Expression of Prolactin Receptors in Rat Reproductive Tissues during Periovulatory Prolactin Imbalance

R. L. Bogorad, T. Kotok,* A. N. Smirnov, V. M. Turovetskii, and O. V. Smirnova

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Indirect immunoperoxidase technique in combination with cytophotometry showed that prolactin imbalance in the periovulatory period is characterized by considerable changes in the expression of prolactin receptors in uterine glands and endometrial stroma (but not in the myometrium) and in the pituitary gland and hypothalamus, while receptor compartmentalization pattern remaines unchanged.

Key Words: *prolactin receptor; prolactin; bromocryptine; periovulatory period; rat reproductive tissues*

Transient periovulatory imbalance of prolactin (PL) is a pathogenetic factor of idiopathic infertility [3]. This imbalance responsible for inadequate response of the follicular apparatus to the hormone modulates the responsiveness of uterine endometrium and hypothalamic neurons and impairs secretory function of the pituitary gland [5,7,8,15].

PL is a powerful regulator of PL receptors, and its regulatory effects are tissue-specific [4,6,9]. Prolactin receptor (PLR) and PLR mRNA were found in the majority of tissues including the ovaries, uterine, pituitary gland, and hypothalamus of different animal species [10]. PLR is a membrane receptor, however, in some tissues and under specific conditions it can be localized to the nucleus, which modifies the spectrum of its effects [2].

The aim of the present study was to identify cells and tissues where the expression and compartmentalization of PLR are most affected by periovulatory PL imbalance. To this end, PLR expression in rat uterine, pituitary gland, and hypothalamus was analyzed by immunocytochemical and cytophotometrical technique after preovulatory administration of PL or bromocryptine (BC), an inhibitor of PL secretion. The data were compared with our previous findings in ovaries [1].

Laboratory of Endocrinology Biological Faculty , M. V. Lomonosov Moscow State University; *Jerusalem Hebrew University. ***Address for correspondence:*** smirnova_@hotmail.com. Smirnova O. V.

MATERIALS AND METHODS

The study was carried out on outbred female rats with regular cycles. Ovine PL dissolved in 0.1 M saline buffered with 0.01 M sodium hydrocarbonate was intramuscularly injected to experimental rats during proestrus at 18.00, 19.00 and 20.00 (100 μg each). BC (Sandoz) dissolved in 50% ethanol in buffered saline and administered subcutaneously in 3 doses (1 mg each) at 18.00 on day 2 of diestrus and at 10.00 and 18.00 during proestrus. The control group received solvent according to the same schedule. The rats were decapitated at 10.00 during the estrus phase. Each group consisted of 4-6 animals.

Blood concentration of PL was determined with a NIDDK rat PRL-RP-3 kit, the concentration of administered PL with a NIDDK ovine/bovine PRL kit, estradiol and progesterone were measured with IMMULITE Estradiol and IMMULITE Progesterone kits, respectively (Diagnostic Products Corporation). Tissue PL was visualized by indirect immunoperoxidase technique as described elsewhere [12].

Cytophotometry was performed in two experimental (treated with anti-PLR antibodies) and two

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control (untreated) sections from each animal. Light transmission was measured on a LUMAM I-3 luminescent microscope at λ=520 nm and ×40 using a 2.5-μ light probe. The intensity of PLR-specific staining was evaluated by optical density (*D*) calculated as $D=\lg(Tc/T)$, where *T* and *Tc* are light transmission in the experimental and control sections, respectively.

In the ovaries, mature postovulatory follicles were analyzed. The granulosa was divided into 3 equal layers (internal, adjacent to the antrum and including the cells of ovum tuberculum, intermediate, and external, adjacent to the theca) and 20 cells in each layer were assayed by cytophotometry. In the uterine, endometrial (glands and stroma) and myometrial cells were assayed separately (20 cells of each type per section). Preliminary analysis of frontal sections of the anterior pituitary and horizontal sections of the hypothalamus revealed a relatively weak homogenous PLR-specific staining in these structures, therefore in each section we analyzed 20 randomly chosen cells.

The data were processed statistically using Statistica software. Statistical significance of differences was assessed using the nonparametric Mann—Whitney *U* test.

