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Effect of Prolactin on Excretory Function of the Liver during the Induction and Relief of Cholestasis in Female Rats

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The effect of hyperprolactinemia on bile excretion from the liver of female rats was shown to increase significantly during obstructive cholestasis. The observed changes were biphasic: initial increase in bilirubin release of into the bile was followed by its reduction. Under conditions of obstructive cholestasis, hyperprolactinemia had a negative effect on the recovery of bile flow rate during decompression of the duct (up to complete cessation of bile flow).

Key Words: *prolactin; obstructive cholestasis; bilirubin; bile flow rate; rat*

Various diseases of the liver accompanied by obstructive cholestasis (OC) depend on sex and some of them, *e.g.*, cholelithiasis, are typical of females. These data provide the basis for a new field of research, female hepatology, intended for studying the positive and negative effects of female sex hormones on the liver [2,9].

Liver sensitivity to prolactin (one of the female sex hormones), evaluated by the number of prolactin receptors, is very high and comparable with the sensitivity of the mammary gland [3,11,13]. There are some data on molecular targets for prolactin in the liver [5,12]. However, little is known about the physiological action of prolactin on bile excretion and other functions of the liver.

Our previous studies showed that the expression of prolactin receptors in the bile duct increases in rats with OC. This state is characterized by significant sex differences in bile bilirubin concentration and bile flow rate during decompression [1,3]. It can be hypothesized that the effect of prolactin on excretory function of the liver becomes more significant during OC.

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To test this hypothesis, the excretory function of the liver in female rats with an abnormal prolactin level was studied during OC and initial period of decompression.

MATERIALS AND METHODS

Experiments were performed on adult outbred female rats (n=110) weighing 190-250 g. The animals were maintained in a vivarium under standard conditions and natural light/dark cycle and had free access to water and food. OC was induced by ligation of the common bile duct for 14 days. The initial post-cholestatic period (IPP) was considered to begin 3 h after decompression of the duct. Persistent hyperprolactinemia was induced by the standard method of pituitary gland transplantation from the donor to the renal subcapsular space in the female recipient [4,8]. This procedure was performed simultaneously or 2 weeks before bile duct ligation. Prolactin concentration in rats was measured by enzyme immunoassay with EIA-4493 kit (DRG). Pituitary gland transplantation was followed by an increase in serum prolactin concentration by 2-3 times (as compared to that in intact animals). Prolactin secretion inhibitor bromocriptine (Bromocriptine-Richter, 1

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tablet contains 2.5 mg bromocriptine) was administered orally through a tube (1 mg/ml physiological saline) for 14 days after bile duct ligation. The rats were divided into 4 experimental groups: group 1, normal prolactin level; group 2, 2-week hypoprolactinemia; group 3, 2-week hyperprolactinemia; and group 4, 4-week hyperprolactinemia. Each group included controls without ligation of the common bile duct (44, 3, 10, and 5 specimens, respectively), OC rats (16, 6, 12, and 10 specimens, respectively), and IPP rats (12, 3, and 4 specimens, respectively).

The bile was collected after 1-h drainage of the common bile duct in females of the non-OC group or specimens with OC (1 h before the induction of OC). The bile present in the dilated duct of OC animals was collected 2 weeks after OC induction. In post-decompression animals, the bile was collected on minutes 150-180 of duct drainage after the release of cholestatic bile (2-week OC).

Total bilirubin concentration in the samples, bile flow rate, and bilirubin excretion were studied as described elsewhere [1].

The results were analyzed by Statistica 6.0 software. The significance of differences was evaluated by nonparametric Mann–Whitney test. The differences were significant at $p<0.05$.

