Effect of Bromocriptine on the Expression of Prolactin Receptors in Liver Cells after Common Bile Duct Ligation

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Immunoperoxidase method combined with cytofluorimetry showed that in contrast to hepatocytes, enhanced expression of prolactin receptors on rat cholangiocytes induced by common bile duct ligation cannot be suppressed by the prolactin secretion inhibitor bromocriptine.

Key Words: *prolactin receptors; rat liver cells; common bile duct ligation; bromocriptine; immunohistochemistry*

Expression of prolactin receptors (PRL-R) in hepatocytes is regulated by a multihormonal assembly, that includes prolactin (PRL), the major positive regulator of PRL-R, sex steroids, insulin, thyroid hormones, and glucocorticoids [2]. Hepatotropic effects of PRL, in particular, induction of ornithine decarboxylase [7], phosphoenolpyruvate carboxykinase [12], and protein kinase C [5], expression of the growth hormone genes [7], stimulation of DNA synthesis [6], and DNA hypomethylation [9] are studied in detail.

Unlike hepatocytes, cholangiocytes express little or no PRL-R in mature rats, but intensely express these receptors during active proliferation (in prepubertal animals [1]) or after common bile duct ligation (CBDL) [3]. The physiological role of increased sensitivity of cholangiocytes to PRL during the prepubertal period remains unclear. Another peculiarity is that the expression of PRL-R in cholangiocytes does not depend on sex steroids [3]. These data suggest different mechanisms of regulation of PRL-R expression in hepatocytes and cholangiocytes. To verify this assumption we studied the effect of PRL on PRL-R expression in rat cholangiocytes after CBDL, since this hormone is known to stimulate the expression of its own receptors in various organs and tissues [2]. Prolactin secretion was specifically inhibited with bromocriptine, a negative regulator of PRL secretion in the pituitary *in vivo* [8].

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MATERIALS AND METHODS

Experiments were carried out on random-bred albino rats of both sexes weighing 200-220 g. The common bile duct was ligated by a standard method described previously [4].

On days 5-13 postoperation, 5 males and 8 females were subcutaneously injected with 10 mg/kg bromocriptine dissolved in 50% ethanol (2 mg/0.5 ml/rat). Control animals (4 males and 8 females) received 0.5 ml 50% ethanol every days. The animals were decapitated on day 14 postoperation. The liver was fixed in 4% paraform for 18-20 h at 4°C and embedded in paraplast; PRL-R were visualized by indirect immunoperoxidase method [11]. To this end, 3-μm-thick sections pretreated with 10 mM sodium periodate and 0.01% sodium borohydrite were incubated with mouse monoclonal antibodies clone U6 (0.1 mg/ml IgG fraction from ascitic fluid, kindly provided by Dr. P. A. Kelly, France) in 0.05

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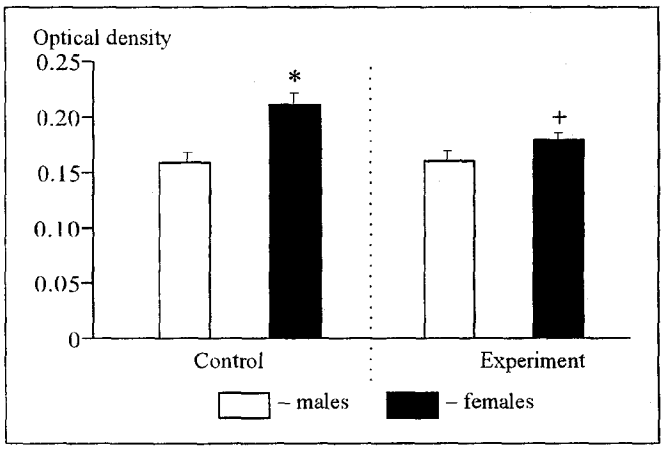

Fig. 1. Effect of bromocriptine on the expression of prolactin receptor in rat hepatocytes 14 days after the common bile duct ligation (cytophotometry). $p<0.05$: *compared with control male rats injected with vehicle, *compared with control female rats injected with vehicle.

