Peculiarities of the Prolactin Receptor Expression in Liver Cells after Partial Hepatectomy

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Changes in the expression of prolactin receptors in the liver in response to partial hepatectomy are studied in gonadectomized male and female rats using the indirect immunoperoxidase method. Experiments reveal the rise of prolactin receptor expression only at the early stages of regeneration (15 and 30 min postoperation). Intense expression of prolactin receptors in vesicular hepatocyte membranes coincides with lipid infiltration and high mitotic activity of hepatocytes 35 h after initiation of regeneration.

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

Prolactin is a systemic regulator of trophic and proliferative processes in the liver. Injection of prolactin leads to induction of some G_1-associated enzymes in hepatocytes. It has been hypothesized that prolactin plays a principal role in the regulation of liver regeneration after partial hepatectomy (PHE) by modulating nuclear protein kinase C activity [5,7,14].

The liver is characterized by high and sex-dependent content of prolactin receptors (PR) [4]. It has been previously found that PR receptors in rat hepatocytes are localized primarily in plasma membrane, cytoplasmic granules, and sometimes perinuclear zone [3,13]. Prolactin receptors have been previously identified in the nuclei of bile duct cell of pubertal rats [2]. The aim of the present study was to analyze tissue and cell localization of PR and the dynamics of their expression in rat hepatocytes during proliferative response to PHE. In order to avoid the effect of sex steroids to the content and cell distribution of PR in hepatocytes [13] experiments were carried out in gonadectomized animals.

MATERIALS AND METHODS

Experiments were performed on male and female albino rats from a mixed population 3 weeks after gonadectomy. Experimental PHE was reproduced by removing the central and left lobes of the liver (66.2±0.2% of the liver). Liver samples (3-4 animals per group) were analyzed 15 and 30 min, 24, 35, 48, and 96 h, and 12 days postoperation. Sham-operated gonadectomized rats served as the control. The degree of regeneration was assessed from the total mass and the content of DNA and protein in the liver 12 days after PHE.

Prolactin receptors were visualized by the indirect immunoperoxidase method [13] using mouse anti-PR monoclonal antibodies (U6 clone) specific for the extracellular domain of the rat liver PR other than the hormone-binding center [10] (generous gift of Dr. P. Kelly, France).

Tissue specimens were fixed in 4% paraform for 20 h at 4°C and embedded in paraplast. Sections (3 μ thick) were treated with 10 mM sodium periodate and 0.01% sodium borohydride and incubated with the monoclonal antibodies (0.1 mg/kg IgG of ascitic fluid) in 0.05 M Tris-HCl buffer (pH 7.6) for 18-20 h at 4°C. Control sections were processed under the same conditions with 0.05 M Tris-HCl on mouse IgG (0.1 mg/ml) in the same buffer. Rabbit anti-mouse (1:10) and donkey anti-rabbit (1:100) 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. These anti-

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sera were depleted with rat serum and incubated with experimental and control sections for 30 min at room temperature. The sections were washed with the buffer after each treatment (5 min, 3 times). Diaminobenzidine was used as a chromogen. Some sections were poststained with hematoxylin. For histochemical visualization of lipids, pre- and postfixed frozen sections were stained with Sudan Red.

RESULTS

As in our previous experiments [3,13], the intensity of PR-positive staining in the liver of castrated males and ovariectomized females was similar and did not depend on the location of a hepatocyte within the hepatic lobule. This parameter increases 15 and 30 min after PHE, while in sham-operated animals it remains practically unchanged. At the later stages of regeneration the intensity of PR-positive staining did nor differ from the control in both castrated males (Fig. 1) and ovariectomized females.

In gonadectomized rats, the PR-positive staining was seen in sinusoidal domains of plasma membranes, intracellular cytoplasmic granules, and sometimes in the perinuclear space of hepatocytes (Fig. 1, *a*). At early stages of liver regeneration induced by PHE (15 and 30 min postoperation) no changes in the subcellular distribution of PR were noted, while the development of regeneration processes was accompanied by considerable changes in PR distribution pattern: intense PR-positive staining appeared in vesicular membranes of hepatocytes 24 h after PHE. The relative portion of PR exposed on vacuoles increased along with the rise in the number of these vacuoles in the hepatocytes of regenerating tissue (Fig. 1, *b*) and attained the maximum 35 h postoperation (Fig. 1, *c*). Forty-eight and 96 h postoperation, the portion of vesicular PR decreased and 12 days after the initiation of regeneration these PR were seen in single cells with minimal number of vesicles (Fig. 1, *d*). The dynamics of PR expression in vacuolar membranes was similar in castrated males and ovariectomized females. Lipid-specific staining of regenerating liver showed that maximum vacuolization (35 h postoperation) coincides with marked steatosis of hepatocytes.

