ONCOLOGY

Secretory Reactions of Cultured Hypophyseal Growth Hormone-Producing Adenoma Cells to Somatoliberin and Somatostatin

I. S. Komolov, A. A. Bulatov, T. A. Osipova, G. P. Elizarova, S. Yu. Serpukhovitin, and S. Yu. Kasumova

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Somatoliberin stimulates secretion of growth hormone and has no effect on secretion of prolactin in primary cultures of hypophyseal adenoma cells obtained from acromegalic patients. A short-term contact of the cells with somatostatin inhibits secretion of growth hormone, while a long-term contact with this hormone inhibits prolactin production. Somatoliberin abolishes the inhibitory effect of somatostatin on the growth hormone secretion and at high concentrations stimulates it.

Key Words: *hypophyseal adenoma; growth hormone; prolactin; somatoliberin; somatostatin; cell cultures*

Generally, hypophyseal tumors producing growth hormone (GH) are benign neoplasms. They consist of adenomatous glandular cells secreting considerable amounts of GH in the circulation, which results in acromegaly, homeostatic disturbances, and serious chronic disease.

Secretion of GH is often accompanied by secretion of prolactin (PL). Growth hormone and prolactin are produced by individual cells types (somatotrophs and mammotrophs, respectively) or by the same cells (somatomammotrophs [9]).

Phylogenetically, GH and PL originate from the same precursor gene [6], which suggests common mechanisms of production and secretion of these hormones and their interaction at the paracrine and autocrine levels.

Increased hormonal production and alterations of receptors associated with lowered inhibitory effect of hypothalamus or formation of abnormal relationships should be accompanied by pathological shifts in hormonal interactions and in the regulation of hormone production.

In order to test this hypothesis we studied secretion of GH and PL by hypophyseal adenoma cells from acromegalic patients and the effects of somatoliberin (GH releasing factor) and somatostatin (GH inhibiting factor) and compared them with the effect of thyroliberin, a hypothalamic prolactotropic and thyrotropic regulator.

MATERIALS AND METHODS

Hypophyseal tumor specimens were obtained upon surgery from 6 patients with acromegaly, treated with 0.25% trypsin, and homogenized. The procedure was described in detail elsewhere [3]. Cells were seeded (10^5 cells/well) into 96-well plates (Flow), and cultures were grown until confluency in medium 199 containing 10% fetal calf serum and antibiotics at

Endocrinology Research Center, Russian Academy of Medical Sciences, Moscow

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TABLE 1. Effects of Somatoliberin and Thyroliberin on Secretion of GH and PL by Cultured Hypophyseal Adenoma Cells (*M±m*, *n*=6)

Culture No.	Incubation time, h	Control	Somatoliberin, ng/ml						Thyroliberin, 10 ng/ml
			0.1	0.5	1	5	10	30	100	
GH concentration in culture medium, ng/ml										
I	1	179.8±12	276±5.3*		368±7.0*		352±10.4**		451±16.7**	224±19.8
	3	251.3±7.6			430±51.7**		585±49.4**		645±48.4**	
	24	1191±36.2						1989±50.1*		
II	1	5500±250	5524±135		5348±664					
III	1	1120±536	16286±1343**		19300±1284*					
IV	1	2355±40	2634±233		3915±440**		3551±332**			136.4±9.6
V	1	124±9.0								
VI	1	105±7.8	137.7±10***	225.4±14*		242±19*	187.5±12.8*			
PL concentration in culture medium, ng/ml										
IV	1	6.02±0.4	5.85±0.87		6.37±0.43		5.55±0.34			6.36±0.4*
V	1	2.9±0.24								

Note. Here and in Table 2: *$p<0.001$, **$p<0.01$, ***$p<0.05$ compared with control.

37°C in an 95% air/5% CO_2 atmosphere. After 4-5 days, the cells were washed with medium 199 and incubated in culture medium containing synthetic preparations of somatoliberin (groliberin, GH releasing factor (1-29)NH_2, Kabi Pharmacia), somatostatin (Serva), or thyroliberin (Institute of Organic Synthesis, Latvia) for 1, 3, or 24 h. After incubation, aliquots of the culture medium were collected and stored at -40°C. The GH and PL contents were determined radioimmunologically using test systems developed at the Endocrinology Research Center on the basis of highly purified hormones and monospecific antisera.

RESULTS

The data on the effect of somatoliberin on the secretion of GH and PL are summarized in Table 1. Somatoliberin significantly increased the GH content in 4 out of 5 cell cultures in the presence of somatotropin. The effect was observed 1 h after its addition, remained at essentially high level for 3 h, and persisted for 24 h after the addition of somatoliberin, being dose-dependent in most cases. Somatoliberin had no effect on the secretion of PL.

