Morphological and Functional Characteristics of Human Pituitary Lactotrophic Adenomas in Cell Cultures

I. S. Komolov, A. A. Bulatov, E. E. Makarovskaya, G. P. Elizarova, S. Yu. Serpukhovitin, S. Yu. Kasumova, and E. I. Marova

UDC 616.832-006.55-076.5

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 118, № 11, pp. 543-546, November, 1994
Original article submitted February 15, 1994

Some features of the morphological cellular structure of prolactin secreting human pituitary adenomas and their secretion of prolactin and somatotropic hormone in primary suspension cultures were investigated. A possible *in vitro* proliferation of lactotrophs was established. The inhibitory effect of somatostatin and its synthetic analog sandostatin, on prolactin secretion in prolactinomas was found to be less than in somatotropic hormone-secreting pituitary tumors.

Key Words: *prolactin; somatotropic hormone; pituitary adenoma*

Lactotrophic adenomas or prolactinomas are the mostly widespread human pituitary tumors. According to Burrow *et al.* [7], they amount to 50% of all pituitary tumors identified at autopsy. According to other authorities [10], 30% of operated pituitary adenomas are prolactinomas. Statistical data on pituitary tumors in subjects under 20 years have put the ratio of prolactinomas at 85.9% [8]. Prolactinoma cells are mainly represented by lactotrophs secreting excessive amounts of prolactin (PRL) into the blood. PRL hypersecretion leads to significant disturbances in hormonal homeostasis, galactorrhea, hypogonadism manifested in menstrual cycle disturbance and infertility in women, and reduced libido and potency, and inhibited spermatogenesis in males [3].

Earlier studies [2,5] on monolayer cultures of pituitary adenoma cells in patients with acromegaly described morphological and functional characteristics of somatotropic adenoma cells, including characteristic features of secretion of somatotropic hormone (STH), as well as PRL, which is synthesized in abundance by adenoma cells. While developing methods of culturing and conducting investigations on lactotrophic adenoma cells, we were confronted with the need to search for other ways of their long-term preservation *in vitro* due to insufficient adhesive ability of these cells and their low survival rate under conditions similar to those used earlier during culturing with somatotropin.

The present work is focused on the isolation and maintenance in primary cultures of cells of pituitary lactotrophic adenomas excised from patients during surgery, a study of the morphological characteristics of these cells, and their hormonal secretion upon the direct action of pituitary regulators: thyroliberin (TRH), somatostatin (SS), and its synthetic analog sandostatin (SDS).

MATERIALS AND METHODS

Tumor tissue was obtained from material removed during surgery on three patients (G, V, and K) with lactotrophic pituitary adenomas with a high PRL level in the serum and a marked clinical picture, and on one patient (B) with mixed lactosomatotropic adenoma and high PRL and STH level in the serum and prevailing clinical symptoms of hyperprolactinemia. The tissue was dissociated and a primary cell suspension was obtained by treatment with 0.25% trypsin followed by me-

Research Center of Endocrinology, Russian Academy of Medical Sciences, Moscow. (Presented by A. N. Konovalov, Member of the Russian Academy of Medical Sciences)

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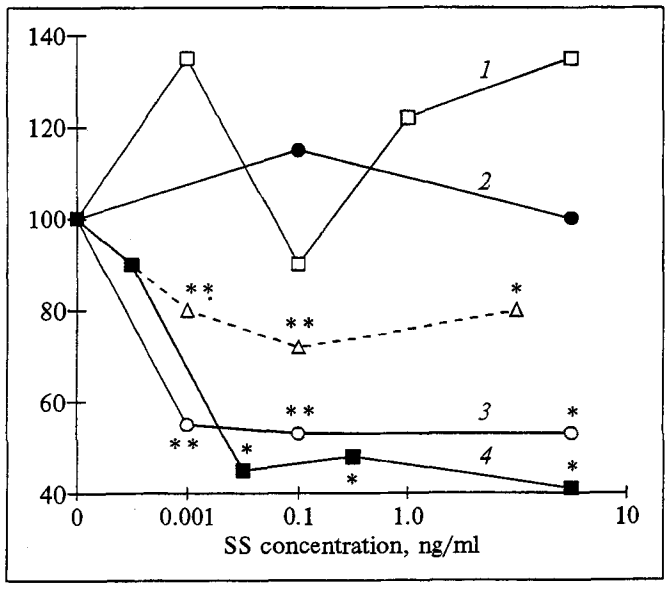

Fig. 1. Effect of SS on 24-hr secretion of PRL (continuous line) and STH (broken line) by primary cell cultures of PRL-secreting (patients G, K and V) and mixed (patient B) pituitary tumors, % of the control. One and two asterisks denote $p<0.05$ and $p<0.01$, respectively. *1* – patient K; *2* – patient G; *3* – patient B; *4* – patient V.

chanical dissociation into single cells. A detailed description of the method was presented earlier [5].

