Prolactine Receptor Expression in Kidney Tissue of Female Rats with Cholestasis: the Effect of Hyperprolactinemia

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Immunohistochemistry with semiquantitative image analysis showed that prolactin receptor in distal renal tubules of female rats is most sensitive to the negative effects of both cholestasis and hyperprolactinemia. The responses of medullary tubules to cholestasis and hyperprolactinemia were less pronounced: decrease and increase in prolactin receptor expression, respectively. Proximal tubules were characterized by stable levels of prolactin receptor expression insensitive to the effects of obstructive cholestasis and hyperprolactinemia. The cholestasis-induced changes in the intensity of prolactin receptor expression were opposite in kidney and liver cells. It is concluded that different parts of the nephron differ by the presence, type, and direction of regulation of prolactin receptor expression in obstructive cholestasis and hyperprolactinemia.

Key Words: *prolactin receptor; hyperprolactinemia; cholestasis; kidney; rat*

Prolactin is a hormone involved in not only regulation of reproduction, but also homeostasis maintenance. It regulates water and salt balance in the kidneys, bladder, intestine, skin, sweat glands, mammary glands, and amniotic fluid of different species. Prolactin regulates secretory activity of ductal structures, including renal tubules and bile ducts of mammals. Osmoregulatory effect on the kidneys was reported in males and females, especially during pregnancy [1,5,8,14]. In some animal species and in humans, prolactin receptor was detected in kidney cells [8,9,11]. Kidney function is substantially changed with cholestasis, which is necessary for elimination of bile components that are normally excreted by the liver [3].

Diseases accompanied by cholestasis are sex-dependent and are more common in females. In this case, pregnancy characterized by elevated levels of prolactin contributes to increased incidence of cholestasis. Cholestasis in pregnant women markedly increases prolactin levels in comparison with normal pregnancy [7,10].

Here we studied whether cholestasis affects renal sensitivity to prolactin evaluated by tissue expression of its receptor in various structures of the kidney in female rats, and whether prolactin can affect it. The liver was used as the reference tissue, changes in prolactin receptor expression in this organ are described previously [2,4].

MATERIALS AND METHODS

We used albino outbred female rats weighing 190-250 g. The animals were kept under standard vivarium conditions at natural illumination regime and free access to water and food. Obstructive cholestasis was modeled by ligation of the common bile duct. The animals were taken in the experiment 14 days after surgery. Persistent hyperprolactinemia was simulated by the classical method of pituitary transplantation under the kidney capsule of a sex-matched recipient [12] simultaneously with bile duct ligation (herein-

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after called hyperprolactinemia) or two weeks before ligation (hereinafter called long-term hyperprolactinemia). Prolactin was measured by ELISA using EIA-4493 kit (DRG). Measurements showed that obstructive cholestasis is accompanied by significant ($p<0.05$) hyperprolactinemia, and pituitary transplantation causes an additional significant ($p<0.05$) 2-3-fold increase in serum prolactin concentration in comparison with the corresponding group without pituitary transplantation.

Localization of prolactin receptor (PRLR) in the renal tissue was determined by indirect immunoperoxidase method using monoclonal antibodies against rat PRLR (clone 6D696, USBio). In each tissue sample, 2 experimental (in the presence of antibodies against PRLR) and 2 control (without antibodies against PRLR) sections were examined. Immunopositive staining for PRLR was analyzed in the proximal and distal tubules of the cortex and in renal medullary tubules as well as in hepatocytes and cholangiocytes of the liver. PRLR expression was measured by semiquantitative computer image analysis of the above structures (50 cells or fragments of tubules of each type for each animal) using ImageJ 1.40 software after conversion of RAW files into TIFF. The intensity of PRLR expression was evaluated by the difference between color intensities in the experimental and control sections in the blue spectrum, which were proportional to the relative concentration of the labeled compounds with values ranged from 0 to 65 536 [13].

Statistical analysis of the data was carried out using Satistica 6.0 software (Statsoft Inc.). Pairwise group differences were detected. The significance of differences was evaluated using non-parametric Mann–Whitney test. Differences were considered significant, if the significance level did not exceed 0.05.

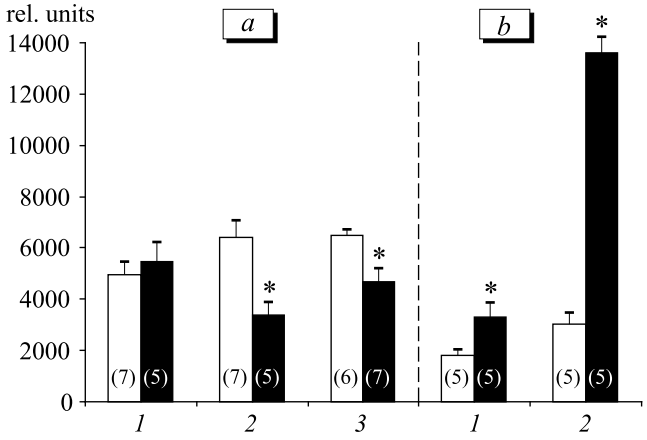

Fig. 1. Effect of obstructive cholestasis on the extent of PRLR expression in proximal tubules (*1*), distal tubules (*2*), and medullary tubules (*3*) of the kidney (*a*); in hepatocytes (*1*) and cholangiocytes (*2*) in the liver (*b*) of female rats. Open bars, normal; dark bars, obstructive cholestasis. *$p<0.05$: in comparison with normal. The number of animals is shown in parentheses.

