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Short communication

TRH synthesis in "mute" thyrotropinomas: cause-effect or coincidence?

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Abstract

In the pathogenesis of thyrotropin (TSH) immunopositive pituitary adenomas, trigger mutagenetic events are well recognized. However, the way towards a clinical significant tumor is followed under the pressure of growth factors, among which the intrapituitary synthesis of releasing factors could bring a significant contribution. In this study, the production of thyrotropin releasing hormone (TRH) and beta TSH chain was evaluated at the mRNA level by *in situ* hybridization and end product level by immunohistochemistry, in 18 patients submitted to neurosurgery for pituitary macroadenomas. The hormonal sampling showed abnormal secretion for FSH in 5 and TSH in 4 patients. Seven cases were immunopositive for TSH, and expressed TSH β mRNA. All but one out of these expressed also TRH mRNA. FSH immunoreactivity was documented in 12/ 18, only one of these being negative for TRH mRNA. Paracrine TRH could contribute to the patogenesis of these "mute" adenomas.

Keywords: transdifferentiation - radioactive *in situ* hybridization - pituitary neoplasia - TSH

Introduction

Thyrotropin secreting tumours with central thyrotoxicosis are reported with the lowest frequency (less than 3% of the pituitary adenomas), the total world number being in 2000 less than 320 [1]. The routine application of immunohistochemical methods in pituitary pathology revealed that a high proportion of the initially considered non-functioning pituitary adenomas are in fact immunopositive for hormones devoid of clinical relevant activity [2], mostly FSH and LH [3], but also TSH. Moreover, it was shown that most of these adenomas were plurihormonal, expressing and secreting free alpha-subunit together with the intact glycoproteic hormones. In some acromegalic patients, together with autonomous growth hormone secretion, there is a co-secretion of TSH (often devoid of clinical relevant effects) and prolactin. This can be explained by the common transcription regulating system for somatotrophs, thyrotrophs and lactotrophs, represented by Pit-1 [4].

Another regulation system shared by thyrotrophs and lactotrophs is expression of TRH receptors. It is

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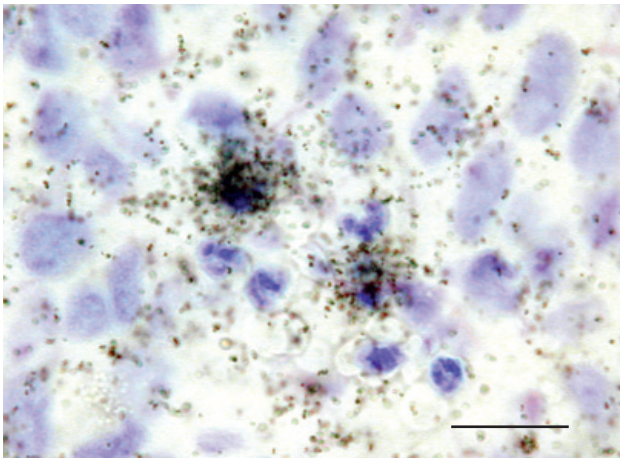

Fig. 1 TRH ISH - S35 a non-functioning pituitary adenoma. Bar = 30μm.

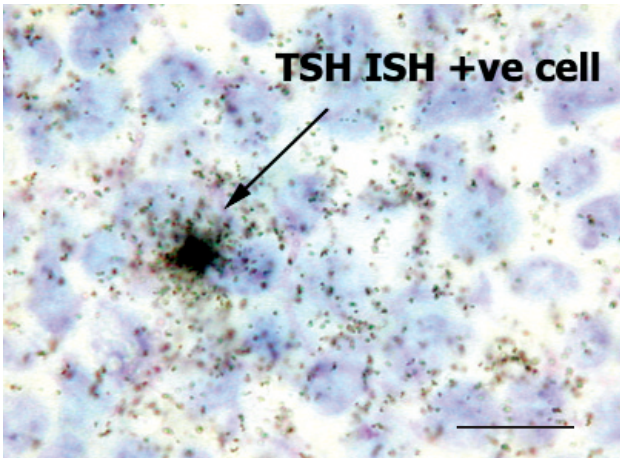

Fig. 2 TSH ISH -S35. Bar = 30μm.

well established that TRH stimulates both categories of cells, effect that becomes clinically relevant in hypothyroidism following thyroidectomy or antithyroid drugs. The pituitary shifts toward lactotroph and thyrotroph hypertrophy and hyperplasia. The normal ultrastructure modifies, producing the so-called thyroidectomy cells: large cytoplasmic processes with dilated endoplasmic reticulum, large lysosomes, small and sparse secretory granules immunoreactive for TSH. In addition, somatotroph cells may undergo transdifferentiation towards TSH secretion, resembling thyroidectomy cells. These are immunoreactive for both GH and TSH. However, these changes are reversible after thyroid hormones substitution [5]. Lack of thyroid hormones trigger these changes through TRH overexpression.

Since the TRH receptors were reported to be normal as both structure and density in pituitary adenomas [6], it was tempting to evaluate the possible paracrine synthesis and release of this peptide in relation to the gonadotrophs and thyrotrophs.

In this study, we investigated the production of thyrotropin releasing hormone (TRH) and β TSH chain at the mRNA level by *in situ* hybridization and end product level by immunohistochemistry, in 18 patients submitted to neurosurgery for pituitary non-functioning macroadenomas.

Patients and methods

The study included 18 patients with clinically nonfunctioning pituitary macroadenomas, aged between 14-73 (44,11 ± 8) classified according to Hardy´s stages in non-invazive (stage II) - 6 cases, locally invasive (III-SSE) - 3 cases and diffuse invasive (IV-SSE) - 9 cases.

