Nuclear Manifestation of Prolactin Receptors in Rat Hepatocytes and Effect of Prolactin

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Ligation of the common bile duct induced intense nuclear expression of prolactin receptors in hepatocytes of male and female rats. Bromocriptin abolished this effect in female, but not in male rats. In males with ectopic pituitary transplants, nuclear and cytoplasmic manifestations of prolactin receptors in hepatocytes decreased. It is concluded that prolactin exerted a sex-dependent effect on the content of prolactin receptors in hepatocyte nuclei in rats.

Key Words: *prolactin receptors; hepatocytes; common bile duct ligation; prolactin; immunohistochemistry, cytophotometry*

Common bile duct ligation (CBDL) in rats induced intense, primarily nuclear expression of prolactin receptors (PRL-R) in bile duct cells, which can be related to the maintenance of cholangiocyte proliferation in response to cholestasis [2].

The nuclear localization of PRL-R in liver cells suggests immediate effect of prolactin (PRL) on nuclear events [5,6]. Obstructive cholestasis affects proliferative and synthetic activities of hepatocytes [7,12]. PRL apart from other factors can regulate these parameters. In the present study we investigate induction of nuclear expression of PRL-R in rat hepatocytes after CBDL and the effect of PRL on this process. Blood concentration of PRL was reduced by administration of the PRL secretion inhibitor bromocriptine (BC) or elevated by transplantation of pituitary grafts beneath the renal capsule [10].

MATERIALS AND METHODS

Experiments were carried out on Wistar rats of both sexes weighing 180-200 g and random-bred male rats weighing 150-180 g. CBDL was reproduced as described previously [3].

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The animals received BC in a dose of 10 mg/kg (0.5 ml in 50% ethanol) or vehicle alone for 13 days starting from day 1 after surgery and sacrificed on day 14 after surgery.

Transplantation of 2 pituitary grafts from male donors beneath the renal capsule of male recipient was performed 2 days before CBDL. Control animals underwent sham operation followed by CBDL. The animals were sacrificed 10 days after CBDL.

Localization of PRL-R in the liver was assayed by immunoperoxidase method [11]. Mouse monoclonal antibodies U5 (kindly provided by P. A. Kelly, INSERM U-34, France) were used as primary antibodies (0.05-0.1 mg/ml IgG from ascitic fluid). Specificity of immunoperoxidase reaction was controlled by replacing primary antibodies on parallel slices with buffer solution.

Cytophotometry was carried out on a Lyumam I-3 microscope (×40, λ=520 nm, 12.5-μ probe). Optical density of hepatocyte nuclei and cytoplasm was measured in 120 points within each section. Two experimental and 2 control sections were analyzed for each animal.

The intensity of nuclear manifestation of PRL-R in hepatocytes varied, being sometimes close to (type I hepatocytes) or far above the cytoplasmic intensity (type II hepatocytes). Cytophotometry was carried out only for type II hepatocytes. Two parameters were

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used: percentage of PRL-R-positive hepatocytes and intensity of PRL-R-specific staining in hepatocytes taking regional peculiarities of this parameter into account. Additionally, the intensity of cytoplasmic expression of PRL-R in hepatocytes was measured in both animal group.

The significance of differences was verified using the Student *t* test.

RESULTS

Unlike clone U5 [1], clone U6 of anti-PRL-R antibodies used as primary antibodies revealed manifestation of PRL-R after CBDL in hepatocyte nuclei, plasma membrane, and cytoplasm. PRL-R-positive staining of hepatocyte nuclei was observed in all compartments of the hepatic lobule, but not in all cells (Fig. 1). In intact animals of both sexes expression of PRL-R was detected only in two compartments.

In all animals, except males with CBDL treated with BC, no significant changes were found in the zonal distribution of hepatocytes with intense nuclear manifestation of PRL-R. At the same time, the percentage of type II hepatocytes tended to increase from pericentral to periportal areas in all groups (Table 1).

Males with CBDL exhibit no zonal variation in the intensity of nuclear manifestation of PRL-R, while in females with CBDL the optical density of specifically-stained hepatocyte nuclei in the pericentral area was considerably decreased. BC markedly decreased the intensity of specific nuclear staining in the pericentral and intermediate areas, but not in the periportal area, and did not eliminate zonal differences of this parameter (Table 2).