The cell suspension was seeded into a 50 ml plastic flask (Novo) and incubated in RPMI-1640 medium (Flow) with the addition of 10% fetal calf serum (Calbiochem), 10 mM HEPES, and penicillin (50 U/ml). The cells in the flask were placed in an incubator at 37°C in an atmosphere of air with 5% CO_2 and kept as suspension cultures. The cell suspension was removed from the flask after three days, centrifuged, resuspended in fresh incubation medium, and seeded either into 24-well plastic macropanels (10^6 cells/well in 1.0 ml medium) or into 96-well micropanels (Flow) at a rate of 10^5 cells/well in 0.1 ml medium.

TABLE 1. PRL and STH Level in Incubation Medium (μg/ml) of Cultured Pituitary Adenoma Cells 24 hours after Its Replacement ($M \pm m$)

Patient	PRL	STH
G	40.56±5.62 (4)	0*
V	9.98±1.86 (4)	0
K	3.56±1.03 (5)	0
B	2.25±0.51 (4)	1.83±0.21 (5)

Note. Asterisk: lower than the sensitivity of the method for hormone determination (<0.2 ng/ml). Here and in Table 2 the number of cultures assayed is given in parentheses.

The macropanels were centrifuged for cell sedimentation and adhesion on coverslips, and then the cells were fixed with paraformaldehyde and stained with hematoxylin and eosin.

Incubation medium (0.1 ml) containing TRH (Institute of Organic Synthesis, Latvia) was applied to micropanel wells for one hour; SS (Serva) or SDS (Sandoz) was applied for 24 hours. After the termination of incubation, aliquots were collected and frozen and stored at -40°C prior to analysis. The PRL and STH level in the incubation medium was determined by means of radioimmunological systems developed at the Institute of Experimental Endocrinology, Endocrinology Research Center, Russian Academy of Medical Sciences, on the basis of highly purified hormones and monospecific antisera [1].

RESULTS

Despite the marked clinical manifestations, pituitary lactotrophic adenomas are usually represented by microadenomas of relatively small size (1-10 mm). This makes it extremely difficult, and in some cases impossible, to perform complex morphological and functional studies on material taken from the same patient.

Histological analysis of the sections from the material obtained during surgery revealed large-cell pituitary adenomas in two cases (patients G and V), and semismall-cell pituitary adenomas in the other two cases (patients K and B), which consisted of diffusely growing tumor cells (mainly eosinophilic cells). They contained large nuclei of a round or oval shape surrounded by cytoplasm with different degrees of eosinophily.

Single dividing cells were found in histological sections of the pituitary adenoma of patient V. Mitotic figures were also observed in the preparations of 3-4-day cultures of the same patient, their percentage being higher than before culturing *in vitro*. This is evidence of the possible enhancement of proliferative activity of tumor lactotrophs in suspension cultures *in vitro*.

The prolactinoma cells differed in the intensity of hormonal secretion by the same number of cells in culture (Table 1). In one case (patient G) hormonal secretion was very high, in another (patient V) relatively high, and in a third case (patient K) relatively low. In contrast to STH-secreting tumors [5], prolactinoma cell cultures in the present study were more homogeneous and, judging by the results of radioimmunological determination of STH, did not contain STH-secreting cells or lactosomatotrophs. Cells of mixed adenoma type

(patient B) produced fairly large amounts of both PRL and STH.

Investigation of the effects of TRH, which is known to be a potent stimulator of thyroid stimulating hormone and PRL [4,6], demonstrated a marked decrease in the sensitivity of prolactinoma cells (Table 2). Earlier studies [2] on lactotroph-containing somatotropinomas showed that TRH stimulated PRL secretion in cell cultures in a dose-dependent manner one hour after addition to the medium, while the present study on lactotrophic adenomas showed that TRH only once brought about a reliable, though small, elevation of the PRL level, and only at a maximal concentration of 10 ng/ml in the incubation medium (Table 2).

These results may indicate that disturbances in the mechanism of dopaminergic action in the process of prolactinoma oncogenesis and, as a result, a sharp and uncontrolled increase in the rate of biosynthesis and secretion of PRL either weaken or completely block the mechanism of pituitary stimulatory regulation of TRH, or lead to the appearance of a clone of tumor lactotrophs insensitive to TRH. This suggests that blocking of TRH action is a protective reaction on the part of the organism which prevents the further rise of the already extremely high PRL level.

