Microheterogeneity of thyroid-stimulating hormone from the pituitaries of euthyroid, hypothyroid and hyperthyroid rats


University of Manchester, Department of Medicine (Clinical Biochemistry), Hope Hospital, Eccles Old Road, Salford M6 8HD, U.K.


RECEIVED 15 January 1992

ABSTRACT

The microheterogeneity of pituitary thyroid-stimulating hormone (TSH) is dependent on variations in the hormone's carbohydrate moieties. In this study, changes in the pattern of heterogeneity have been assessed by chromatofocusing, which separates the isoforms on the basis of their isoelectric points (pI). Rats (n = 6 per group) were either untreated or rendered hypothyroid by including in the drinking water either propylthiouracil (0-05% for 8 weeks) or thyroxine (T4; 4 mg/l for 6 weeks) before they were killed at 16 weeks. On autopsy, serum TSH and total T4 were (means ± s.e.m.): 2 ± 0·3 µg TSH/l and 64 ± 5 nmol T4/l (control); <1 µg TSH/l and 133 ± 6 nmol T4/l (hypothyroid); 58 ± 6 µg TSH/l and 32 ± 6 nmol T4/l (hypothyroid). The pituitaries were individually homogenized and the TSH isoforms separated by chromatofocusing over a pH range of 7-4. Fractions were assayed for TSH by radioimmunoassay. TSH from the control group was distributed into seven major peaks with pI values of (means ± s.e.m., n = 6) 6·9 ± 0·1, 6·6 ± 0·1, 6·2 ± 0·1, 5·8 ± 0·1, 5·5 ± 0·1, 5·2 ± 0·1 and 4·8 ± 0·1; 7 ± 3% of the TSH had a pI of <4·0. Six peaks of TSH were conserved in the hypothyroid group (with pI values of 6·8 ± 0·1, 6·5 ± 0·1, 6·2 ± 0·1, 5·8 ± 0·1, 5·4 ± 0·1 and 5·2 ± 0·1), and 11 ± 4% of the hormone had a pI of <4·0. In contrast to the other two groups, only one major peak (with a pI of 5·8 ± 0·1) was detected in the pituitaries from the hypothyroid group; 13 ± 5% of the TSH had a pI of <4·0. In the pH range of 5·5-6·0, the per cent distribution of TSH was 58 ± 15 (hypothyroid) compared with 17 ± 3 (hypothyroid) and 22 ± 3 (euthyroid). Above pH 6, only 25 ± 13% of the TSH (hypothyroid) was present compared with 46 ± 5% (hypothyroid) and 45 ± 5% (euthyroid). Below pH 5·5, the per cent distribution of TSH was 19 ± 5 (hypothyroid), 37 ± 5 (hypothyroid) and 35 ± 3 (euthyroid). In conclusion, both hyper- and hypothyroidism are associated with changes in the composition of pituitary TSH. This change was most marked in the hypothyroid group, where there was a selective loss of several isoforms of TSH. Journal of Molecular Endocrinology (1992) 9, 245-250

INTRODUCTION

The pituitary glycoprotein hormones, thyroid-stimulating hormone (TSH), luteinizing hormone (LH) and follicle-stimulating hormone (FSH) exist as heterogeneous molecular species in the pituitary and serum of experimental animals and humans (Wide, 1985a,b; Keel, 1989). For example, human pituitary TSH (1st International Reference Preparation (IRP) 68/38) has been separated into 7-11 isoforms depending on the nature of the antibody used for detection (Sergi et al. 1991), while euthyroid rat pituitary TSH has been separated into four isoforms with isoelectric points (pI) ranging from 8·3 to 6·6 (Yora et al. 1979). This heterogeneity relates to the composition of the carbohydrate moieties (Baenziager & Green, 1988), and the biological activity of these molecules is closely related to the extent of glycosylation, especially the sialic acid residues (Berman et al. 1985; Amir et al. 1987; Keel, 1989). Furthermore, the type and extent of glycosylation also appears to influence the antigenic structure of the secreted glycoproteins (Sairam et al. 1990). The existence of microheterogeneity has been well documented for LH and to a lesser extent for FSH. Variations in endocrine status such as gonadal function, stage of cycle or administration of exogenous steroid or gonadotrophin-releasing hormone directly
affect the pattern of gonadotrophin isohormones released from the pituitary (Jia et al. 1986; Padmanabhan et al. 1988). It is becoming clear that the pituitary, in response to the endocrine milieu, alters not only the quantity but also the quality of the gonadotrophins produced. The influence of thyroid status on the charge microheterogeneity of TSH has not been studied in detail and was the purpose of the present investigation. Gross changes in the pattern of heterogeneity of rat pituitary TSH have been assessed by chromatofocusing, a technique which separates isospecies on the basis of their pI values.