The cytoplasmic content of PRL-R had no zonal differences [1]. BC reduced PRL-R-specific staining of hepatocyte cytoplasm in females, but had no effect on this parameter in males (Table 2).

In sham-operated males with CBDL, the percentage of type II hepatocytes in the pericentral area was lower than in the periportal and intermediate areas; we

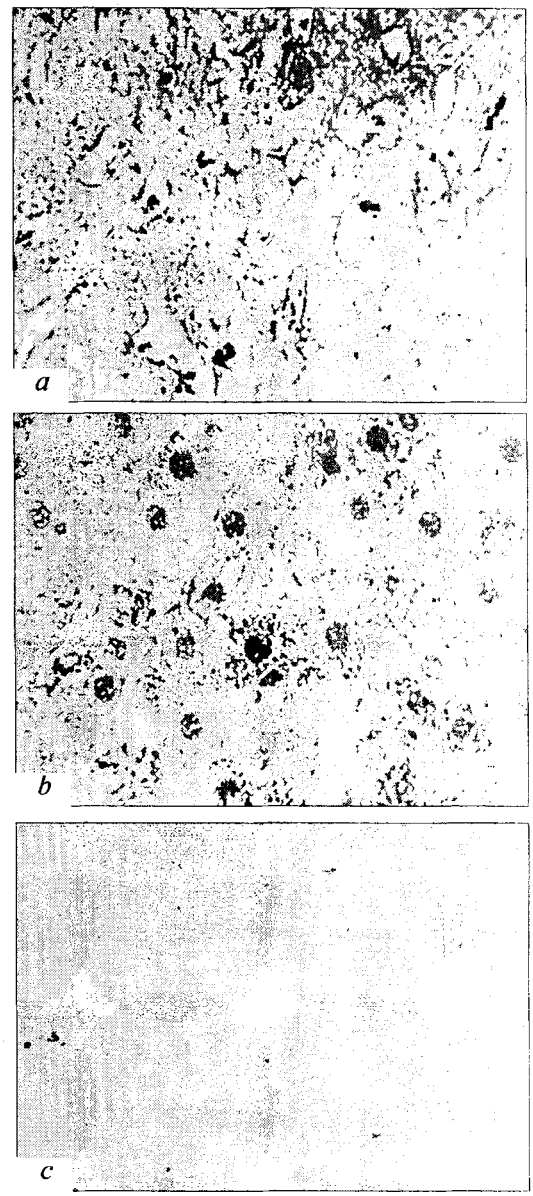

Fig. 1. Immunoperoxidase identification of prolactin receptors in hepatocytes of intact males (*a*) and males with ligation of the common bile duct (*b*, *c*), ×500. Treatment with U5 antibodies (*a*, *b*) or buffer (*c*).

TABLE 1. Effect of BC on Percent of Hepatocytes with Intense PRL-R-Positive Staining of Nuclei (Type II Hepatocyte) in Male and Female Rats 14 Days after CBDL (*M*±*m*)

Area	Percent of PRL-R-positive hepatocytes, %			
	females		males	
	without BC (*n*=4)	with BC (*n*=4)	without BC (*n*=5)	with BC (*n*=5)
Pericentral	59.8±6.98	35.5±7.23	61.2±8.91	58.4±6.31
Intermediate	65.5±2.90	63.5±5.36	66.8±4.92	63.0±1.67
Periportal	67.0±10.79	67.3±3.04	77.4±5.89	70.0±2.17*

Note. *$p<0.05$ compared with the intermediate area in the same group.