RESULTS

After administration of ovine PL, its blood concentration in the early postovulatory period 1.5-fold exceeded the endogenous PL concentration, which remained unchanged ($p>0.1$, Table 1). BC administered at this stage of the estrous cycle 10-fold decreased blood PL concentration ($p<0.05$, Table 1). Administration of exogenous PL significantly reduced blood concentration of estradiol ($p<0.05$), while BC only slightly decreased this index compared to the control. Blood progesterone increased after PL administration ($p<0.05$) and tended to decrease after BC.

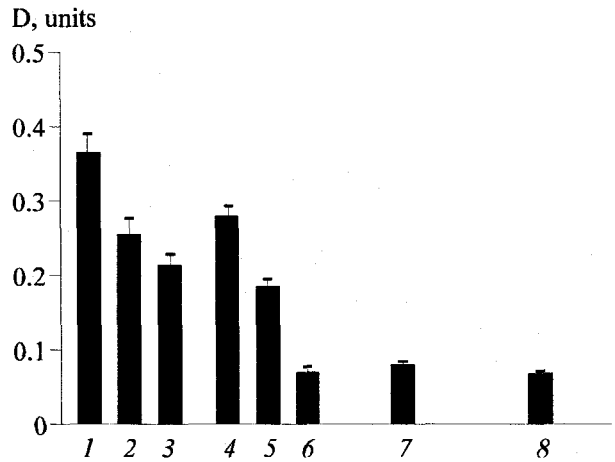

Fig. 1. Expression of prolactin receptors in rat reproductive tissues in early estrus. Internal *(1)*, intermediate *(2)*, and external *(3)* granulosa layers after ovulation; endometrial glands *(4)* and stroma *(5)*, myometrium*(6)*, cells of the anterior pituitary *(7)*, hypothalamic neurons *(8)*.

In control rats, expression of PLR in the early postovulatory period was maximum in ovarian cells, less intensive in the uterine, and weak in pituitary and hypothalamic cells (Fig. 1). The expression of PLR in ovarian cells depended on their location, while in the uterus it depended on the cell type (Fig. 1).

In the control group, PLR expression in the uterine was maximum in endometrial glands, less intensive in stromal cells of the endometrium and minimum in the myometrium (Fig. 2). The response of endometrial glands and stroma to PL and BC differed from that of the myometrium. In the endometrium, PL significantly increased, while BC significantly decreased PLR expression, while in the myometrium BC slight-

TABLE 1. Blood Concentration of Sex Hormones in Experimental and Control Animals (*M±m*, *n*=4-6)

Hormone	Control (PL solvent)	PL	Control (BC solvent)	BC
Rat PL, ng/ml	22.4±1.5	23.9±2.0	19.7±1.7	1.9±0.2*
Ovine PL, ng/ml	—	35.8±2.4	—	—
Estradiol, pg/ml	42.8±4.8	14.7±4.7*	31.8±3.0	23.5±6.9
Progesterone, ng/ml	3.2±0.3	4.9±0.1*	3.5±0.2	2.8±0.3

Note. Here and in Table 2: *$p<0.05$ in comparison with the corresponding control.

TABLE 2. Effect of Periovulatory PL Imbalance on PLR Expression (Optical Density Units) in Rat Pituitary Gland and Hypothalamus (*M±m*)

Tissue	Control (PL solvent)	PL	Control (BC solvent)	BC
Hypothalamus	0.068±0.004	0.087±0.006*	0.080±0.004	0.066±0.004*
Pituitary gland	0.081±0.003	0.070±0.002*	0.058±0.003	0.094±0.003*