In one experiment, hypophyseal adenoma cells (culture II) were resistant to somatoliberin (Table 1). It remains unclear why some cell cultures lost sensitivity to the GH-releasing factor. Previously, we revealed a relationship between the response of mammotrophs to thyroliberin and basal secretion of PL [2]. The effects of thyroliberin were very weak or were not observed in adenoma cells with a high basal level of PL secretion. Presumably, this is not true for somatoliberin. Hormone-secreting activity of resistant cultures was intermediate (5550 ng/ml/h) between that of cultures with low (179.8 ng/ml/h) and high (11,120 ng/ml/h) hormone-secreting activities. It can be assumed that in contrast to PL-secreting cells, some reserves of somatotrophs provide their response to stimulation in the form of GH production and secretion. The possibility that this phenomenon is due to the presence of reserve (accumulation) pool of GH in somatotrophs [12] but not in mammotrophs cannot be ruled out.

The addition of thyroliberin markedly increased the rate of PL release without any statistically significant effect on the GH content of the incubation medium even at comparatively high concentration (10 ng/ml) of the tripeptide stimulator (Table 1). These results are consistent with our previous findings [1]. It should be noted that they argue with some clinical observations that intravenous administration of thyroliberin elevates blood GH content in a considerable number of patients with acromegaly [4]. It

TABLE 2. Effects of Somatostatin and Its Combination with Somatoliberin on Secretion of GH and PL by Cultured Hypophyseal Adenoma Cells (*M±m*, *n*=4-6)

Culture No.	Incubation time, h	Control	Somatostatin, ng/ml			Somatostatin (1 ng/ml)+ somatoliberin (0.1 ng/ml)	Somatostatin (1 ng/ml)+ somatoliberin (10 ng/ml)
			0.1	1	10		
GH concentration in culture medium, ng/ml							
IV	1	2355±40	2227±89	2012±37*	1815±156*	2453±67	2606±55*
V	3	315±10	122±12*	139±11*			
	24	2432±101	1814±200***	1410±79*	1256±71*		
PL concentration in culture medium, ng/ml							
IV	1	6.02±0.4	4.17±0.9	6.29±0.9	4.4±1.5	5.92±0.7	5.96±1.5
V	3	8.48±0.5	4.02±0.5*	3.92±0.28**	4.2±0.18*		
	24	73.9±7.0	44.4±5.1**	23.1±0.7*	20.6±0.74*		

should be noted that predominantly direct effects of regulators are examined *in vitro*. Therefore, the possibility cannot be ruled out that the stimulatory effect of exogenous thyroliberin in patients with somatotropic adenoma is indirect, being mediated by activation by somatoliberin-coupled system of GH regulation. Some researchers believe that thyroliberin-stimulated release of GH in acromegalic patients is associated with ectopic somatoliberin-producing tumors [5].

Previously, we reported that somatostatin inhibits the production of GH and PL [1,2]. In the presence of somatoliberin, the inhibitory effect of somatostatin on GH secretion was observed 1 h after its contact with cells (Table 2).

The inhibitory effect of somatostatin on PL secretion is slower than that on GH secretion. Our results indicate that somatostatin affects primarily the somatotropic but not lactotropic function. However, a long-term contact with somatostatin results in inhibition of PL secretion by cultured tumor cells (Table 2).

The sensitivity of PL-secreting cells to somatostatin, a regulatory factor specific for somatotrophs, increases during the development of GH-secreting hypophyseal adenoma [1]. Presumably, the delayed inhibition is associated with the specific mechanism of action of somatostatin on PL secretion, which is different from that on GH secretion. It cannot be ruled out that inhibition of PL secretion requires realization of the effect of somatostatin in somatotrophs, which initiates an additional paracrine link coupled to the mechanisms of somatostatin action on mammotrophs.

Taken together with published data, our results indicate that PL-producing cells in GH-secreting adenomas differ from mammotrophs in hypophyseal PL-secreting adenomas. They have properties of somatotrophs, although the selectivity of specific hypothalamic factors, including somatoliberin, on hormonal secretion is preserved. This is consistent with the findings of others [10].

Somatoliberin not only abolished the inhibitory effect of somatostatin on GH secretion but in some cases, particularly at high concentrations, also stimulated the release of somatotropic hormone from adenomatous cells (Table 2). The prevalence of activatory effect over inhibitory effect upon the addition of both somatoliberin and somatostatin is probably an important functional peculiarity of adenomatous cells. It is likely that it is associated with the effect of somatoliberin on both secretory and reserve pools of GH in a GH-secreting cell or with different amounts of somatotrophs with the receptors for somatoliberin and somatostatin reacting to these factors by modulations in GH secretion [8,13]. The possible role of somatostatin in preservation and even potentiation of stimulatory effect of somatoliberin on the secretion of somatotropic hormone should be taken into consideration [7,14].

It should be stressed that the spreading of the effect of specific regulators of hormonal secretion in the hypophysis over functionally similar cells is involved in the pathogenesis of GH-secreting hypophyseal tumors. In a developing hypophyseal tumor, disturbances in hormonal regulation are associated with the emergence of bihormonal cells or quantitative redistribution of somatotrophs and mammotrophs. It is likely that endogenous regulatory peptides [11,15] in somatotropic tumors impair autocrine and paracrine links.

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