On the other hand, these results give good grounds for hypothesizing that regulation of PRL secretion by pituitary stimulators occurs in concern with secretion of other pituitary hormones, STH in particular. According to the study of Thapar *et al.* [10], the pituitary cell is regarded as a multifunctional unit capable of producing not one but several biologically active peptides. This concept suggests the existence of interhormonal relationships in the mechanism of biosynthesis regulation and pituitary hormone secretion. Oncogenesis gives rise either to monofunctional clones or to the appearance of secondary hypopituitarism due to pressure on the pituitary by the growing tumor or the frequently occurring hemorrhages, which can also disrupt interhormonal relationships.

Figures 1 and 2 present the results of 24-hr action of SS and SDS on PRL secretion. It can be seen that SS inhibited PRL secretion in only one out of three studied prolactinoma cell cultures (Fig. 1). SDS exhibited a higher activity than SS in respect to lactotrophic function: in two cases it significantly decreased PRL production (Fig. 2). SS and SDS inhibited secretion of both STH and PRL in a dose-dependent manner in the cells of mixed PRL- and STH-secreting pituitary adenoma (patient B). We also earlier demonstrated a high sensitivity

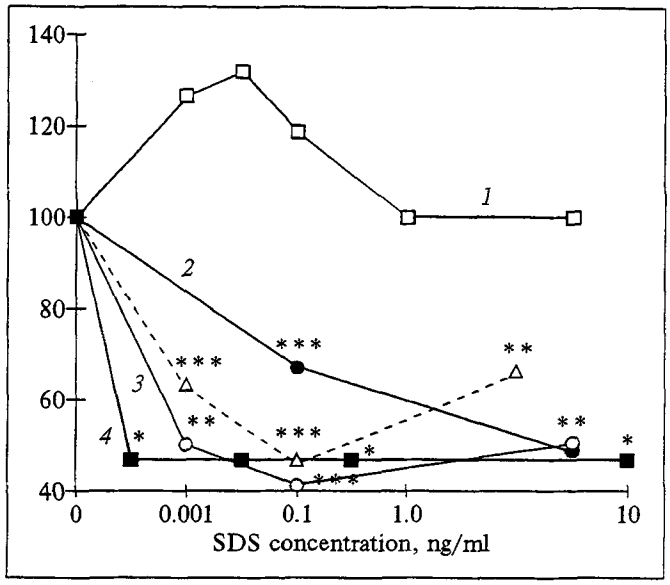

Fig. 2. Effect of SDS on 24-hr secretion of PRL (continuous line) and STH (broken line) by primary cell cultures of PRL-secreting (patients G, K, and V) and mixed (patient B) pituitary tumors. One, two, and three asterisks denote $p<0.05$ and $p<0.01$, and $p<0.001$, respectively. *1* – patient K; *2* – patient G; *3* – patient B; *4* – patient V.

of lactotrophs to SS in STH-secreting adenomas comparable to the reaction of somatotrophs [2].

Although the somatotropic function, rather than lactotrophic function of the intact pituitary is considered to be the main target for hypothalamic SS [11], SS is known for its inhibitory effect on PRL secretion by adenohypophysis cells of different animal species, e.g., *in vitro* in rats [9]. Lactotroph reaction to SS is found to be unstable and is often associated with sex and age characteristics of animals, as well as with the age and cell composition of pituitary primary cultures used in the course of the experiments.

Since the sensitivity of PRL-secreting cells to SS is much higher in STH-secreting and mixed pi-

TABLE 2. Effect of TRH on the PRL Level (μg/ml) in the Culture Medium ($M\pm m$). Incubation with TRH for 60 Min

TRH concentrations	PRL level
Patient V.	
Control	4.62±1.7 (3)
0.05 ng/ml	3.21±0.48 (4)
0.5 ng/ml	5.21±0.75 (3)
5 ng/ml	3.34±0.44 (3)
Patient K.	
Control	0.37±0.03 (5)
0.01 ng/ml	0.37±0.06 (4)
0.1 ng/ml	0.41±0.03 (6)
1 ng/ml	0.38±0.02 (6)
10 ng/ml	0.49±0.01* (6)

Note. Asterisk: $p<0.05$.

tuitary adenomas than in "pure" prolactinomas, the question arises again as to whether PRL interaction with STH is involved in the SS effect on lactotrophic function. Thapar *et al.* [10] believe that the existence of lactosomatotrophs supports the concept of an acidophilic cell line of pituitary tumors, where STH- and PRL-secreting cells are of the same origin. Moreover, PRL mRNA was detected in many somatotrophs and STH mRNA in many lactotrophs, respectively. These results suggest that intracellular events involved in biosynthesis regulation and secretion of STH and PRL may have common features, and SS may produce its effect on both hormones.

Thus, the regulatory mechanisms of pituitary functions by hypothalamic stimulators and inhibitors and their action on different pituitary tumor cells suggest the existence of interactions between hypothalamic regulators, as well as paracrine relationships.

This work was supported by the Russian Foundation for Basic Research (project code 93-04-13147).

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