ISSN 0006-2979, Biochemistry (Moscow), 2006, Vol. 71, No. 2, pp. 178-184. © Pleiades Publishing, Inc., 2006.
Published in Russian in Biokhimiya, 2006, Vol. 71, No. 2, pp. 229-236.

Prolactin Receptors in Rat Cholangiocytes: Regulation of Level and Isoform Ratio Is Sex Independent

R. L. Bogorad[1]*, T. Y. Ostroukhova[1], A. N. Orlova[1], P. M. Rubtsov[2], and O. V. Smirnova[1]

[1]Faculty of Biology, Lomonosov Moscow State University, 119992 Moscow, Russia; fax: (7-495) 939-4309; E-mail: rbogorad@yahoo.com
[2]Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, ul. Vavilova 32, 119991 Moscow, Russia; fax: (7-495) 135-1405

Received February 8, 2005
Revision received April 27, 2005

Abstract—The presence of prolactin receptor and peculiarities of its isoform expression in bile duct cells (cholangiocytes) differentially isolated from rat liver under different conditions were investigated in the present study. Normal cholangiocytes express prolactin receptor at relatively low level comparable to those of some prolactin-dependent tissues. Long receptor isoform is predominant in cholangiocytes but not in hepatocytes. The prolactin receptor level increases significantly under obstructive cholestasis due to evaluation of long and appearance of short isoforms. In rat cholangiocytes, unlike other tissues, the main positive regulators of prolactin receptor expression are cholestasis-induced factors instead of sex hormone and prolactin levels. Long isoform is predominant and induced primarily by cholestasis-induced factors.

DOI: 10.1134/S0006297906020106

Key words: cholestasis, bile duct ligation, RT-PCR, cholangiocyte, prolactin receptor, receptor isoforms

Prolactin is one of the most multifunctional hormones. Several prolactin receptor (PrlR) isoforms have been identified in mammals, and these are shown to be the products of the PrlR pre-mRNA alternative splicing. These PrlR isoforms differ in the length and composition of their intracellular domain [1, 2]. Receptor isoforms (including those of PrlR) possessing different cytoplasmic domains are often characterized by different activity [2]. The sensitivity of a tissue to prolactin and the mode of prolactin-induced response are dependent on both the level of PrlR and the ratio of short and long isoforms of the receptor [3-6].

Liver as a whole organ is a usual object for investigation of the mode of action of PrlR. The interest is based on the high level of PrlR expression as well as on the peculiarities of receptor isoform distribution; liver is one of few tissues where the short isoform of PrlR is predominant [3, 7]. Liver has been the object for many investigations of PrlR expression peculiarities—dependence on sex hormones and prolactin itself [3, 8, 9]. Both the liver level of the PrlR and its isoform ratio are in general distinctive features of hepatocytes, since hepatocytes are the main cellular element of liver [3, 10].

Bile duct cells play a significant role in liver functioning both under normal conditions and under developing pathology. Their role is especially high in bile formation. Prolactin can be suggested to act on cholangiocytes since it takes part in the essential regulation of different biochemical processes, including maintaining of water–salt balance and secretion, in many types of ductal structures [1].

It has been earlier revealed in our laboratory that PrlR is expressed in proliferating cholangiocytes under both normal and pathological conditions [11, 12]. This work has been performed to study the influence of sex hormones, prolactin, and obstructive cholestasis on the level of PrlR and peculiarities of its expression in rat cholangiocytes.

Abbreviations: CBDL) common bile duct ligation; IHC) immunohistochemical staining; PrlR) prolactin receptor; RT-PCR) polymerase chain reaction on the product of reverse transcription.

* To whom correspondence should be addressed.

MATERIALS AND METHODS

Female and male albino mongrel rats (200-250 g) were used in the present work: the experimental groups

Table 1. Experimental groups of rats

Animal sex and virility	Experimental group	Type of analysis
Male and female, mature	intact	IHC, RT-PCR
Male and female, immature*	intact	IHC
Male and female, mature	14 days CBDL/sham-operated	IHC, RT-PCR
Male and female, mature	gonadectomy/ sham-operated	IHC, RT-PCR
Male and female, mature	gonadectomy + 14 days CBDL	IHC, RT-PCR
Male and female, mature	14 days CBDL + daily injections of bromocriptine**/14 days CBDL + daily injections of vehicle	IHC
Male, mature	pituitary transplantation + 14 days CBDL/sham-transplantation + 14 days CBDL	IHC
Male and female, mature	pituitary donors	—

Note: IHC, immunohistochemical staining; RT-PCR, polymerase chain reaction on the product of reverse transcription; CBDL, common bile duct ligation.
* Immature rats, animals at 45th day after birth.
** Dose of bromocriptine injections (see "Materials and Methods").

are represented in Table 1. All basic chemicals for cell isolation and tissue treatment were from Sigma (USA).

