REGULATION OF HORMONAL SECRETION IN PRIMARY CELL CULTURES OF HUMAN SOMATOTROPHINOMAS

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A comparatively new approach to experimental neuroendocrinology, and one which enables the mechanisms of growth and functioning of human pituitary adenomas to be effectively studied, is the use of cultures of cells derived from these tumors. Studies of this kind can provide a basis for discovery of the pathogenesis of the tumor process in the pituitary gland, details of its function, the search for new therapeutic preparations, and the elaboration of rational schemes of treatment of pituitary adenomas.

The aim of the present investigation was to study regulation of hormonal secretion in primary cell cultures of somatotrophinomas. The paper gives the results of a study of the direct effect of hypothalamic regulators — somatostatin (SS) and thyrotrophin-releasing hormone (TRH) — on secretion of somatotrophin (STH) and prolactin (PRL) by somatotrophinoma cells.

METHODS

Tissue of pituitary tumors, obtained at operations from three patients with acromegaly (A, D, and H), aged 24-31 years, was disaggregated into separate cells by the action of 0.25% trypsin solution. A cell suspension was seeded on 96-well plastic micropanels (Flow Laboratories, England) at the rate of 100,000 cells per well, and grown in incubation medium 199 with the addition of 10% bovine fetal serum and 50 U/ml of penicillin in an incubator at 37°C in an atmosphere of air with 5% CO_2. Cell function was studied on 5- to 6-day cell cultures. After a change of medium, incubation medium containing SS (Serva, Germany) or TRH, obtained from the Institute of Organic Synthesis, Riga, was added to the wells for 0.5-1 or 3 h. At the end of incubation aliquots of medium were collected, frozen, and kept at —40°C until required for analysis. Incubation medium with SS or TRH was poured into the wells repeatedly: aliquots of medium were withdrawn after 21-24 h. The content of STH and PRL in the incubation medium was determined by radioimmunoassay using systems developed in the Laboratory of Protein Hormone Standards, All-Union Endocrinologic Research Center, Academy of Medical Sciences, on the basis of the corresponding highly purified hormones and monospecific antisera [1].

RESULTS

High sensitivity of cell cultures of all three somatotrophinomas investigated to SS, as regards the secretion of both STH and PRL, was found (Fig. 1a). Cell cultures of two tumors were exposed to the action of three increasing concentrations of SS: 0.1, 1, and 10 ng/mg ($6 \cdot 10^{-11}$-$6 \cdot 10^{-9}$ M). Maximal lowering of the STH concentration in