TABLE 2. Effect of BC on Intensity of PRL-R Expression in Hepatocyte Nuclei and Cytoplasm in Different Areas of Hepatic Lobule in Male and Female Rats 14 Days after CBDL (*M±m*)

Cell compartment	Area	Intensity PRL-R-positive staining, optical density units			
		females		males	
		without BC (*n*=4)	with BC (*n*=3)	without BC (*n*=4)	with BC (*n*=5)
Nucleus	Pericentral	0.212±0.007	0.157±0.002^{++}	0.257±0.018	0.251±0.026
	Intermediate	0.250±0.006**	0.207±0.015^{++}	0.282±0.029	0.295±0.018
	Periportal	0.250±0.007**	0.225±0.014**	0.292±0.024	0.287±0.018
Cytoplasm	Periportal	0.120±0.011	0.084±0.005^{+}	0.124±0.012	0.127±0.011

Note. $^{+}p<0.05$, $^{++}p<0.01$ compared with the corresponding area in females injected with vehicle. Here and in Table 3 and Table 4 *$p<0.01$ compared with the pericentral area in the same group.

observed a pericentral to periportal gradient of PRL-R-positive staining. Pituitary transplantation considerably reduced the percentage of type II hepatocytes in pericentral and periportal areas, but zonal distribution of this parameter was preserved (Table 3). Pituitary transplantation markedly reduced the expression of PRL-R in hepatocyte nuclei and cytoplasm in all regions of the hepatic lobule (Table 4).

TABLE 3. Effect of Pituitary Transplantation on Percent of Hepatocytes with Intense PRL-R-Positive Staining of Nuclei (Type II Hepatocyte) in Lobule Areas of Male Rats 10 Days after CBDL (*M±m*, *n*=4)

Area	Percent of PRL-R-positive hepatocytes (type II), %	
	CBDL+sham operation	CBDL+pituitary transplantation
Pericentral	53.5±2.6	23.5±9.9^{+}
Intermediate	66.8±3.1*	45.0±8.5
Periportal	74.5±4.4**	49.8±8.4^{+}

Note. $^{+}p<0.05$ compared with the corresponding area in sham-operated rats with CBDL.

TABLE 4. Effect of Pituitary Transplantation on Intensity of PRL-R Expression in Hepatocyte Nuclei and Cytoplasm in Different Areas of Hepatic Lobule in Male and Female Rats 10 Days after CBDL (*M±m*, *n*=4)

Cell compartment, area	Intensity PRL-R-positive staining, optical density units	
	CBDL+sham operation	CBDL+pituitary transplantation
Nucleus		
pericentral	0.213±0.011	0.151±0.013^{+}
intermediate	0.244±0.008*	0.158±0.016^{++}
periportal	0.233±0.007	0.176±0.020^{+}
Cytoplasm	0.107±0.004	0.067±0.005^{+++}

Note. $^{+}p<0.05$, $^{++}p<0.01$, $^{+++}p<0.001$ compared with the corresponding area in sham-operated animals with CBDL.

Thus, CBDL induced intense nuclear expression of PRL-R in rat hepatocytes with a tendency to a zonal gradient of the percentage of PRL-R-positive cells and the absolute intensity of PRL-R-specific staining towards the periportal area. This zonal distribution probably results from different functional activity of hepatocytes in different areas of the hepatic lobule in intact animals [4] and enhanced hepatocyte proliferation primarily in the periportal area under conditions of cholestasis [8].

Surprisingly, regulatory effect of PRL of expression of PRL-R in hepatocytes was different in male and female rats with CBDL (Table 2). It can be hypothesized that in males androgens abolish the positive effect of PRL on PRL-R expression in hepatocytes. Moreover, experiments with pituitary transplantation to male rats demonstrated that excessive production of PRL inhibits expression of PRL-R in hepatocytes of males with CBDL. Similar data were obtained on intact animals [9], hence CBDL does not disturb the direction of the regulation of PRL-R expression.

Contradictory data on nuclear manifestation of PRL-R in hepatocytes of animals with CBDL obtained in the present and previous studies [1] can result from differences in exposed epitopes of PRL-R in cell compartments.

Functional role of CBDL-induced manifestation of PRL-R in hepatocyte nuclei remains unclear. Expression of PRL-R in nuclei can be related to new regulatory functions of PRL realized thought nuclear PRL-R receptors, in particular, regulation of cell proliferation [5].

Thus, CBDL induced intense nuclear expression of PRL-R in rat hepatocytes, primarily in the periportal and intermediate areas, which is positively regulated by PRL in female rats. In male rats with CBDL, physiological concentration of PRL has no effect on PRL-R expression in hepatocyte nucleus and cytoplasm, while high concentrations of PRL inhibit it.

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