EXPERIMENTAL BIOLOGY

Increased Sensitivity of Neonatal Rat Pituitary Cells to Bromocriptine and Melatonin

V. I. Gudoshnikov and V. P. Fedotov

UDC 616.432-018.1-053.31-02:615.252.433]-092.9-07

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 115, № 2, pp. 197-199, February, 1993
Original article submitted 30 July, 1992

Key Words: *pituitary cells; macromolecular synthesis; dopaminergic agonist; melatonin; postnatal development*

It has been established that dopamine, released by tuberoinfundibular neurons into the pituitary portal vein, is the main component of the prolactostatin activity of the hypothalamus. Earlier we showed a pronounced inhibitory effect of dopamine on prolactin secretion in primary cultures of neonatal, prepuberal, and adult rat pituitary cells during a brief (2-3 hours) exposure to bromocriptine [5]. In addition, we revealed a considerable inhibitory effect of the dopaminergic agonist bromocriptine on DNA and total protein synthesis in the adenohypophysis cells [6], as well as on the ^{3}H-thymidine labeling index in lactotroph nuclei and on prolactin secretion by adult rat adenohypophysis cells during prolonged (2-3 days) exposure to melatonin [4]. However, the experiments mentioned did not deal with the age-related peculiarities of chronic bromocriptine influence upon macromolecular synthesis in pituitary cells. Yet the establishment of a dopaminergic influence upon the adenohypophysis (inhibitory dopamine tonus) during the early postnatal period may be one of the inhibitory components affecting not only the release of prolactin, but also the proliferation of adenohypophysis cells, expressed, for instance, in the form of macromolecular synthesis (DNA, RNA, and total protein). Other components of the neurohumoral regulation network influencing the proliferation rate in the adenohypophysis may be the products of the pineal body, specifically, melatonin. For instance, epiphysectomy of adult rats was shown to be followed by a rise of ornithine decarboxylase activity in the adenohypophysis (this enzyme is a key factor in the biosynthesis of the polyamines necessary for maintaining the growth of various tissues) [13]. A transplacental pituitary-inhibitory action of melatonin was also shown, i.e., administration of melatonin to rats on the 17th-20th day of pregnancy led to a reduction of the relative weight of the pituitary in female offspring on the 80th day of postnatal life [15]. It is significant that in our experiments the conditioned medium of cultured pinealocytes inhibited prolactin secretion in primary cultures of adult rat adenohypophysis cells [1].

Here we present findings on the influence of bromocriptine and melatonin on macromolecular synthesis in rat pituitary cells.

Laboratory of Biological Studies of Hormone Compounds, Institute of Experimental Endocrinology, Russian Academy of Medical Sciences, Moscow
(Presented by Yu. A. Pankov, Member of the Russian Academy of Medical Sciences)

MATERIALS AND METHODS

Neonatal (6-7 days old) and adult (90-120 days old) Wistar rats of both sexes served as donors of pitui-

0007-4888/93/0002-0202$12.50

tary tissue. Primary cultures of pituitary cells were established according to Komolov's method [3] in modification [2]. Such primary cultures proved to be good model systems in the study of age-related specific features of the chronic influence of corticosteroids on macromolecular biosynthesis in pituitary cells [2]. Assays of cellular DNA synthesis using ^{3}H-thymidine and total intracellular protein synthesis using ^{14}C-leucine have been described elsewhere [6]. Melatonin (Serva) and bromocryptine (CB-154) (Sandoz, Switzerland) were used in this study. In the study of the chronic effect of melatonin and bromocriptine, the agents were added to 3-day cultures, and the experiment was terminated after the addition of labeled precursors on the 6th day. The short-term (2.5 hours) effect of bromocriptine was studied in a 6-day culture of pituitary cells. The cell cultures were maintained in medium 199 supplemented with 1% fetal calf serum. The results are presented as $M \pm m$; the statistical analysis was performed using Student's t test.

RESULTS

As shown in Fig. 1, bromocriptine in concentrations of 10^{-9}-10^{-7}M weakly, but still in a dose-dependent fashion, inhibits DNA synthesis in neonatal rat pituitary cells. However, in a dose of 10^{-7}M it effectively inhibited DNA synthesis in adult rat pituitary cells. The effect of bromocriptine on total protein synthesis in neonatal and adult pituitary cells was less pronounced, reliable inhibition being recorded in doses of 10^{-8}-10^{-7}M. Short-term (2.5 h) treatment of neonatal pituitary cells with bromocriptine (10^{-7}M) inhibited total protein synthesis but did not affect DNA synthesis (Table 1).

