of membrane receptors of the lymphokine superfamily, to which PR belong [3,13]. It is hypothesized that this is associated with a high rate of internalization and recycling of such receptors and with a possible realization of the hormonal effect not only at the cytoplasmic membrane level but also at the level of the nuclear membrane and the nucleus itself [8,10,13]. In this context, our data on the concentration of PR in the perinuclear area of some hepatocytes surrounding the central veins are significant. It should be noted that a positive immunoreactivity of the nuclear membrane and nuclei of some cells was revealed when the localization of growth hormone, another membrane receptor of this superfamily, was analyzed [8,10].

Prolactin receptors are sex-dependent liver proteins. In females their content in the liver is

considerably higher than in males [2]. The expression of numerous liver proteins, including the sex-dependent ones, is related to hepatocyte localization in the lobule [2,14]. Our results show that in intact female rats PR expression is essentially the same in cells localized in different areas of the lobule. In the absence of estrogens the intensity of PR-specific staining drops evenly in all cells of a lobule, while long-term administration of these hormones leads to a considerable increase in PR expression in all hepatocytes. It can be concluded that estrogens positively regulate the PR content in hepatocytes without changing the number of PR-expressing cells. The mechanism of the stimulatory effect of E_2 on the PR content in hepatocytes is so far unclear. The influence of the receptors may be aimed directly at the hepatocytes [15] or may be realized at the hypothalamic-hypophyseal level, stimulating the secretion of prolactin and its homologs by the pituitary.

Some hepatocytes surrounding the central veins responded to estrogens in a specific manner. In animals with a high estrogen content, their PR-specific staining differed from that of other hepatocytes in the lobule in being much more intense, and PR were present in the perinuclear zone. It is noteworthy that this type of staining is characteristic of the largest, often binuclear, probably, polyploid hepatocytes. In view of the findings that massive influence of estrogens stimulates hepatocyte proliferation, which is most intense in the area adjacent to the central veins [1,7,9], one can assume that the mechanism of estrogen action on these cells differs from the mechanism of their action on other hepatocytes of the lobule and is associated with the direct proliferative effects of sex hormones.

This study was financially supported by the Russian Foundation for Basic Research (project code 93-04-20127).

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