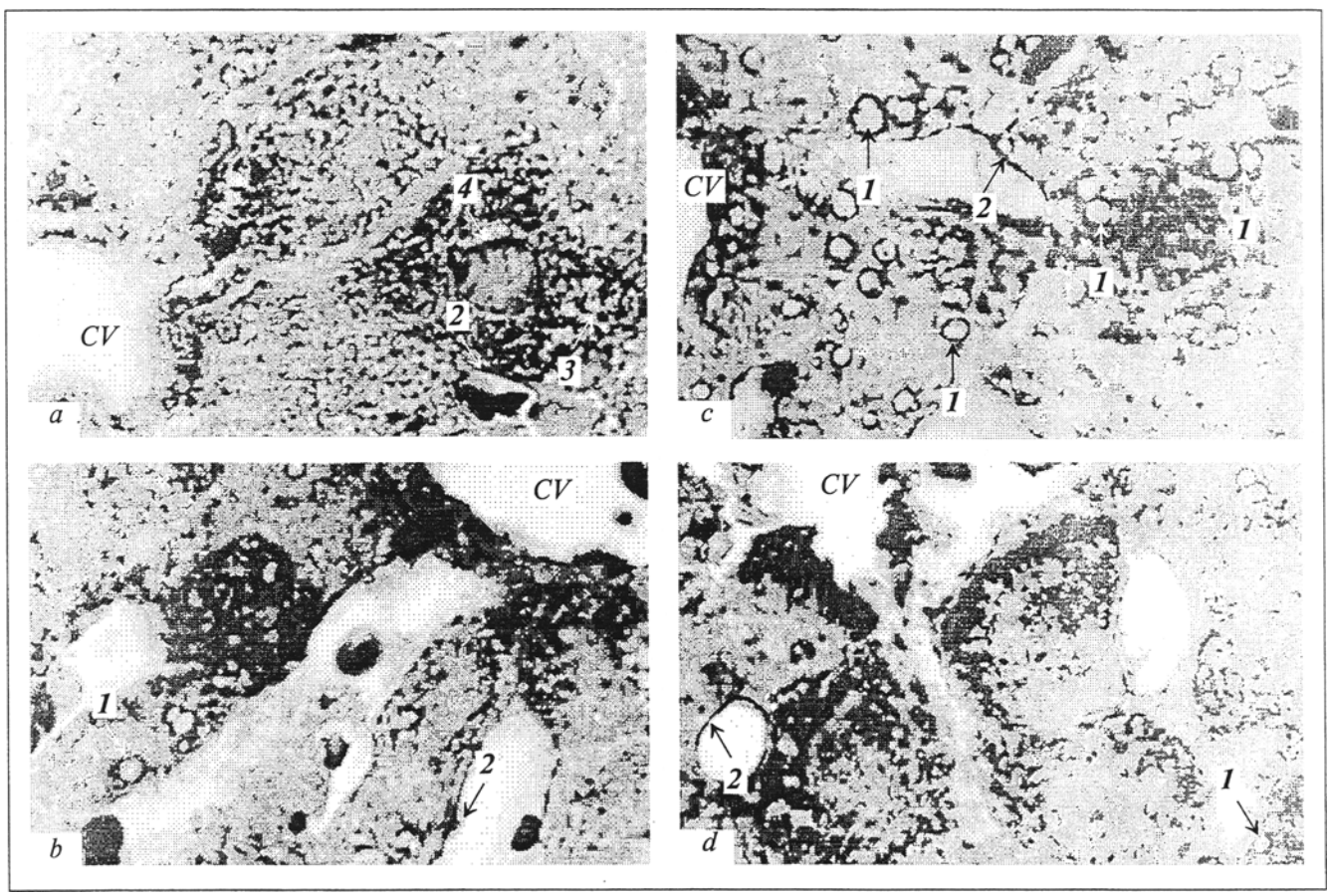

Fig. 1. Immunoperoxidase visualization of prolactin receptors in hepatocytes of castrated male rats after partial hepatectomy: control (*a*), 24 h (*b*), 35 h (*c*), and 12 days (*d*) after initiation of regeneration. Prolactin receptor-positive reaction in vacuolar membranes (*1*), sinusoidal domains of plasma membrane (*2*), cytoplasmic granules (*3*), and perinuclear space (*4*). ×787.5. *CV*: central vein of hepatic lobule.