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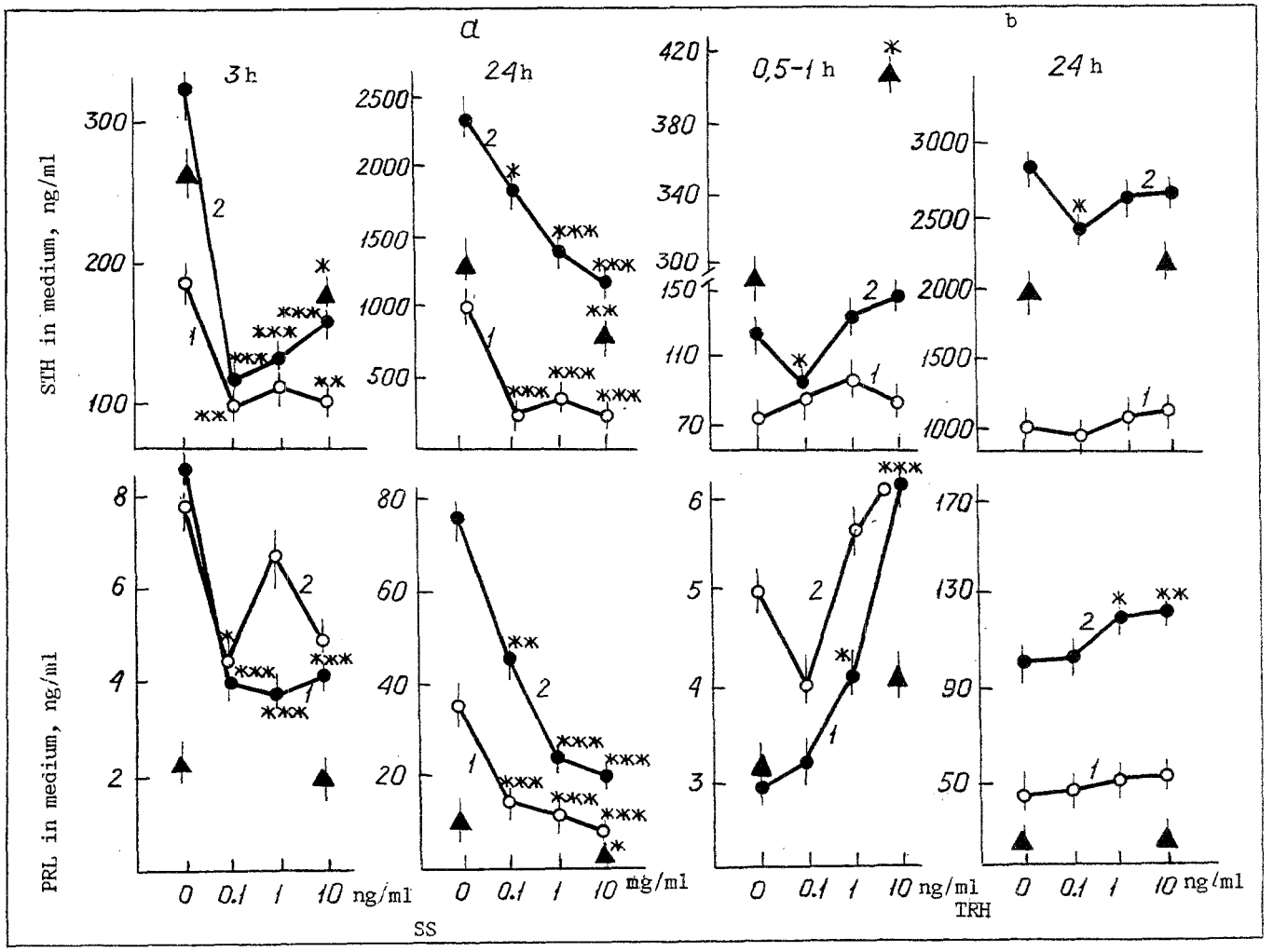

Fig. 1. Secretion of STH and PRL by 5- to 6-day cultures of human somatotrophinoma cells: a) effects of SS; b) of TRH (*p < 0.05, **p < 0.01, ***p < 0.001).

TABLE 1. Effect of Intravenous Injection of TRH (500 μg) on Serum Levels of STH (ng/ml) and PRL (μU/ml) (patient D)

Time after injection of TRH, min	STH	PRL*
0	126,75	258,8
15	100,95	785,7
30	104,30	704,9
60	125,80	417,2
120	95,0	280,8

Note. *) PRL was determined by radioimmunoassay using a kit supplied by the World Health Organization.

the medium compared with the control was observed 3 h after addition of SS to the incubation medium in a concentration of 0.1 ng/ml. A similar effect also was found in relation to PRL secretion. Changes in the state of STH and PRL secretion after exposure to SS for 24 h were closely similar on the whole to effects observed after incubation for 3 h, although the sensitivity of the cells to SS, under conditions of a long exposure, was clearly reduced. For instance, in cell cultures obtained from patient A, the maximal inhibitory effect on STH and TRL secretion occurred in the presence of higher SS concentrations, whereas in cell cultures from patient D, the percentage lowering of the level of the hormones in the medium compared with the control, following long-term exposure to SS, was less than during exposure to SS for 3 h. In cell cultures from patient H, there was no response of the lactotrophs to SS, but somatotrophs continued to react.

Some workers [2] have described the suppressing effect of SS on STH secretion in cell cultures when used in concentrations 10-100 times higher than in our experiments. In one such study [5] even very high doses of SS (1 μg/ml), inhibiting STH release in vitro in four of six somatotrophinomas studied, did not affect mRNA synthesis. Having described disparity between the action of SS on STH secretion and mRNA synthesis, the authors cited state that there is no direct connection between receptor and transcription mechanisms of regulation of hormonal secretion in the pituitary gland.

