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Immunoreactive thyroliberin (TRH) precursor forms in human hypothalamus and anterior pituitary tissues

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Immunoreactive (IR) proTRH forms were characterized in human hypothalamic tissue with two antisera raised against a hepta- and a decapeptide containing the TRH progenitor sequence (-Gln-His-Pro-Gly-). A similar study was performed in human normal and adenomatous anterior pituitaries, tissues in which TRH synthesis has been previously suggested. IR-proTRH was found in all the samples ranging from 42–775 fmol/mg proteins. Size exclusion chromatography identified a major 25–35 kDa form and a minor 4–8 kDa form. The existence of the major form was confirmed by immunoblotting. The results suggest that both human hypothalamic and normal or adenomatous anterior pituitary tissues synthesize similar IR-proTRH forms.

TRH; Precursor; Human hypothalamus and anterior pituitary

1. INTRODUCTION

Thyroliberin (TRH) is a tripeptide, initially identified in mammalian hypothalamus, that is widely distributed throughout the body and is synthesized in extrahypothalamic tissues [1–3]. It has also been observed in the rat anterior pituitary and in the human normal and adenomatous pituitary [4–6] and its synthesis by these tissues has been envisaged [7–9]. Like other peptides, TRH is produced through the processing of a high molecular weight precursor. The structure of this preproTRH has been deduced from the cDNA sequence established in the xenope [10,11], rat and man [12,13]. Human preproTRH contains six TRH progenitor sequences (-Gln-His-Pro-Gly-), each flanked by pairs of basic amino acids. Precursor forms of different molecular weights (2.5–52 kDa) have been partially characterized in rat and mouse brain by immunochemical methods [14,16].

This work provides evidence for the presence of IR-proTRH forms in human hypothalamus, normal and tumoral anterior pituitaries. Their partial characterization was performed by size exclusion chromatography, immunoenzymatic assay and immunoblotting, using two antisera against extended TRH progenitor sequences.

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2. MATERIALS AND METHODS

2.1. *Tissues*

Six human pituitary adenomas (2 prolactinomas, 2 GH-secreting and 2 non-secreting adenomas) were collected in the operating room after trans-sphenoidal adenomectomy and frozen in liquid nitrogen until use. One normal pituitary and fragments of one hypothalamus were obtained 6 h post-mortem from 2 patients presenting no neuroendocrine disorder.

2.2. *Extractions and size exclusion chromatography*

Tissue fragments were immersed in 1 M acetic acid at 95°C for 5 min (1:10, w/v), then homogenized in a Potter apparatus and centrifuged at 5,000 × *g* for 30 min at 4°C as previously described [14]. The pellet was stored at −20°C until protein assay while the supernatant was lyophilized. The lyophilized extracts were resuspended in 1 M acetic acid and filtered through a G_{50} sephadex column (100 × 1.6 cm, Bio-Rad) equilibrated and eluted with 1 M acetic acid at 4°C, at a rate of 12 ml/h. 2 ml fractions were collected, aliquoted and lyophilized for proTRH and TRH assays.

2.3. *RIA of TRH*

RIA of TRH was performed as previously described [17]. The minimum amount of TRH that was detected was 4 fmol.

2.4. *Immunoenzymatic assay of proTRH forms*

This was performed according to [18] using a rabbit antibody directed against the P10 peptide, an extended TRH progenitor sequence (Ser-Lys-Arg-Gln-His-Pro-Gly-Lys-Arg-Phe). Amounts of IR-proTRH were expressed in terms of IR-P10. The sensitivity threshold of the assay was 2 fmol.

2.5. *Immunoblotting of proTRH forms*

The acidic extracts were analyzed by sodium dodecyl sulfate, 14% polyacrylamide gel electrophoresis [19]. Proteins were transferred to an immobilon P membrane for 18 h in 25 mM Tris, 192 mM glycine and 20% methanol, pH 8.3 [20]. The membrane was saturated for 90 min in 50 mM Tris buffer, 9% NaCl, pH 7.4, containing 30% skimmed milk. The immunological reactions were performed using a rabbit antibody directed against the P7 peptide, another extended TRH pro-

genitor sequence (Gln-His-Pro-Gly-Lys-Arg-Phe), at a 1:1,000 dilution for 24 h at 4°C. The antigen–antibody complex was revealed with ^{125}I-labelled anti-rabbit immunoglobulin G antibody (Amersham). The membrane was exposed to film (Hyperfilm βmax, Amersham) for 5 days.

3. RESULTS

3.1. *TRH and IR-P10 contents*

As previously described [9], the amount of TRH widely varied from one adenoma to another. TRH contents in the normal anterior pituitary and the adenomas were in the same range, 17 and 3 to 83 fmol/mg proteins, respectively (Table I). It was much higher in the hypothalamus (1,802 fmol/mg proteins).

