Role of Prolactin in the Regulation of Some Liver Cell Functions after Ligation of Common Bile Duct

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In rats with ligated common bile duct, the inhibitor of prolactin secretion bromocriptine inhibited proliferation of bile ducts in males, reduced Na^+ concentration in bile in females, and elevated blood bilirubin. In males with pituitary transplants, proliferation of bile ducts increased. It was concluded that the effects of prolactin on cholangiocyte proliferation and bile ion content after ligation of the common bile duct are sex-dependent.

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

Some liver pathologies, in particular diseases associated with bile duct obstruction are accompanied by proliferation of ductal structures. In experimental animals this reaction can be induced by common bile duct ligation (CBDL) [9].

Cholangiocyte hyperplasia induced by CBDL is accompanied by intense expression of prolactin receptors (PRL-R) [2]. This study was aimed at investigation of the effect of prolactin (PRL) on liver cells under conditions of cholestasis induced by CBDL. 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. These grafts produced large amount of PRL due to the absence of the hypothalamic inhibitory control [8].

We studied the effects of PRL on bile duct proliferation and bile ion content. Functional activity of liver cells in CBDL and after administration of BC was assessed by serum bilirubin concentration.

MATERIALS AND METHODS

Experiments were carried out on outbred and Wistar rats of both sexes weighing 150-220 g. CBDL was reproduced as described previously [4].

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During 9-13 days after surgery the animals received BC in a dose of 10 mg/kg (0.5 ml in 50% ethanol. Control animals received equivalent volume of vehicle according to the same scheme. All animals were 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 on day 10 after CBDL.

Liver samples were fixed in 4% paraformaldehyde for 18-20 h at 4°C and embedded in paraplast; 3-μ sections were stained with hematoxylin and proliferation of bile ducts was evaluated microscopically by the area occupied by bile ducts in the total section area measured using a calibration grid.

The concentrations of Na^+ and K^+ were measured by flame photometry, the concentration of HCO_3^- was determined by titration with 0.1 N HCl after extraction of lipids with 2-fold volume of chloroform.

The concentration of bilirubin in the blood was determined using Bio-La-Test Bilirubin kits (Lachema).

Significance of differences was assessed using Mann—Whitney test or precise one-tailed Fisher test.

RESULTS

After CBDL, BC inhibited proliferation of bile ducts in males 1.6-fold and had no effect on this parameters in females (Fig. 1, *a*). Elevated blood concentration of

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TABLE 1. Effect of BC on Concentration of Inorganic Ions in Bile 14 Days after CBDL (*M±m*)

Concentration, mmol/liter	Females		Males	
	without BC (*n*=15)	with BC (*n*=14)	without BC (*n*=8)	with BC (*n*=7)
Na^+	148.75±4.11+	127.78±2.75*	122.80±5.43	134.40±4.14
K^+	4.80±0.23	5.01±0.23	4.35±0.18	4.66±0.11
HCO_3^-	33.32±1.63	29.49±1.30	33.11±1.76	32.51±0.91

Note. $p<0.01$: *compared with the control, +compared with males.

PRL produced by pituitary transplants in males stimulated proliferation of bile ducts 1.6-fold (Fig. 1, *b*).

Bile concentration of Na^+ in females after CBDL increased in comparison with males. BC eliminated differences in bile Na^+ content between males and females due to the absence of the response in males (Table 1). The concentration of K^+ and HCO_3^- were similar in males and females with CBDL, did not differ in control males and females, and remained unchanged after injection of BC. In males with pituitary grafts bile ion content did not differ from the control (sham-operated animals) (Table 2).

Animals with CBDL had high serum bilirubin concentration. Injection of BC induced further increase in this parameter most pronounced in females (83 and 57%, respectively). In females this rise was due to accumulation of both conjugated and unconjugated bilirubin (1.8- and 2-fold, respectively, in comparison with the control), whereas in males hyperbilirubinemia was due to accumulation of unconjugated bilirubin (3-fold), while the content of conjugated bilirubin was practically unchanged (Table 3).