RESULTS

In females with normal liver function, the increase or decrease in prolactin concentration was not followed by variations in bilirubin concentration in the bile ($p>0.01$, Table 1). After 2-week hyperprolactinemia, bilirubin concentration in the bile in OC animals increased significantly ($p<0.05$) compared to control rats and insignificantly compared to OC rats, while bromocriptine caused an insignificant decrease in bilirubin concentration in the bile (Table 1). In light of this, a special scheme of the experiment was used: bilirubin concentration in the bile of each rat was

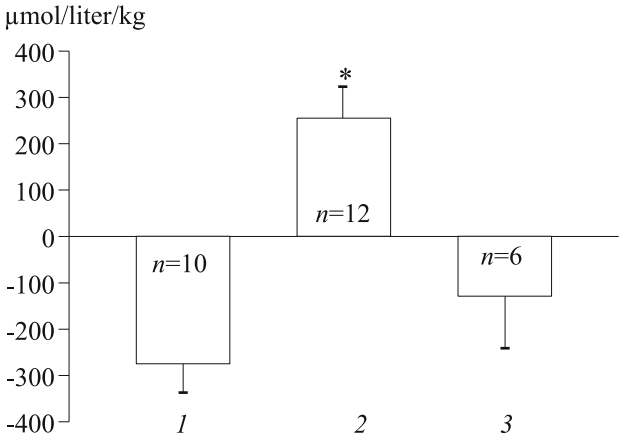

Fig. 1. Degree and directionality of changes in bilirubin concentration in the bile from OC females with normal (*1*), increased (*2*), and decreased levels of prolactin (*3*, *M*±*SEM*). The difference between bilirubin concentrations in cholestatic and normal bile was estimated individually in each animal. The measurements were performed before and 14 days after common bile duct ligation. *$p<0.001$ compared to the difference in females with normal prolactin level. Here and in Fig. 2: *n*, number of animals.

TABLE 1. Effect of Prolactin on Bilirubin Concentration in the Bile from Female Rats of Various Groups (*M*±*SEM*)

Group	Bilirubin concentration in the bile, μmol/liter/kg		
	untreated	hyperprolactinemia	hypoprolactinemia
Intact	470.6±26.8 (44)	505.9±32.7 (10)	314.3±42.1 (3)
OC	467.9±98.1 (16)	617.7±70.2 (12)+	236.1±88.5 (6)
IPP	2241.5±237.5 (12)	1861.2±477.2 (4)	1555.3±215.3 (3)

Note. Here and in Tables 2 and 3: number of animals is shown in brackets. +$p<0.05$ compared to intact females.

TABLE 2. Effect of Prolactin on Bilirubin Excretion Rate in Female Rats of Various Groups (*M*±*SEM*)

Group	Bilirubin excretion rate, nmol/kg/min		
	untreated	hyperprolactinemia	hypoprolactinemia
Intact	4.0±0.2 (43)	3.9±0.4 (10)	2.3±0.3 (3)+
IPP	30.5±3.5 (12)	21.4±5.8 (4)	19.7±1.9 (3)

measured before the induction and relief of cholestasis. The degree and directionality of variations in bilirubin concentration in the bile of cholestatic animals were evaluated from the difference in these parameters. In all females with normal prolactin level, bilirubin concentration in the bile decreased during OC (compared to baseline) and the difference in bilirubin concentrations was negative (Fig. 1). Against the background of 2-week hyperprolactinemia, the concentration of bilirubin in OC animals increased, the difference in bilirubin concentrations was positive and significantly differed from that observed in animals with normal prolactin level ($p<0.001$, Fig. 1). Administration of bromocriptine had little effect on this parameter (Fig. 1).

No intergroup differences were found in bilirubin concentration in the bile of IPP females ($p>0.1$). Individual variations in pigment concentration in the bile of OC animals and IPP specimens with various levels of prolactin were similar ($p>0.1$).

A significant decrease in bile flow rate was found during the initial period of decompression in rats with OC and 2-week hyperprolactinemia. However, bile flow rate did not change in animals with normal liver function (Fig. 2). Bromocriptine had little effect on bile flow rate in rats of various groups.

Induction of hypoprolactinemia reduced the rate of bilirubin excretion in bile of females with normal liver function. Bilirubin excretion rate in females with various concentrations of prolactin did not differ during the initial period of decompression (Table 2).

OC was accompanied by a 2-fold increase in the relative weight of the liver in females with various concentrations of prolactin. Three hours after decompression, the relative weight of the liver was reduced by 13 and 23% in females with normal prolactin level and 2-week hyperprolactinemia, respectively.

