SECRETORY ACTIVITY OF LACTOTROPHS AND ITS REGULATION BY HYPOTHALAMIC HORMONES IN PRIMARY PITUITARY CELL CULTURES FROM RATS OF DIFFERENT AGES

V. P. Fedotov, V. I. Gudoshnikov, I. S. Komolov, and V. V. Abramova

UDC 616.432-018.1-092.4]-092.9-07

KEY WORDS: pituitary gland, cell cultures, prolactin, hypothalamic hormones, postnatal development.

The authors' previous research showed that primary cultures of adenohypophyseal cells of adult rats are convenient objects with which to study the fine details of regulation of prolactin (PRL) secretion and proliferation of lactotrophs in the mature pituitary gland by hypothalamic hormones [3, 5]. The establishment of the lactotrophic function of the pituitary in rats takes place in the perinatal and early postnatal periods and is not completed until sexual maturity [10, 12]. It was shown previously in our laboratory that adenohypophyseal cell cultures from immature female rats contain and secrete much less electrophoretically detectable ^{14}C-labeled PRL than adult female cells [4].

Particular interest attaches to the study of formation of hypothalamic regulation of PRL secretion in ontogeny. Although the content of thyrotrophin-releasing hormone (TRH) and somatostatin (SS) in the neonatal rat hypothalamus is low, and begins to rise progressively only in the first 3 weeks of postnatal development [13, 14], the concentration of TRH receptors in the neonatal rat pituitary is even higher than in adult animals [6], and binding of SS by the pituitary almost reaches the adult level by the 7th day after birth [9]. The dopamine content in the young rat hypothalamus rises from 207.9 to 259.6 mg/g tissue from birth until the 5th postnatal day [11]. Investigations conducted on primary cultures of rat adenohypophyseal cells in the postnatal period of development have demonstrated increased reactivity of the somatotrophs of neonatal rats to the action of TRH [15] and lowered reactivity to the action of SS [8]. Responses of lactotrophs to these hypothalamic hormones have not been investigated. The aim of the investigation was to study secretory activity of lactotrophs and particular features of its regulation by hypothalamic hormones in pituitary cell cultures from rats of different ages throughout the period of postnatal development.

METHODS

The method of preparing primary cultures of Wistar rat adenohypophyseal cell cultures was described previously [2]. Some cultures from whole pituitary glands of neonatal rats (aged 5 days) and cultures of adenohypophyseal cells from sexually immature males and females (aged 24-28 days), pubertal males and females (35-40 days), and adult female rats (90-120 days) were used. Isolated pituitary cells were seeded in 96-well microtitration panels (Flow Laboratories, England) with a high density [$(1.0\text{-}1.2)\cdot 10^5$ cells per well] and grown in medium 199 with 10% fetal calf serum (Calbiochem AG, Switzerland). On the 4th-5th day of culture the pituitary cells were washed with serum-free medium and then incubated for 3 h in medium 199 containing 2 mg/ml of bovine serum albumin, with the addition of the various hypothalamic hormones. TRH was obtained from the Laboratory of Protein Hormone Chemistry (Head, Professor Yu. P. Shvachkin), Institute of Experimental Endocrinology, All-Union Endocrinologic Research Center,

Laboratory of Biological Research into Hormonal Compounds, Institute of Experimental Endocrinology, All-Union Endocrinologic Research Center, Academy of Medical Sciences, Moscow. (Presented by Academician Yu. A. Pankov, Academy of Medical Sciences.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 113, No. 4, pp. 402-404, April, 1992. Original article submitted June 5, 1991.