Melatonin in a dose range of 10^{-8}-10^{-6}M induced a weak, dose-dependent decrease of DNA synthesis in neonatal rat pituitary cells but failed to affect this parameter in adult cells (Fig. 2). It produced a biphasic effect on total protein synthesis by neonatal rat pituitary cells: synthesis was slightly enhanced for a dose of 10^{-8}M, slightly inhibited for a dose of 10^{-6}, and unaffected for 10^{-7}M. It is also significant that melatonin given in the dose range of 10^{-8}-10^{-6}M did not cause any significant variations in total protein synthesis by adult rat pituitary cells.

Thus, the results obtained revealed a higher sensitivity of neonatal rat pituitary cells to the cytostatic action of bromocriptine when compared with adult cells. Moreover, melatonin exhibited aregulatory effect upon macromolecular synthesis only in neonatal cells. It is worthwhile comparing these results with the data obtained by others. The level of dopamine in the peripheral blood of fetuses of rats in late pregnancy has been shown to exceed that in the

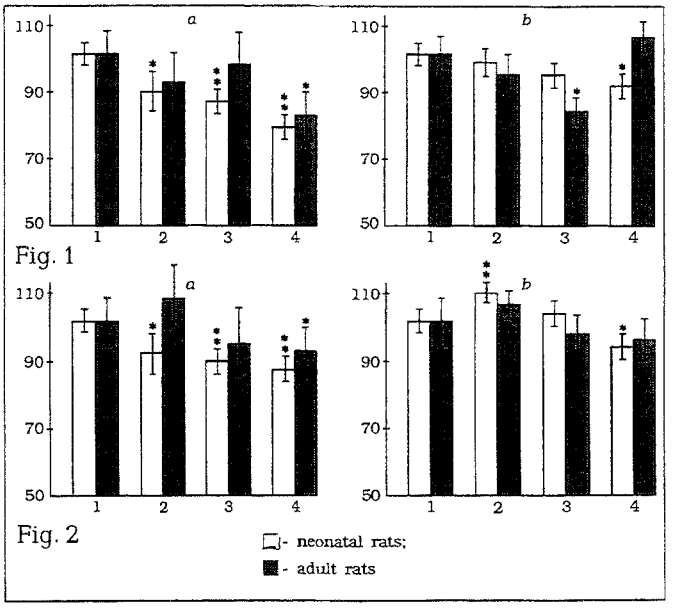

Fig. 1. Effect of bromocriptine on DNA (*a*) and total protein (*b*) synthesis in cultured pituitary cells taken from rats of different age. 1) control; 2, 3 and 4) bromocriptine in doses 10^{-9}M, 10^{-8}M, and 10^{-7}M, respectively. Here and in Fig. 2: ordinate: labeled precursor incorporation (percent of control; background levels of biosynthetic activity are the same as in [2]); n = 12 in control groups and 5–6 in experimental groups; one asterisk: $p<0.05$, two asterisks: $p<0.001$ when compared with respective control; duration of bromocriptine exposure is 3 days.

Fig. 2. Effect of melatonin on DNA (*a*) and total protein (*b*) synthesis in cultured pituitary cells taken from rats of different age. 1) control, 2, 3, and 4) melatonin in doses 10^{-8}M, 10^{-7}M, and 10^{-6}M, respectively.

maternal blood. During the first days post partum the level of dopamine in the systemic circulation does not decrease, whereas the concentrationS of noradrenalin and adrenalin fall during that period [7]. It is supposed that the sorce of dopamine circulating in the fetal blood may be extra-adrenal chromaffin tissue and/or intensively fluorescing small cells situated in the upper cervical ganglia and carotid bodies. Significantly, the functional activity of such cells, unlike the extra-adrenal chromaffin tissue, does not require glucocorticoid, activation, this apparently being responsible for the resistance of cells to involution during the stress-hyporeactive phase in the early postnatal period (see references in [7]). It is also important that the lactotrophic function of neonatal rats shows a high sensitivity to dopamine and its agonist *in vitro* [5,8] as well as to the dopamine antagonist *in vivo* [11]. Hence, in the neonatal period and, to a much lesser degree, in adults the dopaminergic pathway may participate in the regulation not only of prolactin, but also of the proliferative activity in the pituitary cell population as a whole; moreover, the cytostatic reaction requires precisely a chronic influence of inhibitory dopaminergic tonus on the pituitary (see Table 1 and Fig. 1).