The significant response of tumor cells secreting PRL to SS suggests that their sensitivity to this hypothalamic regulator, which is specific for somatotrophs but not for lactotrophs, is increased with the development of an adenoma in the pituitary gland, and it indicates the existence of definite interaction between regulation of STH and PRL in somatotrophinomas. Incidentally, according to data in the literature [10], an increase in sensitivity of somatotrophs to bromocryptine and of lactotrophs to SS may be observed in cells of pituitary tumors secreting STH and PRL.

In many organs and systems which have elements of endocrine and nervous tissue in their composition, the presence of thyrotrophin-releasing hormone (TRH) has been found, and on those grounds it has been regarded as a multipurpose regulator of secretion of various hormones. A role for TRH in stimulating release of thyrotrophin and PRL is known [3]. In our own experiments, with short-term exposure (0.5-1 h), TRH in concentrations of 1 and 10 ng/ml ($3 \cdot 10^{-10}$-$3 \cdot 10^{-8}$ M) significantly increased the release of PRL into the medium by tumor cells (Fig. 1b), i.e., the response of the lactotrophs of human pituitary adenoma cells in culture to TRH may be preserved, just as in normal pituitary cells. With an exposure of 24 h, TRH significantly but weakly increased PRL accumulation in the medium only by cells of one of the adenomas studied (patient A). The possibility cannot be ruled out that the initial incubation of the cells with TRH for 0.5-1 h led to refractoriness of the cells to the subsequent addition of a new portion of the stimulator to the culture.

Some workers are of the opinion that TRH may be an inducer of STH secretion in man and animals [4, 7], but experiments on rat adenohypophyseal cell cultures showed that TRH did not affect STH production whether in the short term or in the long term [12]. In some cases it actually inhibited STH secretion slightly during superfusion of pituitary cells [6]. A raised blood level of STH has been described in patients with acromegaly [8], but other workers [9] detected a reaction of this kind in only some patients. It is claimed that there are forms of acromegaly that are sensitive or resistant to TRH.

We found no clear, unequivocal effect of TRH on the somatotrophic function of tumor cells. In one of three cases of short-term exposure to TRH (patient N) a significant increase was found in the STH concentration in the medium. In another case (patient A), on the other hand, exposure of the cells to TRH in low concentration (0.1 ng/ml) for 1 and 24 h was accompanied by a small but significant decrease in STH secretion (Fig. 1).

The effect of intravenous injection of TRH (patient D) on serum STH and PRL levels is shown in Table 1. Comparison with the data in Fig. 1b shows that the effects of TRH on STH and PRL secretion in vivo and on cultures of cells of this patient coincided: in both cases PRL secretion rose but STH secretion was virtually unchanged.

The study of the direct action of TRH in experiments by other workers in vitro showed that when human pituitary tumor cells were maintained in culture the secretory reaction of the somatotrophs to this tripeptide was significant in only 60% of tumors studied, although the sensitivity of the lactotrophs to TRH was observed in all cases [9].

The low sensitivity of the somatotrophs of pituitary tumors of patients with acromegaly to TRH, on the one hand, may indicate that TRH is evidently a natural regulator of STH secretion. Another possible explanation may

be the quite high basal level of STH secretion by tumor cells observed in our experiments, when additional stimulation appeared to be ineffective.

Meanwhile, a more recent communication [11] describing the presence of TRH in the tissue of certain human pituitary tumors, together with the results described above, still leaves open the question of the role of TRH in the regulation of hormone secretion of adenoma cells and the participation of this tripeptide in the pathogenesis of pituitary tumors.

On the whole, our investigations have shown that somatotrophinoma cells in primary cultures remain capable of regulating somatotrophic and lactotrophic functions by hypothalamic hormones (SS and TRH), and that they constitute an adequate model in vitro for the study of the pathogenesis of pituitary neoplasia.

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