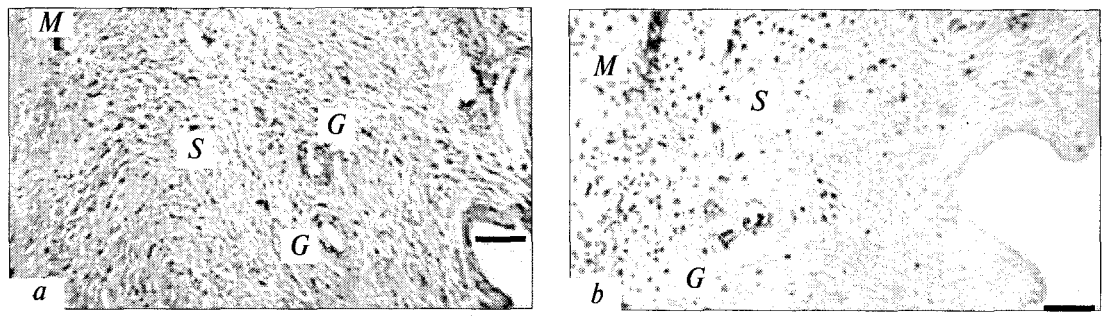

Fig. 2. Immunoperoxidase identification of prolactin receptors in different cell types of rat uterine in early estrus. Positive staining of prolactin receptors: *a*) with U5 monoclonal antibodies to rat prolactin receptors; *b*) without antibodies. *G:* cells of endometrial glands; *S:* cells of endometrial stroma; *M:* cells of myometrium.

ly, but significantly enhanced PLR expression and PL had no effects of this process (Fig. 3).

In hypothalamic neurons, PL significantly stimulated, while BC significantly inhibited PLR expression. In contrast to other reproductive tissues, expression of PLR in pituitary cells decreased after administration of PL and increased after injections of BC (Table 2).

None of the studied groups showed changes in receptor compartmentalization.

Thus, in the early postovulatory period the intensity of PLR expression decreased in the following order: ovaries—uterine—pituitary gland—hypothalamus. Heterogeneity of PLR expression in the pituitary gland and hypothalamus was not definitely revealed, because of its relatively low intensity, however in the ovaries and uterine PLR expression varied in different cells. In different layers of the ovarian granulosa this heterogeneity was associated with the presence of two cell subpopulations depending on oocytes and theca [1]. In the uterine PLR were most intensely expressed in the endometrial glands and stroma and less intensely in the myometrium cells, which is associated with endometrium activation during the secretory phase of estrous cycle and agrees with the data obtained on other animal species [6,14].

It was found that periovulatory imbalance of PL is not associated with the appearance of PLR in the nuclei of reproductive tissue cells, *i.e.*, did not modify signal transduction type, but changes the intensity of PLR expression in these tissues [2].

We have previously shown that in postovulatory follicles, PLR are sensitive to PL only in the granulosa layer adjacent to the theca. On the contrary, unovulated follicles in the early estrus show sensitivity to PL in all layers of the granulosa [1].

Normally, PLR expression in glandular and stromal cells of the endometrium increases during the second half of the secretory phase due to stimulation by high PL concentrations produced by decidual cells and by elevated progesterone [6]. In our study, the periovulatory PL imbalance and resulting shifts in progesterone concentration (Table 1) induced much earlier changes in PLR expression, which can desynchro-