Common bile duct ligation (CBDL), gonadectomy, and sham operations were performed as described earlier [12, 13]. Animals were used for PrlR detection or for further manipulations 14 and 30 days after surgery, respectively.

In some experiments, two donor pituitaries were transplanted into the kidney capsule of each recipient animal for elevation of circulating prolactin level. In two days recipient animals underwent CBDL.

The preliminary investigations (by RT-PCR) showed that sham-operated female and male animals do not differ from correspondent intact group of rats in either PrlR mRNA level or in the ratio of PrlR isoforms in cholangiocytes (one-way ANOVA test, $p > 0.6$).

Bromocriptine was injected daily after the CBDL as 50% ethanol suspension of bromocriptine (Gedeon Richter, Hungary) (10 μg per kg of animal weight) to decline the level of circulating prolactin. The control animals were injected with the vehicle.

The cholangiocytes were isolated as intrahepatic bile duct units [14] for polymerase chain reaction analysis. Briefly, the liver was perfused through the portal vein with Ca^{2+}/Mg^{2+}-free Hanks' balanced salt solution containing 0.7 mM EDTA and then perfused with collagenase buffer containing 0.05% collagenase, 40 mM HEPES, and 5 mM $CaCl_2$ (pH 7.2-7.4). The portal tissue was mechanically dissociated from hepatocytes, minced, and then incubated at 37°C with solution A (α-MEM containing 0.05% collagenase, 0.006% DNase, and 0.033% pronase). The solution A was replaced by solution B (α-MEM containing the same enzymes excluding pronase, which was replaced by 0.036% hyaluronidase). The resulting suspension was subsequently filtrated through 100 and 30 μm nylon net filters (Millipore, USA). Bile duct units retained on the 30 μm net were monitored by light microscopy to control for their purity and isolation quality; bile duct fragments appeared as tubule-like structures (Fig. 1). Cholangiocyte phenotype was additionally confirmed by RT-PCR for revealing the cDNA of cytokeratin 19 (an epithelial cell marker), and hepatocyte contamination was monitored by RT-PCR with primers specific for cDNA of glucose-6-phosphatase (hepatocyte marker) [15] (Fig. 2). The portion of viable cells was >95% as assessed by trypan blue exclusion immediately after bile duct isolation.

Total RNA was isolated from bile duct fragments using phenol–chloroform extraction [16]. The degree of RNA integrity and its purity were verified by agarose gel electrophoresis (the ratio of 18S and 28S rRNA) and spectrophotometry.

cDNA was synthesized using a SuperscriptIIRT kit (Invitrogen, USA) after denaturing and annealing 465 ng of total RNA with 1.5 μg $(dT)_{15}$ (Syntol, Russia). The reaction was performed according the manufacturer's manual.

Polymerase chain reaction (PCR) was performed in 25 μl of incubation mixture containing 1× buffer for

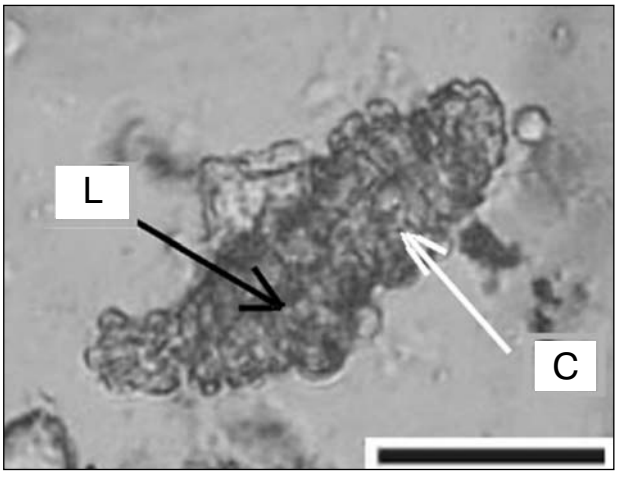

Fig. 1. Isolated bile duct units, non-fixed and non-stained. L, lumen; C, cholangiocyte; bar, 25 μm.