The values of IR-P10 concentrations in the anterior pituitary tissues were relatively grouped except in one prolactinoma (case 1, Table II) in which the concentration was much higher (775 fmol/mg proteins) than in the other samples (120–241 fmol/mg proteins). By contrast, IR-P10 content was lower in the hypothalamus (42 fmol/mg proteins).

The ratio, TRH/IR-P10, was far lower in tumoral and normal pituitaries (Table II, 0.02–0.53 and 0.14, respectively) than in the hypothalamus (43.95).

3.2. *IR-P10 after elution on Sephadex G_{50}*

Two peaks of IR-P10 were constantly observed after gel filtration of acidic extracts from all the tumoral and normal anterior pituitaries as well as from the hypothalamus (Fig. 1). The first peak eluted at a position corresponding to a molecular weight of 25–35 kDa. The second peak corresponded to a 4–8 kDa molecular weight. The respective sizes of the two peaks were variable from one adenoma to the other. The surface of the 25–35 kDa peak was larger than that of the 4–8 kDa peak in most adenomas, as well as in the normal anterior pituitary and the hypothalamus. However in two adenomas (cases 1 and 5), the major peak consisted of the 4–8 kDa form (Table II).

3.3. *Immunoblotting*

The immunoblotting of acidic extracts of the hypothalamus and two adenomas (cases 4 and 5), using the anti-P7 antibody, revealed the presence of a strongly immunoreactive band corresponding to a molecular weight of 37 kDa (Fig. 2). This band was absent when the anti-P7 antibody pre-absorbed with P7 was used.

4. DISCUSSION

Our results are the first evidence for the presence of immunoreactive, high molecular weight TRH precursor forms (25–35 and 4–8 kDa) in human tissues. The antisera used in this study were developed to identify TRH precursor forms in murine brain [16]. These antisera