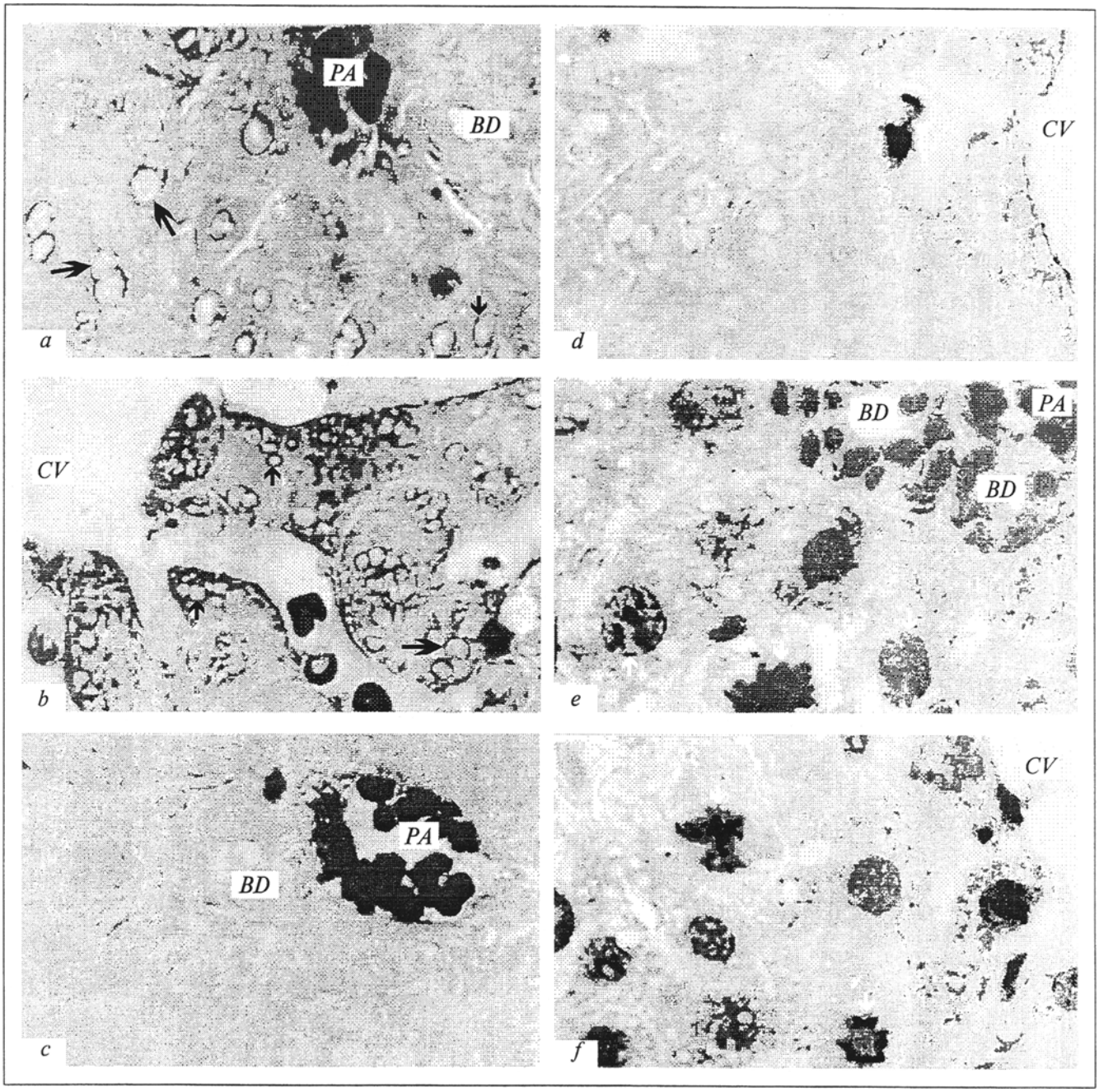

Fig. 2. Immunoperoxidase visualization of subcellular distribution of prolactin receptors in periportal (*a*) and pericentral (*b*) hepatocytes of castrated male rats 35 h after partial hepatectomy. Immunoperoxidase staining of control sections of periportal (*c*, *e*) and pericentral (*d*, *f*) zones of a hepatic lobule with (*c*, *d*) and without (*e*, *f*) hematoxylin poststaining. Prolactin receptor-positive reaction in vacuolar membranes is indicated by black arrows. Nuclei of mitotically active hepatocytes are indicated by white arrows. ×787.5. *BD*: bile duct; *PA*: portal artery; *CV*: central vein.

An equal PR expression in different zones of the lobule persisted throughout the regeneration period. Thirty-five hours after PHE, the periportal and pericentral zones of the lobule were characterized by intense expression of PR on vesicular membranes and PR-positive staining of the cytoplasm and sinusoidal domains of the plasma membrane (Fig. 2). As seen from Fig. 2, *e* and *f*, this stage of liver regeneration is characterized by a great number of mitoses in hepatocytes. However, there is no PR-positive staining in the perinuclear zone of hepatocytes and in the nuclei of bile duct cells throughout the observation period (Figs. 1 and 2).

The chosen time intervals reflect different stages of proliferative response of hepatocytes to PHE. At the initial stages (15 and 30 min after PHE), the liver is very sensitive to various regulatory factor stimulating cell division [8,9,15]. The enhanced expression of

PR in hepatocytes presumably reflects the involvement of PR into the initiation of regeneration. The late prereplicative period (24 h after PHE) is characterized by reduced synthesis of many tissue-specific proteins [9], however, the expression of PR in hepatocytes remains unchanged. This implies that prolactin participates in the regulation of hepatocyte proliferation.