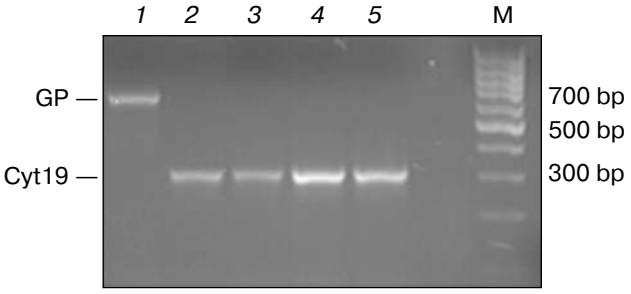

Fig. 2. PCR monitoring of rat cholangiocyte purity. Lanes: *1*) hepatocytes isolated from liver of mature female rat; *2-5*) five cholangiocytes isolated from liver of intact mature female rat (*2*), intact mature male rat (*3*), mature female rat with common bile duct ligation (CBDL) (*4*), mature male rat with CBDL (*5*). M, DNA length markers (numbers represent length of fragment in bp). Amplification products: GP (glucose-6-phosphatase) and Cyt19 (cytokeratin 19).

Taq DNA polymerase, 100 μM dNTP (Fermentas, Lithuania), 0.5 unit Taq polymerase (Dialat, Russia), and 2 μl of RT reaction aliquot. Probes contained 20 pmol of each required oligonucleotides (Syntol, Russia) (Table 2). PCR was run usually for 33 cycles using the following regimen: 94°C, 30 sec; 60°C, 30 sec; 72°C, 45 sec. We performed PCR for revealing of cDNA fragments of cytokeratin 19 and glucose-6-phosphatase simultaneously to control for hepatocyte contamination. The PCR products were visualized by agarose-gel electrophoresis.

We developed conditions for semiquantitative PCR assay for PrlR level earlier [3, 17]. PCR specificity was verified by hybridization of amplified fragments separated by agarose gel electrophoresis and transferred onto Hybond-N with γ-^{32}P-labeled primer Rc and also by sequencing of cloned amplification products of PrlR isoform cDNA into pBluescriptIIKS plasmid.

We then performed semiquantitative PCR assay for PrlR under the following conditions: each probe contained primers Baf, Bar, Rf, and Rc (to estimate the total PrlR mRNA level), or Baf, Bar, Rf, Rs, and Rl (to estimate the ratio of PrlR isoforms); β-actin mRNA served as an internal standard in both cases. Rf and Baf labeled with [γ-^{32}P]ATP using polynucleotide kinase was added into each probe at the amount of 1 pmol. Preliminary results demonstrated the exponential phase from the 25th to 33rd cycles of amplification of β-actin and both PrlR isoforms [17]. For competitive kinetic analysis of product accumulation, PCR was run in duplicate, and the PCR products were separated by polyacrylamide gel electrophoresis. The results were quantified by measurement of product radioactivity. The region of reliable PCR product detection and linear increment logarithm of incorporated isotope radioactivity was from the 29th to 33rd cycle of amplification (Fig. 3). The PrlR quantity was expressed as arbitrary units (ratio of ^{32}P isotope radioactivity incorporated into PCR products in reactions for cDNA of PrlR and β-actin).

Tissues were embedded into paraplast and sliced into 3 μm sections. Immunohistochemical staining of sections

Table 2. Oligonucleotide primers used in PCR analysis

Oligonucleotide	Gene name	Nucleotide sequence	GeneBank Ac. number
Rf	prolactin receptor	f 5'-gtagatggagccaggagagttc-3'	M57668
Rc		r 5'-ggtggaaagatgcaggtcatcat-3'	
Rf	prolactin receptor	f 5'-gtagatggagccaggagagttc-3'	M57668
Rl	long isoform	r 5'-accagagtcactgtcgggatct-3'	
Rf	prolactin receptor	f 5'-gtagatggagccaggagagttc-3'	M19304
Rs	short isoform	r 5'-tgagtctgcagcttcagtagtca-3'	
Baf	β-actin	f 5'-atctggcaccacaccttctacaatgagc-3'	NM031144
Bar		r 5'-actcctgcttgctgatccacatctgc-3'	
Cytf	cytokeratin 19	f 5'-tgcgcgacaacatccttggc-3'	X81449
Cytr		r 5'-gtggaatccacctccacact-3'	
GPf	glucose-6-phosphatase	f 5'-caagagactgtgggcatcaatc-3'	NM_0130978
GPr		r 5'-gctggcaaagggtgtagtgt-3'	

Note: f, forward primer; r, reverse-strand primer.