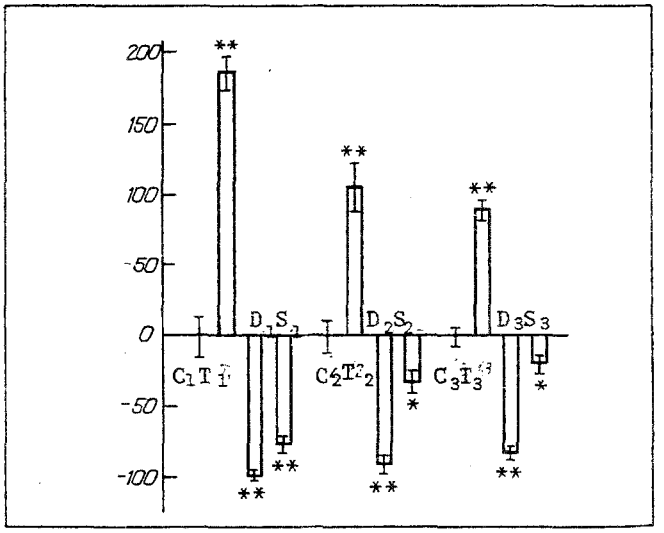

Fig. 1. Changes in prolactin secretion caused by hypothalamic hormones (in % of corresponding control, M ± m) in primary cultures of pituitary cells from postnatal rats. Designation of groups: C) control; T, D, S) TRH (40 mg/ml), dopamine (200 ng/ml), and SS (20 ng/ml); indices 1, 2, and 3 denote groups of neonatal rats, sexually immature males, and adult females, respectively. *$p < 0.05$, **$p < 0.001$. Compared with corresponding control. n = 5-10 in a group.

TABLE 1. Basal PRL Secretion (M ± m) in Primary Pituitary Cell Cultures from Rats in the Postnatal Period of Development (n = 10 in the group)

Groups of animals donating pituitary glands	PRL in medium, mg/ml	p
Neonatal rats	18,2±2,2	<0,001
Sexually immature females	984±74	
Sexually immature males	1594±82	>0,05
Pubertal males	1749±86	
Pubertal females	3026±166	
Adult females	3493±193	>0,05

Note. Significance of differences between paired age groups shown in each experiment.

Academy of Medical Sciences of the USSR. Other products used were dopamine hydrochloride (Sigma, USA) and somatostatin tetradecapeptide (Serva, Germany). Prolactin in the incubation medium was determined by a homologous version of radioimmunoassay, developed by the authors [1]. The results were subjected to statistical analysis by Student's *t* test.

RESULTS

Table 1 gives the results of measurement of basal PRL secretion in primary pituitary cell cultures. Secretory activity of the lactotrophs in cultures from neonatal rats can be seen to be 50-190 times lower than in cultures of pituitary cells of the other age groups studied (sexually immature, pubertal, and adult). It is important to note that because of the high seeding density and the use of growth medium with a sufficiently high serum concentration, by the time

of incubation with hypothalamic hormones a state close to that of a continuous monolayer was achieved, so that there was no need to calculate the results per unit mass of total protein in the well. On the other hand, experiments of which the results are given in this paper were planned so that cell cultures of different age groups in each of the experiments were introduced in parallel rows of the same 96-well panel, thus reducing to the minimum the possible contribution of variability from one experiment to another.

Changes in secretory activity of lactotrophs of different age groups under the influence of hypothalamic hormones are shown as percentages of the corresponding control values in Fig. 1. The first point to note is the absence of any qualitative differences in the effects of the bioregulators on PRL secretion. Quantitative differences were found mainly when pituitary gland cultures from neonatal and sexually immature rats were compared. For instance, TRH stimulated PRL secretion by 186 and 106% in the groups of neonatal rats and sexually immature males, respectively. Dopamine inhibited secretory activity of lactotrophs in the two above-mentioned groups by 99.5 and 90.7%, respectively. Somatostatin reduced PRL release from cells of immature males by 33% and inhibited more effectively the secretory activity of lactotrophs of neonatal rats (reduced by 76%).

The experiments thus revealed high reactivity of the pituitary cells of neonatal rats to the action of TRH, dopamine, and SS, despite the low basal PRL secretion. The results are in agreement with those of investigations conducted by other workers both in vivo and in vitro. Judging by the results of the reaction of hemolytic plaque formation and the immunocytochemical method, PRL-secreting and PRL-containing cells account for only 5 and 10%, respectively, of the total population in the adenohypophysis of 5-day-old rats. These proportions were increased several times when the rats attained maturity [7]. Levels of immunoreactive PRL in the blood plasma were low in newborn rats and began to rise only after the 6th day of postnatal life [12]. Radioimmunoassay also revealed a low PRL content in the neonatal rat pituitary and a progressive increase in its value during early postnatal development [10].