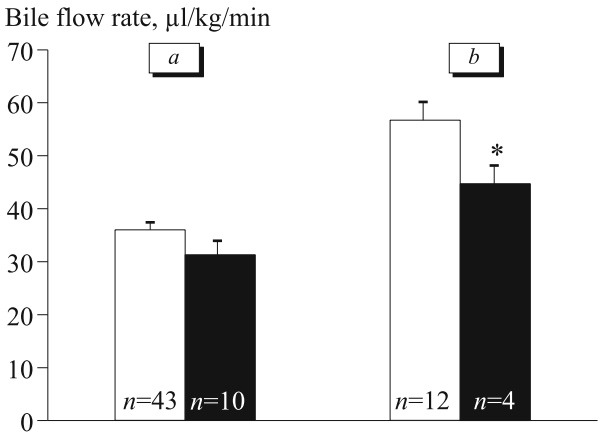

Fig. 2. Effect of prolactin on bile flow rate in female rats under normal conditions (*a*) and 3 h after the relief of cholestasis (*b*, *M±SEM*). Light bars, females with normal prolactin level; dark bars, females with hyperprolactinemia. *$p<0.05$ compared to females with normal prolactin level.

The increase in the duration of hyperprolactinemia to 4 weeks (common bile duct ligation after 2-week hyperprolactinemia) had a surprising effect. In hyperprolactinemic animals without ligation of the bile duct, bile excretion from the liver remained practically unchanged. In animals with induced OC, bilirubin was not detected in the bile under these conditions or its concentration decreased significantly, which led to the appearance of "white bile"; dilated bile duct (above the site of ligation) contained a considerable amount of caseous masses and mucus. Bile flow did not recover (Table 3).

Our results indicate that the effect of hyperprolactinemia on bile excretion from the liver increases significantly in female rats with OC. The observed changes are biphasic in nature and depend on the duration of hyperprolactinemia: initial increase in bilirubin excretion with the bile was followed by its decrease.

TABLE 3. Effect of Prolactin on Bile Excretion in the Liver of Female Rats of Various Groups (*M±SEM*)

Group	Bilirubin concentration in the bile, µmol/liter/kg	Bile flow rate, µl/kg/min	Bilirubin excretion rate, nmol/kg/min
Intact	470.6±26.8 (44)	36.0±1.4 (43)	4.0±0.2 (43)
Intact+4-week hyperprolactinemia	577.0±68.0 (5)	30.6±2.6 (5)	3.5±0.5 (5)
OC	467.9±98.1 (16)	–	–
OC+4-week hyperprolactinemia	68.3±26.2 (10)*	–	–
IPP	2241.5±237.5 (12)	56.7±3.7 (12)	30.5±3.5 (12)
IPP+4-week hyperprolactinemia	No recovery of bile flow		

Note. *$p<0.001$ compared to females with OC.

In animals with OC, hyperprolactinemia has a negative effect on the recovery of bile flow rate during decompression of the duct (up to complete cessation of bile flow).

The increase in bilirubin excretion with the bile under conditions of short-term hyperprolactinemia (despite obstruction of the common bile duct) is probably determined by the effect of prolactin on the expression and/or subcellular distribution of multidrug resistance protein 2 (MRP2), one of the major transporter proteins, which plays a role in the transport of bilirubin and other endotoxins and exotoxins in bile [6]. Previous studies showed that the expression of this protein is higher in the liver of female rats. It is positively regulated by pituitary hormones of the family of somatotropic hormones [10]. These data suggest that prolactin is a female sex hormone of the pituitary gland, which regulates MRP2 concentration. The decrease in MRP2 expression in hepatocytes during OC [6] is probably less pronounced in specimens with high concentration of prolactin. It cannot be excluded that under conditions of OC prolactin inhibits the expression of multidrug resistance protein 3 (MRP3) mediating bilirubin transport from the liver to the blood [6].

With increasing the duration of hyperprolactinemia, the effects of prolactin on other factors regulating bile flow are observed (*e.g.*, mucus secretion by the epithelium of bile ducts). This process results in mechanical obstruction of bile flow. The appearance of "white bile" can be related to both increased degradation of bilirubin with the bile and changes in the directionality of bilirubin transport [7].

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