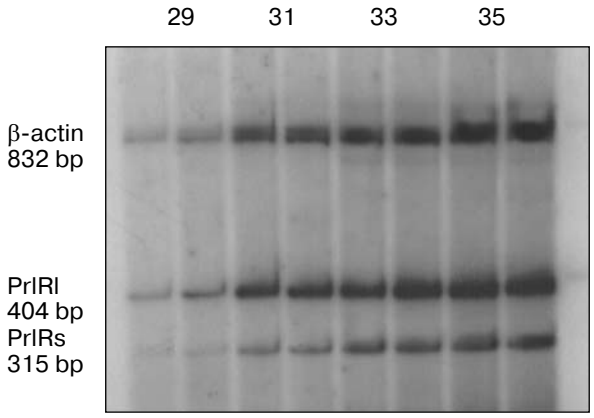

Fig. 3. Semiquantitative assay of mRNA of short and long prolactin receptor (PrlR) isoforms by RT-PCR. Radioautographs of PCR products (cDNA of cholangiocytes of rat under obstructive cholestasis) separated in 5% polyacrylamide gel. The number of PCR cycles is given at the top. Positions and lengths of amplification products for cDNAs of β-actin and short and long isoforms of PrlR are shown.

with anti-PrlR antibodies (clone U5, kindly provided by Drs. V. Goffin and P. Kelly, France) and cytophotometry were done as described previously [18]. The degree of cell PrlR-positive immunostaining is represented as arbitrary units (a.u.) of optical density.

Values for cytophotometry and RT-PCR results of PrlR level were expressed as mean a.u. ± SD. Statistical significance was assessed by one-way Kruskal–Wallis ANOVA test for multiple comparison followed by Mann–Whitney U-test, with a significance level of $p < 0.05$.

RESULTS

PrlR mRNA in the cholangiocytes of intact mature male and female rats is detected at low level. The PrlR-positive immunostaining is not pronounced in cholangiocytes of intact male and female rats, in contrast to hepatocytes (Fig. 4, a and b). However, we have revealed PrlR mRNA in cholangiocytes of intact animals of both sexes by RT-PCR, and its level has been found to be low and sex-independent.

While in mature rats the PrlR-positive immunostaining of cholangiocytes is very low, cholangiocytes at the earlier stages of postnatal development are characterized by elevated sex-independent PrlR-positive staining (Fig. 4, c and d), and this level is strongly correlated with cholangiocyte high proliferative capacity [19].

Influence of obstructive cholestasis on cholangiocyte PrlR level and its isoform ratio are sex-independent. The induction of bile duct cell proliferation by CBDL promotes the appearance of intensive PrlR-positive immunostaining in cholangiocytes of both male and female rats. An interesting feature of PrlR-immunostaining in proliferating cholangiocytes is pronounced nuclear localization of the positive signal (Fig. 4, e and f).

The obstructive cholestasis induced by CBDL leads to 3.0-3.4-fold increase in PrlR mRNA level in cholangiocytes of both male and female rats (Table 3).

Known regulators of PrlR do not affect its level in cholangiocytes under normal and cholestatic conditions. Since the cholangiocytes of intact mature male and female rats have been shown to posses no difference in PrlR level, unlike most other tissues, we investigated the influence of the main known hormonal regulators (sex hormones and prolactin) on PrlR level in bile duct epithelial cells.

The RT-PCR analysis has shown that the gonadectomy does not change the PrlR mRNA level in cholangiocytes of both intact rats and animals with obstructive cholestasis. It has been verified that gonadectomy of male and female rats with CBDL does not influence the PrlR-positive staining of cholangiocytes. Further, either increase or decrease in systemic prolactin level does not significantly change the intensity of PrlR-positive immunostaining in cholangiocytes. This level is 0.21 ± 0.03 a.u. of optical density for all tested groups; the differences between groups do not exceed 5% (Kruskal–Wallis ANOVA test, $p > 0.7$).