In experiments on incubated half pituitary glands the reaction of the lactotrophs to the stimulating effect of TRH (100 mg/ml) was observed with effect from the 18th day of pregnancy, but the intensity of this reaction decreased toward the 8th day of postnatal life. At the same time, the dopaminergic agonist apomorphine reduced PRL secretion by the incubated pituitary glands as early as on the 19th day of pregnancy. This inhibitory effect of the dopamine agonist was potentiated toward the 5th postnatal day [10]. In experiments in vivo the response of the lactotrophs to pimozide, which blocks dopaminergic receptors, was observed as early as on the 3rd postnatal day; the response to pimozide increased until the 35th postnatal day [12].

Our investigations on primary monolayer cultures of pituitary cells confirm the high reactivity of the lactotrophs of neonatal rats to the action of TRH and dopamine. The high response of the lactotrophs of neonatal rats to SS, which we found, is particularly interesting. As has already been stated above, experiments on primary cultures of pituitary cells [8] have demonstrated the low reactivity of the somatotrophs of newborn rats to SS compared with prepubertal and adult animals. Thus our results, together with the data in [8], are evidence of reciprocal changes in responses of the lactotrophs and somatotrophs to SS in the early stages of postnatal development.

It follows from our findings that lactotrophs are present in the population of pituitary cell cultures from neonatal rats in comparatively small numbers, as is confirmed by the low level of basal PRL secretion. However, the mechanisms of PRL synthesis and secretion in these few cells correspond precisely to those in the lactotrophs of rats in a later period of development. This is shown by the adequate or even enhanced reactivity of cultures from neonatal animals under the influence of hypothalamic regulators. These properties of the lactotrophs of neonatal rats, found in the present investigations, correlate well with data in the literature on the high concentration of TRH receptors [6] and active binding of SS [9] by the pituitary gland of rats during the 1st days after birth.

In conclusion, it must be emphasized that primary monolayer cultures of pituitary cells obtained from animals of different ages, and grown in parallel rows of the same 96-well microtitration panel, proved to be useful objects with which to study the postnatal development of the mechanisms of regulation of the lactotrophic function of the pituitary gland.

LITERATURE CITED

1. V. V. Abramova and V. P. Fedotov, *Probl. Éndokrinol.*, No. 2, 68 (1982).

2. I. S. Komolov, I. Fazekas, L. G. Morozova, et al., *Probl. Éndokrinol.*, No. 2, 60 (1978).
3. I. S. Konolov, V. P. Fedotov, D. Rappau, et al., *Byull. Éksp. Biol. Med.*, No. 4, 481 (1988).
4. H. A. Perez-Arce, "Effect of estradiol on pituitary lactotrophic function in vivo and in vitro," Dissertation, Candidate of Medical Sciences, Moscow (1979).
5. V. P. Fedotov and I. S. Konolov, *Vest. Akad. Med. Nauk SSSR*, No. 2, 87 (1983).
6. A. Banerjee and C. Prasad, *Endocrinology*, **110**, 663 (1982).
7. H. T. Chen, *Endocrinology*, **120**, 247 (1987).
8. L. Cuttler, J. B. Welsh, and M. Szabo, *Endocrinology*, **119**, 152 (1986).
9. A. Enjalbert, L. Tapia-Arancibia, M. Reutort, et al., *Endocrinology*, **110**, 1634 (1982).
10. O. Khorram, L. R. De Palatis, and S. M. McCann, *Endocrinology*, **115**, 1698 (1984).
11. T. Ohkura and N. Hagino, *Neuroendocr. Lett.*, **8**, 39 (1986).
12. S. R. Ojeda and S. M. McCann, *Endocrinology*, **95**, 1499 (1974).
13. C. Oliver, R. L. Eskay, and J. C. Porter, *Biol. Neonate*, **37**, 145 (1980).
14. P. Walker, J. H. Dussault, G. Alvarado-Urbina, et al., *Endocrinology*, **101**, 782 (1977).
15. J. B. Welsh, L. Cuttler, and M. Szabo, *Endocrinology*, **119**, 2368 (1986).