RESULTS

High intensity of PRLR expression significantly exceeding that in liver cells of different types ($p<0.05$; Fig. 1) was revealed in all renal structures of intact female rats. PRLR expression in the proximal tubules was slightly lower than in the distal and medullary tubules ($p<0.05$). Specific immunostaining was mainly localized in the cytoplasm and plasma membranes of tubular cells, though nuclear immunoreactivity was observed in the medullary tubules (Fig. 2). This is

TABLE 1. Effect of Hyperprolactinemia on PRLR Expression in Different Renal Structures under Normal Conditions and in Obstructive Cholestasis in Female Rats (*M*±*SEM*)

Experimental condition	PRLR expression, rel. units		
	without hyperprolactinemia	hyperprolactinemia	long-term hyperprolactinemia
Proximal tubules			
Normal	4945±510 (n=6)	5548±1051 (*n*=3)	5432±1090 (*n*=3)
Obstructive cholestasis	5452±768 (*n*=5)	4320±408 (*n*=4)	5586±881 (*n*=5)
Distal tubules			
Normal	6397±670 (*n*=6)	3325±805* (*n*=3)	6182±553 (*n*=3)
Obstructive cholestasis	3376±510^{+} (*n*=5)	3586±722 (*n*=4)	3977±702 (*n*=5)
Medullary tubules			
Normal	6483±230 (*n*=5)	5003±545 (*n*=3)	8930±568* (*n*=3)
Obstructive cholestasis	4689±526^{+} (*n*=5)	5120±784 (*n*=4)	7621±124* (*n*=3)

Note. $p<0.05$ in comparison with: *corresponding group without hyperprolactinemia; $^{+}$corresponding normal group.