Long PrlR isoform mRNA predominates in rat cholangiocytes. The long PrlR isoform is revealed to be the only PrlR isoform in the cholangiocytes of mature intact rats, in contrast to hepatocytes. The amplification product of short isoform cDNA does not become apparent under ethidium bromide staining even after the 40th cycle (i.e., the short isoform is not practically expressed

Table 3. Level of PrlR mRNA in rat cholangiocytes of different experimental groups

Group	PrlR mRNA level, a.u. ± SD	
	without obstructive cholestasis	with obstructive cholestasis
Female	0.32 ± 0.06	1.08 ± 0.31*
Male	0.32 ± 0.05	0.92 ± 0.09*
Gonadectomized females	0.28 ± 0.08	1.26 ± 0.22*
Gonadectomized males	0.27 ± 0.04	0.95 ± 0.15*

Note: Level of prolactin receptor mRNA expressed in arbitrary units (a.u.) is represented as the ratio to β-actin mRNA (see "Materials and Methods").

* Level of significance of $p < 0.05$: cholangiocytes of rats with obstructive cholestasis vs. rats without obstructive cholestasis (4-5 animals in each experimental group).

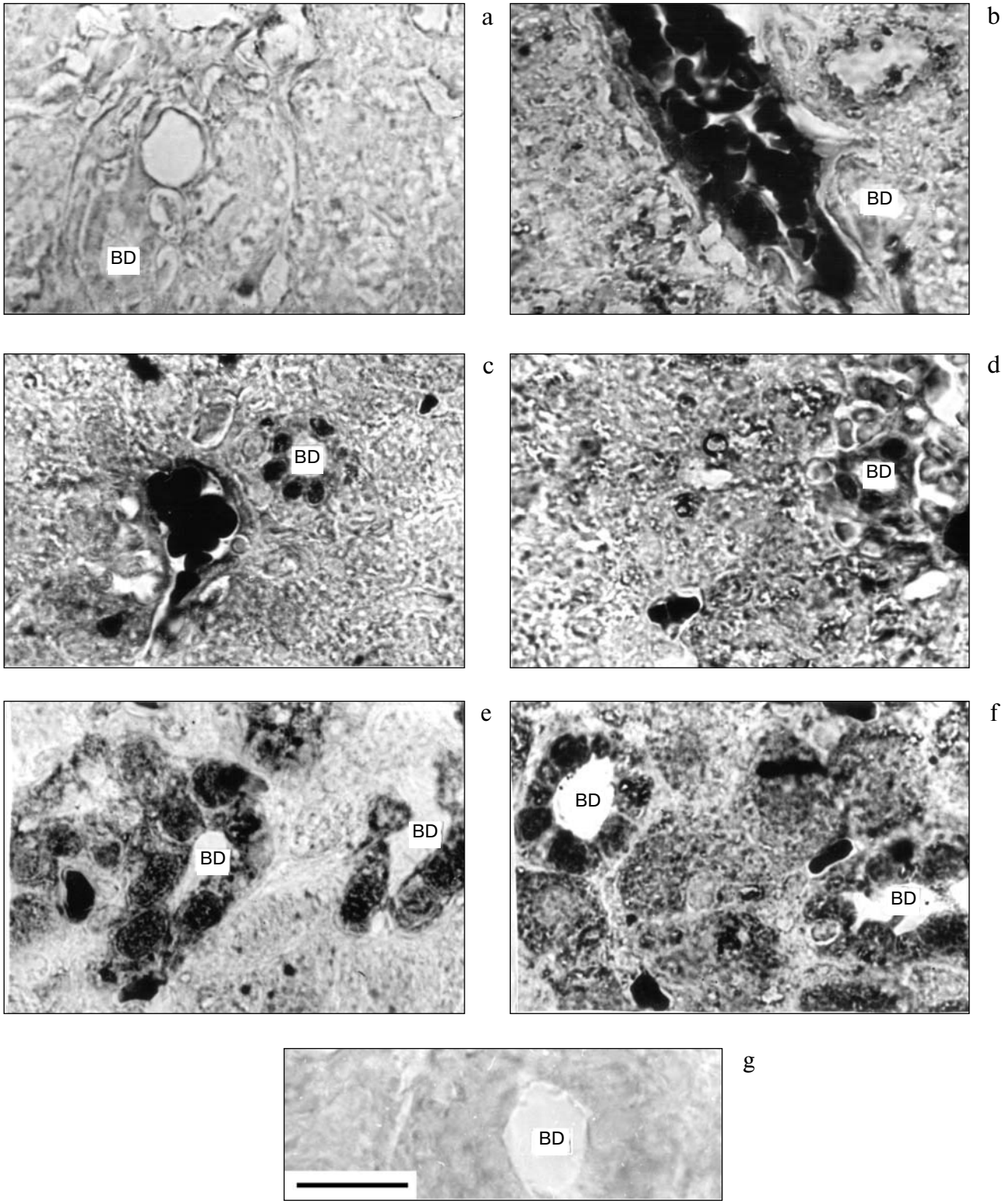

Fig. 4. Immunocytochemical identification of prolactin receptor in liver tissue of different experimental groups (see Table 1): a, b) mature rats; c, d) immature rats; e, f) mature rats after 14-day CBDL; a, c, e) males; b, d, f) females; g) negative control for immunohistochemical staining of prolactin receptor: anti-prolactin receptor antibodies were omitted. BD, bile ducts; bar, 25 μm.

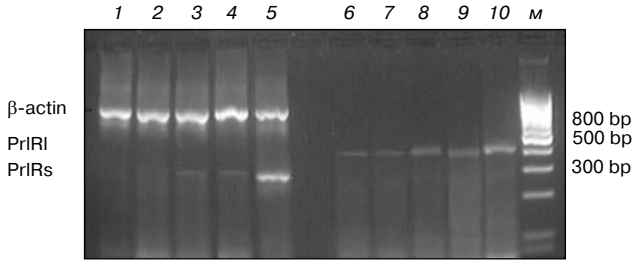

Fig. 5. Identification of the mRNA of prolactin receptor isoforms in isolated cholangiocytes of different experimental groups revealed by RT-PCR (40th cycle of PCR). Lanes: *1-5*) PCR for short PrlR isoform; *6-10*) PCR for long PrlR isoform. Cholangiocytes of intact mature male rat (*1*, *6*), of intact mature female rat (*2*, *7*), of mature male rat after 14-day CBDL (*3*, *8*), of mature female rat after 14-day CBDL (*4*, *9*); *5*, *10*) hepatocytes of mature female rat. M, DNA length markers (numbers represent length of fragment in bp); PrlRl, long isoform of prolactin receptor; PrlRs, short isoform of prolactin receptor.