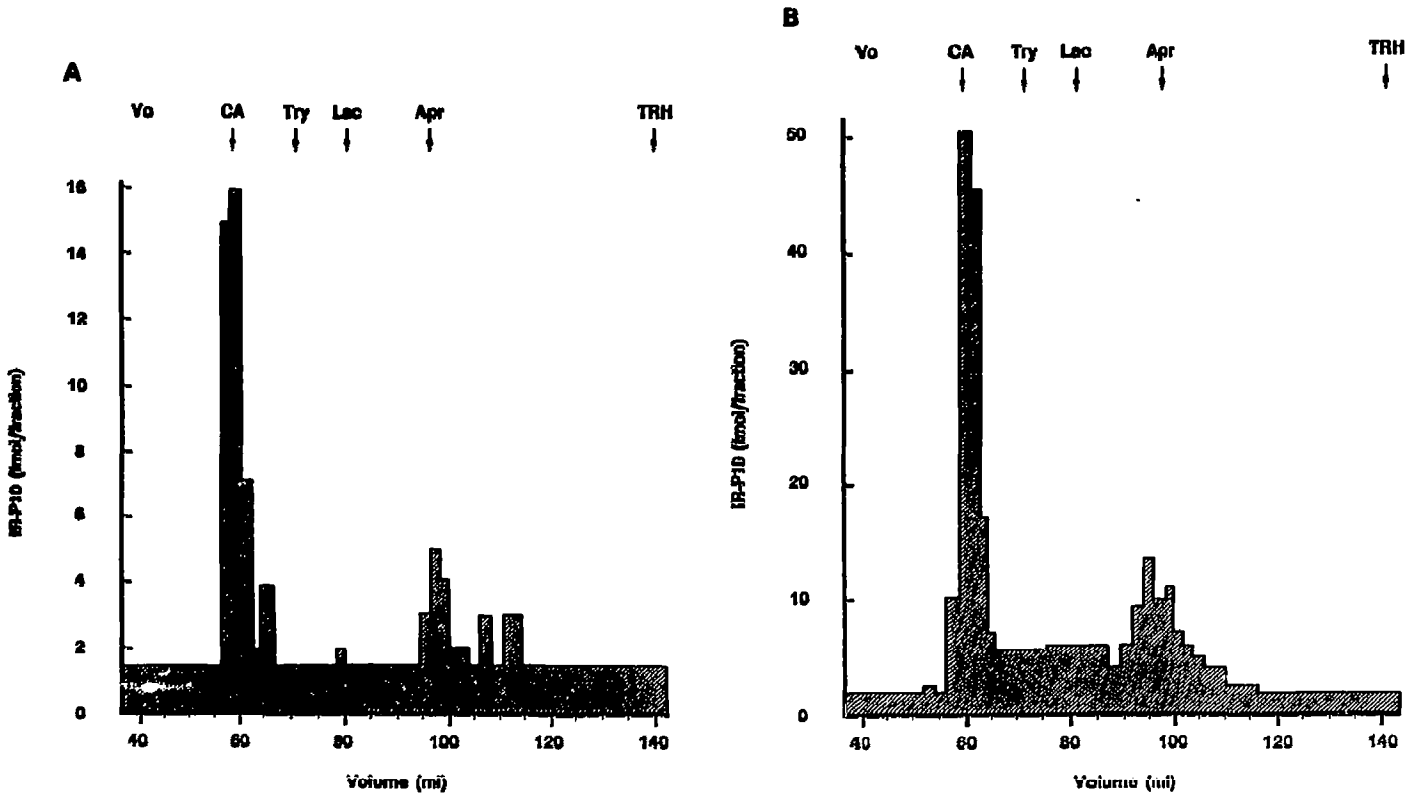

Fig. 1. IR-P10 in G_{50} Sephadex chromatography fractions corresponding to 2 mg of proteins of crude extract. (A) human hypothalamus (case 8). (B) human non-secreting adenoma (case 6). The molecular weight markers shown are: CA, carbonic anhydrase, 29 kDa; Try, trypsinogen, PMSF-treated, 24 kDa; Lac, α-lactalbumin, 14.2 kDa; Apr, Aprotinin, 6.5 kDa; and [^{125}I]THR. Vo, void volume.

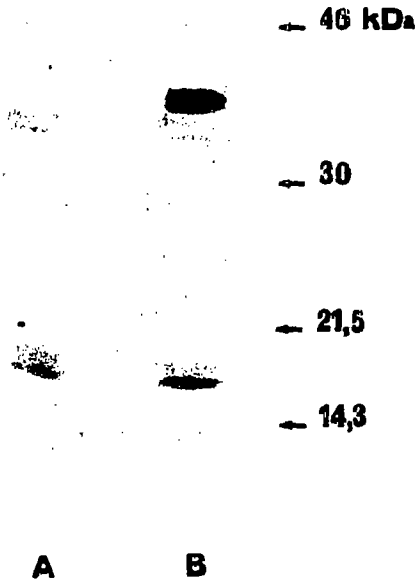

Fig. 2. Immunoblotting of a human non-secreting adenoma (case 5) performed with anti-P7 antibody (lane B) or preabsorbed anti-P7 antibody (lane A). Molecular weight markers: ovalbumin, 46 kDa; carbonic anhydrase 30 kDa; trypsin inhibitor, 21.5 kDa; and lysozyme, 14.3 kDa.

have been shown [17] not to cross-react with TRH itself, with connecting peptides resulting from proTRH processing [21], or with TRH-Gly, the direct precursor of TRH [3]. The cross-reactivity of the antisera with both human and murine TRH precursors resulted from extended sequence homologies between the two precursors, above all at the level of the TRH progenitor sequences and the surrounding amino acids [12,13]. The two antisera used here were raised against such highly homologous regions. The 25–35 kDa form is compatible with that of the 242-residue protein that could be encoded by the human preproTRH mRNA [13]. The 4–8 kDa form might represent an intermediate step of the processing yielding a product containing a progenitor sequence still attached to a connecting peptide, although an artefactual degradation of the large form cannot be excluded.

In addition to significant amounts of TRH, anterior pituitary tissues contained IR-proTRH forms similar to those found in the hypothalamus. However, the TRH/IR-proTRH ratio was much higher in the hypothalamus than in the anterior pituitary tissues. The sharp increase of the TRH/IR-proTRH ratio that has been reported in the mouse hypothalamus during fetal development might reflect ontogenic alterations of the processing of proTRH [16].

To conclude, our data identify the human normal and tumoral anterior pituitary as a novel site of TRH synthesis and suggest that the maturation process of proTRH in the anterior pituitary is different from that occurring in the hypothalamus.

Table I

TRH and IR-P10 contents in human hypothalamus, normal and adenomatous anterior pituitary

	IR-P10*	TRH*	TRH / IR-P10
Adenomas (n = 6)	285 ± 243	42 ± 31	0.21 ± 0.20
Normal anterior pituitary (n = 1)	120	17	0.14
Hypothalamus (n = 1)	42	1,802	43.95

*Results expressed in fmol/mg proteins.

Table II

IR-P10 contents in human anterior pituitary tissues and hypothalamus: distribution into the two peaks after size exclusion chromatography

	IR-P10 in crude extracts*	1st peak (25–35 kDa*)	2nd peak (4–8 kDa)*
Prolactinomas			
# 1	775	135	550
# 2	234	139	88
GH-secreting adenomas			
# 3	241	163	54
# 4	163	135	23
Nonsecreting adenomas			
# 5	158	33	73
# 6	139	66	36
Normal anterior pituitary			
# 7	120	44	70
Hypothalamus			
# 8	42	25	16

*Results expressed in fmol/mg proteins.

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