It is well known that hepatocyte proliferation in response to PHE is accompanied by reduction of granular and extension of smooth endoplasmic reticulum, which results in dilation of cisternae and vacuolization of hepatocytes. Similar changes have been observed in hepatocytes after injection of somatotropic hormone and prolactin [1,12,14]. Moreover, early stages of regeneration are characterized by a considerable shift of liver metabolism toward enhanced utilization of carbohydrates and reduced catabolism of lipids. This leads to accumulation of lipid droplets adjacent to membranes of smooth endoplasmic reticulum and to the formation of autophagocytic lipid-containing vacuoles [1]. In our experiment, intense expression of PR in vesicular membranes coincided with marked steatosis of hepatocytes. The role of PR exposed on vesicular membranes remains unclear. It is strongly improbable that these PR are stored before being transported to the plasma membrane of newly formed hepatocytes. It cannot be excluded that PR-bearing membranes of smooth endoplasmic reticulum are additional targets for prolactin in proliferating cells. The possibility of intracellular traffic of prolactin and other peptide hormones to various intracellular targets is now intensively discussed [6,11]. It can be hypothesized that these processes underlie the prolactin-mediated regulation of detoxificating function and lipid and carbohydrate metabolism in the liver [4]. This promotes accumulation of energy substrates necessary for repair processes.

We previously observed a perinuclear localization of PR in pericentral hepatocytes of estrogenized rats. Since estrogens are proliferation inductors in hepatocytes, the perinuclear localization of PR can be assumed as a marker of mitotically active hepatocytes [3,13]. However, we did not observe enhanced PR-positive staining of the perinuclear space in different zones of the lobule. No enhanced expression of PR typical of pubertal animals [2] was detected in the nuclei of bile duct cells.

Thus, the enhanced expression of PR was observed only at the initial stages of regeneration, while changes in their intercellular distribution (intense expression of the vacuolar membranes) coincided with lipid infiltration and high mitotic activity of hepatocytes.

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