in cholangiocytes of mature rats) (Fig. 5). The mRNA of short PrlR isoform has been showed to appear in cholangiocytes under conditions of obstructive cholestasis. It is accompanied by significant increase in long PrlR mRNA level, and thus the ratio of short and long isoforms becomes 0.31-0.48. This ratio is also sex-independent. Gonadectomy does not influence this ratio, which may serve as additional evidence of sex hormone independent manner of PrlR alternative splicing in rat cholangiocytes.

Thus, obstructive cholestasis sex-independently induces the increase in long PrlR isoform level approximately 2.5-fold and also leads to appearance of short isoform in rat cholangiocytes (Table 4).

DISCUSSION

In the present work, cholangiocytes of mature animals are shown to have a lower level of PrlR mRNA than hepatocytes (especially in female rats) and the ratio of its short and long isoforms in cholangiocytes is opposite to hepatocytes (the long form predominates in bile duct cells). On the other hand, PrlR level and isoform ratio in cholangiocytes of mature animals are similar to those parameters in various other tissues, which are dependent on prolactin (e.g., pituitary, hypothalamus, kidney, etc.) [7].

The mode of regulation of PrlR expression in cholangiocytes has been found to be unusual compared with other types of prolactin target tissues: the PrlR level in bile duct cells does not depend on either animal sex hormones or prolactin. We have also revealed high PrlR level under conditions of high proliferative activity of cholangiocytes (in immature rats as well as in mature rats with obstructive cholestasis). The PrlRs are not the only receptors whose level in proliferating cholangiocytes increases under obstructive cholestasis: it has been earlier found that the same trend is a characteristic feature for receptors for secretin [20] and estrogen [21]. Thus the cholangiocytes magnify its sensitivity to various regulatory factors including prolactin under obstructive cholestasis. The increased cholangiocyte susceptibility to prolactin may play a role both in direct regulation of bile duct cells functions and in maintaining the action of other factors on these cells.