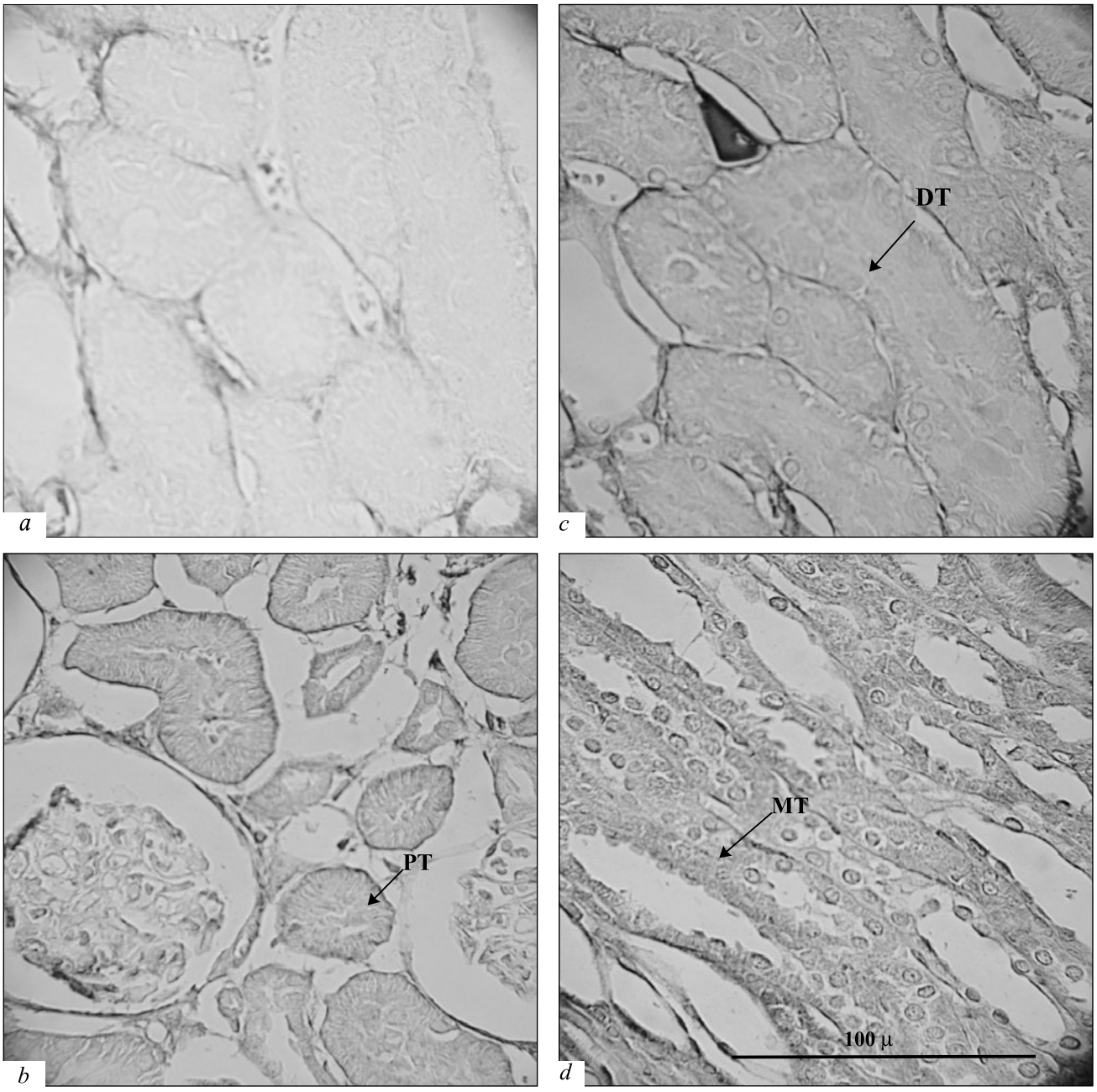

Fig. 2. Immunoperoxidase identification of PRLR in various renal structures of intact female rat. Control without first antibody (*a*); staining with monoclonal antibodies against rat PRLR (*b*, *c*, *d*). Arrows show proximal tubules (PT), distal tubules (DT), and medullary tubules (MT).

consistent with high levels of PRLR mRNA in human kidney inferior only to these in the uterus and mammary gland [9].

Obstructive cholestasis markedly (2-fold) decreased PRLR expression in distal tubules ($p<0.05$) and less markedly (1.3-fold) in the medullary tubules ($p<0.05$), but had no effect on the expression of this protein in the proximal tubules (Fig. 1). In contrast, the liver showed enhanced (4.5-fold) PRLR expression in cholangiocytes and moderate (1.8-fold) in hepatocytes ($p<0.05$; Fig. 1) under these conditions. Such reciprocal changes during cholestasis are known for some other liver and kidney proteins, in particular, those involved in regulation of the excretion of metabolic wastes [15,6]. The subcellular localization of immunopositive staining did not vary significantly in different parts of the kidney during obstructive cholestasis.

Hyperprolactinemia most heavily and negatively influenced the extent of PRLR expression in renal distal tubules and liver cholangiocytes. Such an effect was observed only under high initial level of receptor expres-

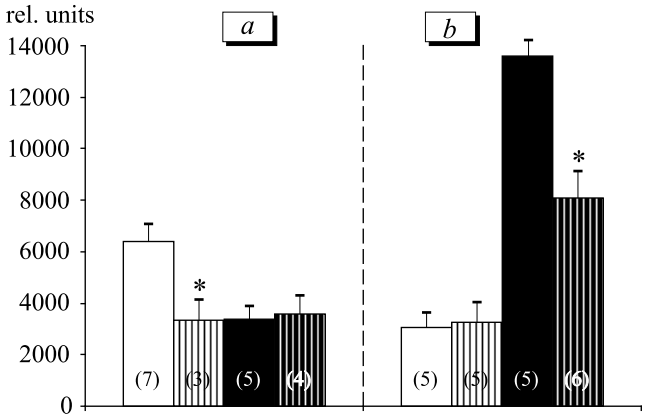

Fig. 3. Effect of hyperprolactinemia on PRLR expression in renal distal tubules (*a*) and cholangiocytes in the liver (*b*) of normal female rats (light bars) and rats with obstructive cholestasis (dark bars). Shaded bars: corresponding group against the background of two-week hyperprolactinemia. *$p<0.05$ in comparison with the corresponding group without hyperprolactinemia. The number of animals is shown in parentheses.

sion, *i.e.* in normal distal tubules and cholangiocytes during obstructive cholestasis (Fig. 3). On the contrary, medullary tubules showed increased intensity of PRLR expression during long-tern hyperprolactinemia, both under normal conditions and in obstructive cholestasis. Neither in normal, nor in cholestasis hyperprolactinemia of different duration significantly affected the level of PRLR in renal proximal tubules, (Table 1).

Thus, we have demonstrated differential levels of PRLR expression in different parts of the nephron and presented evidence that the possibility, type, and direction of regulation of PRLR expression during cholestasis and hyperprolactinemia varied in different parts of the nephron. Proximal tubules showed stable levels of PRLR insensitive to the impact of both obstructive cholestasis and hyperprolactinemia. Distal tubules are most susceptible to the impact of both cholestasis and hyperprolactinemia (both produce a negative effects on PRLR expression). Obstructive cholestasis and hyperprolactinemia had less pronounced opposite effect on the medullary tubules decreasing and increasing expression of prolactin receptor, respectively. Of particular importance is the fact that PRLR expression in cells of the kidney and liver showed opposite reaction to cholestasis. This may indicate significantly altered mechanisms of prolactin action under cholestasis. We revealed high, differentially regulated level of PRLR expression in non-reproductive organs, liver and kidney [1,2,4] that provides insight into less researched effects of prolactin on homeostasis under normal and pathological conditions.

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