M Tris-HCl buffer (pH 7.6) at 4°C for 18-20 h. Control sections were incubated with 0.05 M Tris-HCl buffer under the same conditions. Experimental and control sections were incubated with bridge (rabbit antimouse antiserum depleted with rat serum and diluted 1:200) and developing (peroxidase-labeled goat antirabbit antibodies, 1:200) antibodies (both from Sigma) for 30 min at room temperature; 3,3'-diaminobenzidine was used as the chromogen substrate.

Cytofluorimetry was performed in a Lyumam-I3 photometric microscope (×40) at l=520 nm using light probes with diameters 12.5 and 2.5 μm for hepatocytes and cholangiocytes, respectively. Optical density of hepatocytes and cholangiocytes was measured in 120 and 40 points per section; for each animal 2 sections were analyzed.

The data were processed statistically using Statistica 4.3 software. Significance of differences was verified using the Mann—Whitney test.

RESULTS

In operated animals injected with the vehicle, PRL-R-specific staining of hepatocytes revealed sex-related differences, being 1.3-fold more intense in females ($p<0.05$). Bromocriptine injected for 9 days reduced PRL-R expression in hepatocytes in females ($p<0.05$ compared with intact females) but not in males, abolishing these sex-related differences ($p>0.1$, Fig. 1). It should be noted that bromocriptine equally reduced the intensity of PRL-R-specific staining within the hepatic lobule (Table 1).

In both bromocriptin-treated and control males and females subjected to CBDL, PRL-R expression in proliferating cholangiocytes considerably surpassed that of hepatocytes (Fig. 2). Neither in females nor in males bromocriptin affected the PRL-R expression in cholangiocytes ($p<0.05$); no sex-related differences in the intensity of PRL-R positive staining in cholangiocytes were noted ($p<0.05$) (Figs. 2 and 3).

The observed suppressive effect of bromocriptine on PRL-R expression in hepatocytes of female rats