We have earlier shown that prolactin participates in stimulation of cholangiocyte proliferation after CBDL [11]. Taking into account that the level of long isoform increases under CBDL, this effect seems to be mediated by activation of the Jak/STAT cascade. The same cascade

Table 4. Level of PrlR isoform mRNA in rat cholangiocytes of different experimental groups

Group	PrlR isoform mRNA level, a.u. ± SD			
	without obstructive cholestasis		with obstructive cholestasis	
	short isoform	long isoform	short isoform	long isoform
Female	n.d.	0.10 ± 0.03	0.11 ± 0.03*	0.28 ± 0.03*
Male	n.d.	0.10 ± 0.01	0.11 ± 0.01*	0.24 ± 0.07*
Gonadectomized females	n.d.	0.08 ± 0.04	0.11 ± 0.03*	0.30 ± 0.04*
Gonadectomized males	n.d.	0.09 ± 0.03	0.10 ± 0.04*	0.21 ± 0.04*

Note: Ratio of short and long PrlR isoforms is represented as arbitrary units (a.u.); n.d., not detected by PCR.

* Level of significance of $p < 0.05$: cholangiocytes of rats with obstructive cholestasis vs. rats without obstructive cholestasis (4-5 animals in each experimental group).

is activated by interleukin-6 and insulin-like growth factor I, which has been shown to stimulate cholangiocyte proliferation [22].

Also, prolactin may be considered as a factor participating in the maintaining of normal bile duct structure and polarity of cholangiocytes. The intercellular junctions are shown to be dramatically altered under obstructive cholestasis, e.g., the level of connexins decreases [23]. Prolactin has been shown to increase the connexin levels, e.g., connexin 32 in mammary gland [24] and connexin 43 in pancreas islets [25]. These connexins are the main proteins of gap junctions of hepatocytes and cholangiocytes, respectively [23, 26].

Prolactin increases the level of estrogen receptors in various cell types [27, 28], and cholangiocytes may be one of them. The known stimulatory effect of estrogen on cholangiocyte proliferation [21] may be supposed to be potentiated by increase in prolactin action.

In summary, the mature rat cholangiocytes express PrlR at a level comparable with that of some prolactin-dependent tissues [18, 29]. The predominance of the long PrlR isoform in mature cholangiocytes is also typical for most of such tissues. Cholangiocytes of mature rats differ from hepatocytes by parameters of PrlR expression and by sensitivity of PrlR level to obstructive cholestasis induced factors. Moreover, cholangiocytes differ from almost all other types of cells by the absence of sex hormone and prolactin influence on PrlR level. These data may be evidence for differential regulation by prolactin of various liver functions, which are mediated by diverse liver cells. Furthermore, the investigations of the particular role of PrlR in rat cholangiocytes demand the revelation of machinery for regulation of PrlR expression, e.g., usage of one of the described PrlR gene promoters, possibly another than used in hepatocytes [30].