Melatonin synthesis begins in the pineal body of rats only in the second week after birth [9]. It is well known that melatonin (10^{-9}-10^{-7} M) inhibits the secretion of luliberin-stimulated luteinizing hormone and cyclic adenosine- and guanosine-monophosphate production in pituitary cells cultured *in vitro* and in later period of neonatal rats (up to 15th day), but is absolutely ineffective in later periods (at the age of 20-30 days as well as in adults) [10,14].

The source of melatonin for suckling rats may be the maternal milk [12]. Thus, the revealed inhibitory effect of melatonin on DNA synthesis in neonatal but not adult rat pituitary cells shows that, as in the case of dopaminergic tonus, the contribution of the pineal body hormone to the regulation of pituitary cell proliferative activity may be restricted to the neonatal period; however, the restriction is relative regarding the dopaminergic mechanism and absolute for melatonin.

In conclusion, we would point out that pituitary cell primary cultures obtained from rats of various age have repeatedly proven their high efficiency as model systems in the study of the formation of hormonal regulation of the functional and proliferative activity of the adenohypophyseal cells during postnatal ontogenesis [2,5]. They have also proved to be extremely useful in disclosing the age-related features of the effect of neurohumoral factors on the pituitary cells, both in short-term assays, e.g., in the presence of the catecholamines serotonin and acetylcholine (data in press) and in assays with prolonged (chronic) exposure (the results of this report). Therefore, the development of this research direction, including the combination of chronic exposure to comparatively stable hormones (e.g., steroid and thyroid hormones), followed by short-term treatment with more labile factors (peptides, neurotransmitters), promises to be fruitful. Evidently, such an approach would help in the modeling of the maturation of the regulation of function and proliferation in the rat pituitary, a process which involves an increase in the hormone secretion of the peripheral endocrine glands in the early postnatal period.

TABLE 1. Effect of Short-Term (2.5 h) Exposure to Bromocriptine (10^{-7}M) on DNA and Total Protein Synthesis in Cultures of Neonatal Rat Pituitary Cells

Group of rats	Incorporation of label (percent of control level)	
	^{3}H-thymidine	^{14}C-leucine
Control	100±2.37	100±1.86
Bromocriptine	98.9±3.86	79.8±2.45
	$p>0.05$	$p<0.01$

Note: Background levels of biosynthetic activity were within 2000-3800 cpm per well.

REFERENCES

1. V. I. Gudoshnikov, I. S. Komolov, I. Fazekash, *et al.*, *Probl.Endokrinol.*, **34**, № 1, 64 (1988).
2. V. I. Gudoshnikov and V. P. Fedotov, *Ibid.*, **38**, № 1, 61 (1992).
3. I. S. Komolov, L. G. Morozova, I. Fazekash, *et al.*, *Bull.Eksp.Biol.*, **85**, № 2, 215 (1978).
4. I. S. Komolov, V. P. Fedotov, D. Rappai, *et al.*, *Ibid.*, **105**, № 4, 481 (1988).
5. V. P. Fedotov, V. I. Gudoshnikov, I. S. Komolov, *et al.*, *Ibid.*, **113**, № 4, 402 (1992).
6. V. P. Fedotov, V. I. Gudoshnikov, and A. Ya. Kurishko, *Farmakol. i Toksikol.*, № 5, 57 (1988).
7. N. Ben-Jonathan, *Life Sci.*, **23**, 39 (1978).
8. O. Khorram, L. R. DePalatis, and S. M. McCann, *Endocrinology*, **115**, 1698 (1984).
9. D. C. Klein, M. A. A. Namboodiri, and D. A. Auerbach, *Life Sci.*, **28**, 1975 (1981).
10. J. E. Martin and C. Sattler, *Endocrinology*, **105**, 1007 (1979).
11. S. R. Ojeda and S. M. McCann, *Ibid.*, **95**, 1499 (1974).
12. S. M. Reppert and D. C. Klein, *Ibid.*, **102**, 582 (1978).
13. G. Scalabrino, M. E. Ferioli, D. Modena, *et al.*, *Ibid.*, **111**, 2132 (1982).
14. J. Vanecek and L. Vollrath, *Ibid.*, **126**, 1509 (1990).
15. M. K. Vaughan, G. M. Vaughan, and W. K. O'Steen, *J.Endocrinol.*, **51**, 211-212 (1971).