These findings indicate that PRL stimulated proliferation of cholangiocytes in male rats. At the same time, BC and pituitary transplantation had no effect on the expression of PRL-R in cholangiocytes in animals with CBDL [1]. It can be hypothesized that the effect of BC is related to inhibition of PRL secretion in the pituitary, rather that to regulation of PRL-R expression in cholangiocytes. The observed sex-related diffe-rences in these effects are probably due to different rhythms of PRL secretion in the pituitary of males and females [3] and to the influence of other sex-related factors on cholangiocyte proliferation.

TABLE 2. Effect of Pituitary Transplantation on Concentration of Inorganic Ions in Male Bile after CBDL (*M±m*)

Concentration, mmol/liter	Transplantation (*n*=8)	Sham operation (*n*=5)
Na^+	131.24±5.59	130.50±3.53
K^+	5.26±2.75	4.58±1.40
HCO_3^-	30.65±1.41	28.99±2.88

The decrease in Na^+ concentration in males treated with BC attests to a stimulating effect of PRL on Na^+ biliary secretion. Previous data demonstrated the involvement of PRL into regulation of canalicular bile formation [7]. Theoretically, PRL can regulate secretion of acid-independent fraction on the bile by modulating both hepatocyte and cholangiocyte function. Although we did not identify the cells responding to RPL, the presence of PRL effect only in female rats suggests in favor of the formation of these differences at the hepatocyte level. These differences can be related to a higher expression of PRL-R in female hepatocytes [10] (whereas expression of PRL-R in cholangiocytes after CBDL was the same in males and females [1]) and to sex-related differences in hepatocyte enzymes responsible for bile production [5,6].

Increased concentration of total and direct bilirubin in BC-treated rats attests to impaired hepatocyte function in the absence of PRL in animals with CBDL and confirms the important role of RPL in the regulation of bilirubin absorption and metabolism by liver cells.

Thus, our experiments showed that expression of PRL in proliferating cholangiocytes is primarily associated with stimulation of their proliferative activity controlled by PRL, most pronounced in males. Moreover, in females PRL stimulated accumulation of Na^+

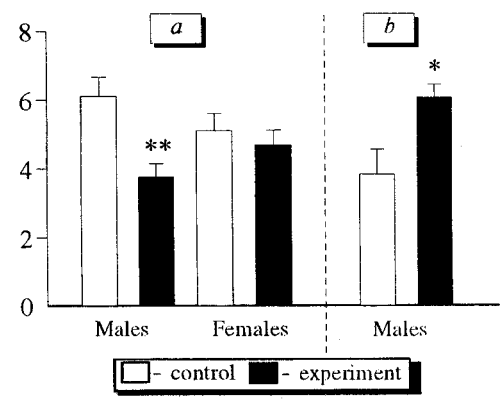

Fig. 1. Effect of bromocriptine (*a*) and pituitary transplantation (*b*) on bile duct proliferation in rats after ligation of the common bile duct. Ordinate: area occupied by bile ducts on liver sections, %. *$p<0.05$, **$p<0.01$ compared with the control.

TABLE 3. Effect of BC on Serum Bilirubin Concentration 14 Days after CBDL (*M*±*m*)

Bilirubin, μmol/liter	Females		Males	
	without BC (*n*=14)	with BC (*n*=11)	without BC (*n*=13)	with BC (*n*=14)
Total	69.54±11.54	130.47±22.88**	55.03±14.38	86.29±12.95*+
Unconjugated	21.71±4.69	43.44±6.46**	9.77±2.32++	30.38±4.59***+
Conjugated	47.83±9.71	87.04±17.64*	45.2612.75	55.91±8.88+++

Note. *$p<0.05$, **$p<0.01$, ***$p<0.001$ compared with the control; +$p<0.05$, ++$p<0.01$, +++$p<0.001$ compared with females.

in the bile and plays an essential role in bilirubin absorption and metabolism in animals after CBDL.

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