REFERENCES

1. Bole-Feysot, C., Goffin, V., Edery, M., Binart, N., and Kelly, P. A. (1998) *Endocrine Rev.*, **19**, 225-268.
2. Smirnova, O. V., and Bogorad, R. L. (2004) *Biochemistry (Moscow)*, **69**, 351-363.
3. Bogorad, R. L., Zenkova, T. Y., Rubtsov, P. M., and Smirnova, O. V. (2004) *Biochemistry (Moscow)*, **69**, 1114-1122.
4. Perrot-Applanat, M., Gualillo, O., Pezet, A., Vincent, V., Edery, M., and Kelly, P. A. (1997) *Mol. Endocrinol.*, **11**, 1020-1032.
5. Das, R., and Vonderhaar, B. K. (1995) *Mol. Endocrinol.*, **9**, 1750-1759.
6. Duan, W. R., Farmer, T. G., Albarracin, C. T., Zhong, L., and Gibori, G. (1997) *Endocrinology*, **138**, 3216-3221.
7. Nagano, M., and Kelly, P. (1994) *J. Biol. Chem.*, **269**, 13337-13345.
8. Jolicoeur, C., Boutin, J. M., Okamura, H., Raguet, S., Djiane, J., and Kelly, P. A. (1989) *Mol. Endocrinol.*, **3**, 895-900.
9. Sakaguchi, K., Ohkubo, T., Sugiyama, T., Tanaka, M., Ushiro, H., and Nakashima, K. (1994) *J. Endocrinol.*, **143**, 383-392.
10. Yokoyama, Y., Kitchens, W. C., Toth, B., Schwacha, M. G., Bland, K. I., and Chaudry, I. H. (2003) *J. Appl. Physiol.*, **95**, 2530-2536.
11. Orlova, A. N., Smirnov, A. N., and Smirnova, O. V. (1999) *Byul. Eksp. Biol. Med.*, **127**, 573-575.
12. Smirnova, O. V., Petrashchuk, O. M., and Smirnov, A. N. (1998) *Byul. Eksp. Biol. Med.*, **125**, 66-70.
13. Alpini, G., Lenzi, R., Zhai, W. R., Slott, P. A., Liu, M. H., Sarkozi, L., and Tavolini, N. (1989) *Am. J. Physiol.*, **257**, G124-133.
14. Mennone, A., Alvaro, D., Cho, W., and Boyer, J. L. (1995) *Proc. Natl. Acad. Sci. USA*, **92**, 6527-6231.
15. Alpini, G., Phillips, J. O., Vroman, B., and LaRusso, N. F. (1994) *Hepatology*, **20**, 494-514.
16. Chomczynski, P., and Sacchi, N. (1987) *Analyt. Biochem.*, **162**, 156-159.
17. Bogorad, R. L., Smyslova, V. S., Rubtsov, P. M., and Smirnova, O. V. (2002) *Mol. Biol. (Moscow)*, **36**, 71-73.
18. Bogorad, R. L., Kotok, T., Smirnov, A. N., Turovetskii, V. M., and Smirnova, O. V. (2000) *Byul. Eksp. Biol. Med.*, **129**, 608-611.
19. Alpini, G., Lenzi, R., Sarkozi, L., and Tavoloni, N. (1988) *J. Clin. Invest.*, **81**, 569-578.
20. Alpini, G., Ulrich, C. D., 2nd, Phillips, J. O., Pham, L. D., Miller, L. J., and LaRusso, N. F. (1994) *Am. J. Physiol.*, **266**, G922-928.
21. Alvaro, D., Alpini, G., Onori, P., Perego, L., Svegliati Baroni, G., Franchitto, A., Baiocchi, L., Glaser, S. S., Le Sage, G., Folli, F., and Gaudio, E. (2000) *Gastroenterology*, **119**, 1681-1691.
22. Alvaro, D., Gigliozzi, A., and Attili, A. F. (2000) *J. Hepatol.*, **33**, 333-340.
23. Fallon, M. B., Nathanson, M. H., Mennon, A., Saez, J. C., Burgstahler, A. D., and Anderson, J. M. (1995) *Am. J. Physiol.*, **268**, C1186-1194.
24. Miyoshi, K., Shillingford, J. M., Smith, G. H., Grimm, S. L., Wagner, K. U., Oka, T., Rosen, J. M., Robinson, G. W., and Hennighausen, L. (2001) *J. Cell. Biol.*, **155**, 531-542.
25. Collares-Buzato, C. B., Leite, A. R., and Boschero, A. C. (2001) *Pancreas*, **23**, 177-185.
26. Bode, H. P., Wang, L., Cassio, D., Leite, M. F., St-Pierre, M. V., Hirata, K., Okazaki, K., Sears, M. L., Meda, P., Nathanson, M. H., and Dufour, M. H. (2002) *Hepatology*, **36**, 631-640.
27. Tessier, C., Deb, S., Prigent-Tessier, A., Ferguson-Gottschall, S., Gibori, G. B., Shiu, R. P., and Gibori, G. (2000) *Endocrinology*, **141**, 3842-3851.
28. Norstedt, G., Wrange, O., and Gustafsson, J. A. (1981) *Endocrinology*, **108**, 1190-1196.
29. Kotok, T., Bogorad, R. L., Smirnov, A. N., Turovetskii, V. B., and Smirnova, O. V. (2000) *Russ. J. Develop. Biol.*, **31**, 117-123.
30. Hu, Z. Z., Li, Z., and Dufau, M. L. (1996) *J. Biol. Chem.*, **271**, 10242-10246.