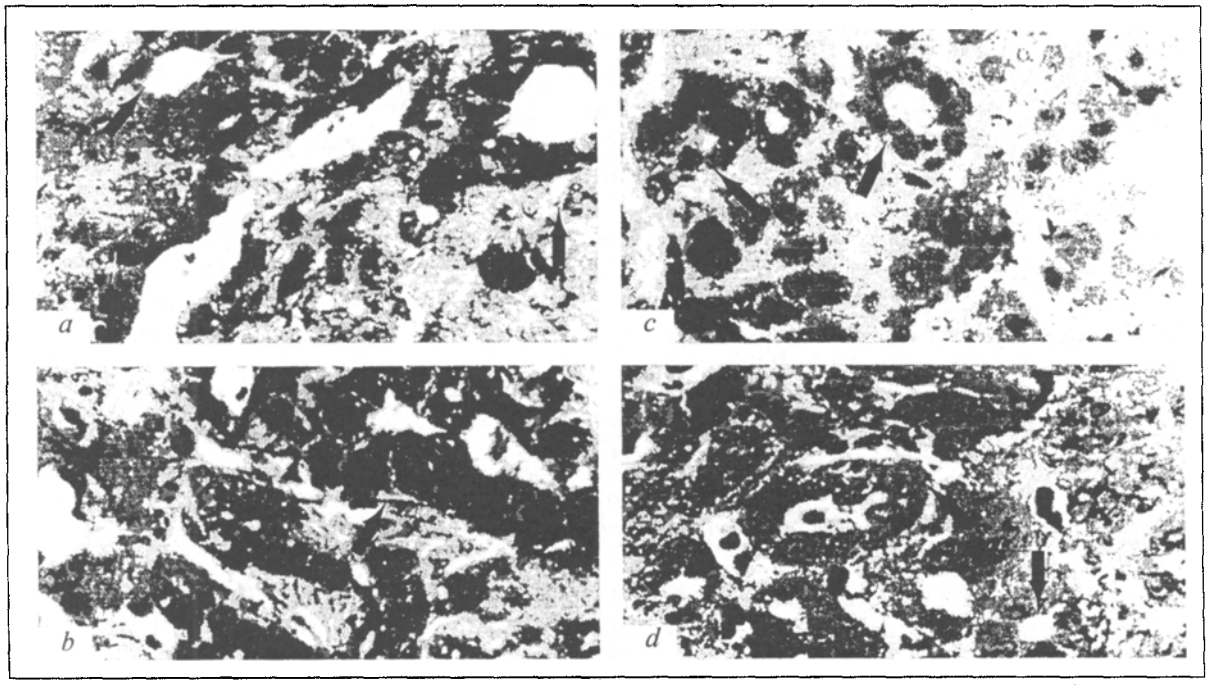

Fig. 2. Immuneperoxidase visualization of prolactin receptors in peroportal cells of hepatic lobules 14 days after ligation of the common bile duct in male (*a,c*) and female (*b,d*) rats. Injected with vehicles (*a,b*) and bromocriptine (*c,d*). Bile ducts are indicated by arrows. ×787.5.

TABLE 1. Effect of Bromocriptine of PRL-R Expression in Hepatocytes in Different Zones of Hepatic Lobule 14 Days after Ligation of the Common Bile Duct (*M±m*)

Inhibitor	Intensity of PRL-R-specific staining		
	central zone	intermediate zone	periportal zone
Vehicle	0.209±0.010	0.218±0.008	0.203±0.005
Bromocriptine	0.174±0.008*	0.183±0.006*	0.181±0.009*

Note. *$p<0.05$ compared with the corresponding hepatic lobule zones of rats receiving vehicles.

is consistent with published data on positive regulation of PRL-R in hepatocytes with PRL [2]. The fact that bromocriptine in our experiments had no effect of PRL-R-specific staining in hepatocytes of males can be attributed to its initially low expression in these cells [3,11]; under these conditions, the down-regulating effect of PRL depletion in males cannot be detected.

These findings in combination with previously reported data that PRL-R expression in proliferating cholangiocytes does not depend on sex hormones [3] suggest that PRL-R gene expression in proliferating cholangiocytes is controlled by a special promotor regulated by local CBDL-induced factors rather than by hormonal signals. Changes in the composition of secreted bile, elevated ductal pressure, inflammatory infiltration, and necrosis can serve as primary inductors for this promoter [10]. Increased sensitivity of cholangiocytes to PRL after CBDL is probably associated with the involvement of PRL in the maintenance of high proliferative potential of these cells. Previous studies demonstrated a mitogenic effect of PRL on hepatocytes [5,7]. Similarly to secretin, prolactin can play a role in stimulation of secretory activity of the ductal hepatic system [3].

Thus, we demonstrated that in rats subjected to CBDL bromocriptine, an inhibitor of PRL secretion, suppresses PRL-R expression in hepatocytes of females, and has no effect on the number of PRL-R in proliferating cholangiocytes in rats of both sexes.

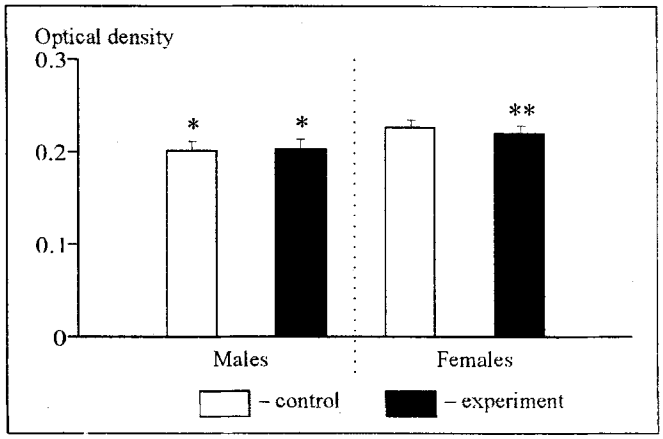

Fig. 3. Effect of bromocriptine on the expression of prolactin receptor in rat cholangiocytes 14 days after the common bile duct ligation (cytophotometry). *$p<0.05$, *$p<0.01$ compared with hepatocytes of the corresponding group.

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