The patients were admitted in the Department of Endocrinology at the University of Medicine and Pharmacy in Bucharest, on the basis of visual field disturbances, amenorrhoea or radiological changes on the sellar X-ray, in order to be evaluated for pituitary disease.

Extension of the macroadenoma was established using coronal CT scan, following Hardy´s criteria [7]. The hormonal level was assessed for TSH, FSH LH by simultaneously sampling blood and cerebrospinal fluid.

The patients were submitted to neurosurgery after the initial clinical and hormonal evaluation.

Except one case, they underwent transcranial hypophysectomy. All the patients were informed about the study and gave their informed consent before enrolling. The Ethical Committee of the "Carol Davila" University of Medicine and Pharmacy approved the study protocol.

The pituitary tumor tissue was used for immunohistochemistry (IHC) and *in situ* hybridization (ISH) studies after fixation into buffered PFA and criosectioning. The IHC was performed as previously described [8], using the avidin-biotin complex method with polyclonal primary antibodies (kindly provided by Dr. A.F. Parlow, NHPP) against anterior pituitary hormones in the following dilutions: TSH 1:2k, FSH 1:1k, LH 1:1k, PRL 1:3k, GH 1:2k.

The *in situ* hybridization for TRH mRNA and βTSH mRNA was performed using synthetic oligonucleotide probes The TRH oligonucleotide probe corresponded to nucleotides bases 117-152 in exon 2 of the pro-TRH gene. The beta TSH oligoprobe was complementary to bases 93-122 from exon 1 in the TSH β subunit gene [9]. The probes were custom synthesised by Pharmacia Biotech, St Albans, UK and labeled with S^{35}-ATP

according to our protocol [10]. The slides were hybridized overnight at 42°C. After stringency washes, the sections were dried and exposed upon emulsion for 6 weeks, then developed and counterstained. Comparative analysis was performed for the IHC and ISH pictures using an Axioscop Zeiss microscope equipped with LUCIA image analysis system.

Results and discussion

Among the 18 nonfunctioning pituitary adenomas, only 3 adenomas were totally negative on all the hormones tested IHC. Twelve out of 18 were immunopositive for FSH in >10% cells, and in 7 out of these, significant immunostaining for TSH was documented. Furthermore, all the TSH immunoreactive adenomas were positive for TSH β mRNA by ISH on serial sections (Fig. 1), as expected. In addition, TRH mRNA ISH (Fig. 2) documented the tumoral TRH synthesis in 11 cases, as follows: all but one TSH immunoreactive adenomas and all but one FSH secreting tumors were positive for TRH mRNA. In the other 6 cases, with low FSH immunoreactivity, there was no TRH mRNA synthesis and the IHC detected LH secretion in 2 and PRL in 1, the remaining 3 adenomas being totally negative.

The simultaneously sampling of cerebrospinal fluid/ blood in 18 patients demonstrated an abnormal pattern (*i.e.*: hormonal CSF/blood ratio above 1) in 5 non-functioning adenomas for FSH, 4 for LH, 4 for TSH, despite the absence of any clinical signs of pituitary hyperthyroidism. However, there was not a complete correspondence between the hormonal and morphological picture.

It is well known that TRH can trigger FSH and LH discharge from gonadotropinomas, but not from normal gonadotroph cells [11]. On the other hand, usually 400 μg i.v. TRH can not stimulate the TSH discharge from a TSH–oma with central hyperthyroidism [12]. However, "mute" thyrotropinomas behave differently. In our series, TRH triggered TSH, as well as discrete LH release, as shown in table 1 for one 38 yrs. male patient with an invasive grade IV SSE pituitary nonfunctioning adenoma (Table 1).

The synthesis of TRH inside the pituitary adenoma could stimulate the proliferation as well as hormonal production of TSH and gonadotropins.

Table 1. Morphological and hormonal data in patient NF1, male, 38 yrs., (IV-SSE). TRH test, 400 μg i.v. indirectly shows the presence of functional TRH receptors. TRH mRNA intense positive. +++ = more than 20% cells positive, CSF = cerebrospinal fluid, IHC = immunohistochemistry, ISH = *in situ* hybridization.

	Serum	**CSF**	Condition
TSH (IHC) +++	3,5 mU/l	2,2 mU/l	Basal
TSH (ISH) +++	12,6 mU/l	2,3 mU/l	TRH 0.4 mg
FSH (IHC) +++	16,9 U/l	39,6 U/l	Basal
	18 U/l	39,8 U/l	TRH 0.4 mg
LH (IHC) +++	1,5 U/l	6,9 U/l	Basal
	1,69 U/l	8,64 U/l	TRH 0.4 mg

Several groups reported the TRH release from primary pituitary tumor cell cultures. The shortest bioactive neuropeptide can induce electrophysiological changes when infused in primary TSH or PRL tumor cell cultures [13]. The TRH receptors are normal in both number and structure, in these tumors. However, the fact that gonadotropins respond to TRH shows an abnormal expression of its receptors on FSH & LH producing cells. The involvement of TRH as a potential pathogenic molecule is possible only as a facilitating co-factor, which can stimulate the proliferation and synthesis of TSH, acting on the background random mutations. Which are the factors involved in the balance between the role of transdifferentiation (a reversible process) encountered in hypothyroidism and the single way mutagenenesis, towards the development of an expansive tumor, remains to be established.

In conclusion, many non-functioning (so-called "mute") pituitary adenomas produce glycoproteic hormones, among which TSH is fundamental. The intrapituitary TRH synthesis could significantly contribute to TSH expression by these adenomas.

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