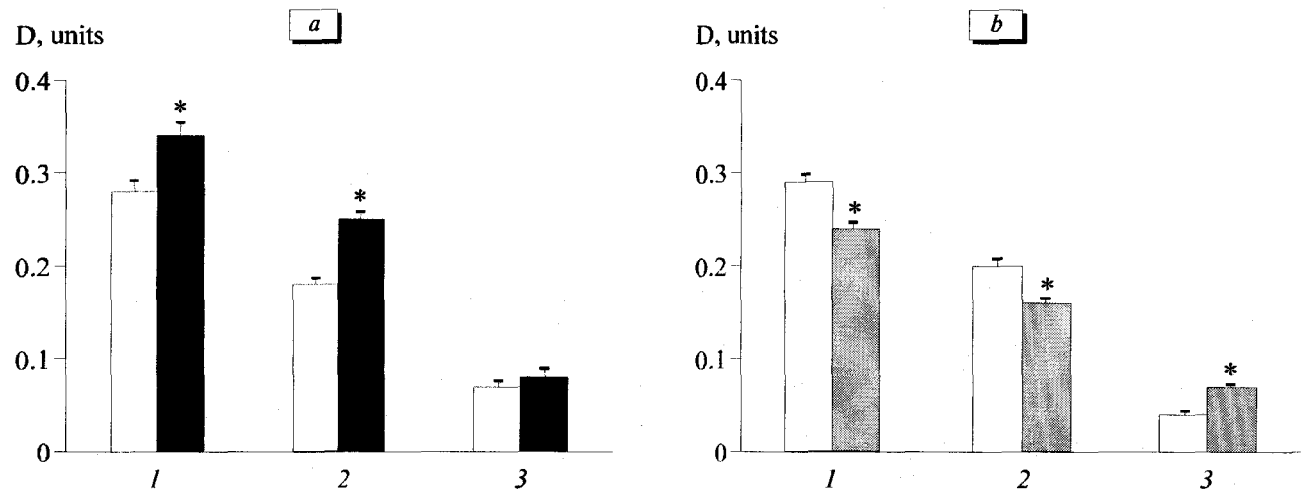

Fig. 3. Effect of prolactin (*a*) and bromocryptine (*b*) on the expression of prolactin receptors in the early postovulatory period in cells of endometrial glands (*1*), stroma (*2*) and myometrium (*3*). Open bars: control; filled bars: prolactin; shaded bars: bromocryptine. *$p<0.05$ compared to the control.

nize functional changes in endometrial glands and stroma and the time of implantation. Our findings show that the level of PLR in the myometrium is not modulated by PL and, therefore, is less sensitive to periovulatory PL imbalance.

This up-regulation of PLR in the hypothalamus induced by PL was described in previous studies [9]. Down-regulation of PLR observed in the pituitary gland was previously reported for other tissues and for the pituitary gland under different conditions [4,10]. It is known that these regulatory shifts depend on PL dose [14]. PL regulates secretion of luteinizing and follicle-stimulating hormone by modulating PLR density in the hypothalamus and pituitary gland [11,13]. Periovulatory PL imbalance induces opposite changes in PLR expression in these tissues and modifies secretion of gonadotropins, thus affecting the rate and degree of luteinization of granulosa cells and disturbing progesterone secretion.

It can be concluded that periovulatory PL imbalance is associated with changes in the sensitivity to this hormone not only in the ovarian follicular apparatus, but also in the uterine endometrium, pituitary gland, and hypothalamus. Therefore, the negative effect of PL imbalance on fertility is aggravated by autoregulation of PLR expression in reproductive organs.

REFERENCES

1. T. Kotok, R. L. Bogorad, A. N. Smirnov, *et al., Ontogenez,* **31,** No. 2, 144-151 (2000).
2. O. V. Smirnova, *Biol. Membrany,* **1,** No. 2, 199-211 (1999).
3. K. Asukai, T. Uemura, and H. Minagushi, *Fertil. Steril.,* **60,** No. 3, 423-427 (1993).
4. R. Di Carlo, C. Bole-Feysot, O. Gualillo, *et al., Endocrinology,* **136,** No. 10, 4713-4716 (1995).
5. K. Ginsburg, *Endocrinol. Metab. Clin. North. Am.,* **21,** No. 1, 85-104 (1992).
6. R. Jones, H. Critchley, J. Brooks, *et al., J. Clin. Endocrinol. Metab.,* **83,** No. 1, 313-316 (1998).
7. Y. Liu, X. Peng, Y. Cheng, *et al., Hum. Reprod.,* **2,** No. 12, 2748-2755 (1997).
8. P. Mohankumar, S. Mohankumar, L. Arbogast, *et al., Proc. Soc. Exp. Biol. Med.,* **217,** No. 4, 461-465 (1998).
9. G. Muccioli and R. Di Carlo, *Brain Res.,* **663,** No. 2, 244-250 (1994).
10. M. Nagano and P. Kelly, *J. Biol. Chem.,* **269,** No. 18, 13337-13345 (1994).
11. X. Pi and D. Grattan, *J. Neuroendocrinol.,* **11,** No. 9, 693-705 (1999).
12. O. Smirnova, O. Petracshuk, and P. Kelly, *Mol. Cell. Endocrinol.,* **105,** No. 1, 77-81 (1994).
13. D. Tortonese, J. Brooks, P. Ingelton, and A. McNeily, *Endocrinology,* **139,** No. 12, 5215-5223 (1998).
14. L. Tseng and J. Mazella, *Semin. Reprod. Endocrinol.,* **17,** No. 1, 23-27 (1999).
15. S. Wardlaw, J. Kim, and C. Matera, *J. Neuroendocrinol.,* **9,** No. 2, 81-85 (1997).