MATERIALS AND METHODS

Male Sprague–Dawley rats (six per group, 8 weeks old) were maintained under a cycle of 12 h light: 12 h darkness, and standard chow (Biosure, Manea, Cambs, U.K.) was available ad libitum. Hypothyroidism was induced by adding n-propylthiouracil (PTU, 0.05%; BDH, Poole, Dorset, U.K.) to the drinking water for a period of 8 weeks before the rats were killed. Hyperthyroidism was induced by the presence of L-thyroxine (T$_4$, 4 mg/l; Sigma, Poole, Dorset, U.K.) in the drinking water for 6 weeks before they were killed. The control group of rats was given tap water. All animals were killed at 16 weeks and blood samples were taken for analysis of thyroid hormones. The anterior pituitary glands were removed and washed in Dulbecco’s minimum essential medium (NBL, Washington, Tyne and Wear, U.K.). The pituitaries were then frozen in n-hexane cooled by a freezing mixture of methanol and cardice. Tissues were stored at −70°C until TSH extraction.

Pituitary TSH extraction

Individual rat pituitaries were homogenized on ice for 10 s in NaCl (50 mmol/l)–Tris (15 mmol/l) and phenylmethylsulphonylfluoride (PMSF; 0.1 mmol/l, pH 7.2). The homogenate was centrifuged at 1000 g for 30 min at 4°C and the supernatant removed and stored at −20°C. The pellet was rehomogenized and centrifuged and the supernatant stored. The procedure was performed four times in total. To ascertain the total pituitary content of TSH, the pellet was homogenized twice more in HCl (0.1 mol/l) containing PMSF (0.1 mmol/l) and the supernatants were saved. For chromatofocusing experiments, the four salt extracts were pooled and used as the sample. Recovery of TSH in the salt extract was 97 ± 3% of the total extractable (by salt and acid) TSH.

Preparation of buffers

All buffers were prepared in distilled, deionized water and were filtered through a 0.2 μm bottle filter. The start buffer used for chromatofocusing on a Mono P column (4 ml; Pharmacia, Milton Keynes, Bucks, U.K.) was Bis–Tris (7.3 mmol/l, pH 7.0–7.2) and the limit buffer was Polybuffer (1:35 dilution at pH 3.8–4.0; Pharmacia). A saturated solution of iminodiacetic acid was used to titrate buffers to the correct pH and all buffers were degassed for 4 h prior to use.

Chromatofocusing

A Mono P column was used in conjunction with a fast-performance liquid chromatographic system (Pharmacia). After equilibration of the column with >20 ml start buffer, the desalted sample containing 1 μg TSH was loaded. Limit buffer was pumped at a flow rate of 0.5 ml/min to generate the pH gradient and 1.0 ml fractions were collected into tubes containing 0.05 ml 2% (w/v) bovine serum albumin solution (in water). The run was continued until the pH of the eluent was the same as that of the limit buffer. After completion of the pH gradient, 0.5 ml 2 M NaCl were injected onto the column and eluted with start buffer (2–3 column volumes). Fractions were again collected for estimation of TSH with a pI of <4 (salt peak). Recovery of TSH after chromatofocusing was 94 ± 7% (n = 18). All fractions were stored at −20°C until assayed.

Assay of rat TSH

Materials for the radioimmunoassay (RIA) of rat TSH were obtained from the National Hormone and Pituitary Program, Bethesda, MD, U.S.A. and included lyophilized rat pituitary standard (NIADDK-rTSH RP-2), rat TSH antigen (NIADDK-rTSH-I-8) for iodination and rat TSH antiserum (NIADDK-anti-rTSH-S-5). All fractions generated from one chromatofocusing run (n = 90–140) were assayed in the same TSH RIA. Every tenth sample and each of four quality control samples were assayed in duplicate in each assay. From these replicate estimations, intra- and interassay coefficients of variation (C.V.) were <8% and <12% (n = 25) respectively over the range 3–30 mg/l.

Assay of T$_{4}$

Thyroxine was assayed by standard RIA using an antiserum raised in sheep (Scottish Antibody Production Unit, Carluke, Strathclyde, U.K.) and
125I-labelled T4 (Amersham International plc, Amersham, Bucks, U.K.). Intra- and interassay C.V. values for this assay were <7% and <10% respectively over the range 10–300 nmol/l.

Statistical analysis

A peak in the chromatofocusing profile was defined as having an ascending or descending limb with a point(s) which was at least 24% (i.e. three times the intra-assay C.V.) greater than the preceding nadir.

Levels of significance were estimated by Student’s t-test.

RESULTS

At death, the mean body weight for the hypothyroid group of animals (353 ± 21 g) was reduced (P < 0.001) compared with weights for the euthyroid (539 ± 36 g) and hyperthyroid (462 ± 24 g) groups (Fig. 1). Mean serum concentrations of TSH (μg/l) and total T4 (nmol/l) were 2 ± 0.3, 64 ± 5 (euthyroid); 58 ± 6, 32 ± 6 (hypothyroid) and <1, 133 ± 6 (hyperthyroid) respectively. Chromatofocusing profiles of TSH for individual pituitaries from euthyroid, hyperthyroid and hypothyroid rats (two per group) are presented in Fig. 2. Group data presented as per cent pituitary TSH compared with pH interval are illustrated in Fig. 3. TSH from the control group was distributed into seven major peaks. Six peaks of TSH were conserved in the hypothyroid group. In contrast to the other two groups, only one major peak (pI 5.8 ± 0.1) was consistently detected in the pituitaries from the hyperthyroid group (Table 1). In the pH range 5.5–6.0, the per cent distribution of TSH was 58 ± 15 (hypothyroid) compared with 17 ± 3 (hypothyroid) and 22 ± 3 (euthyroid). Above pH 6, only 25 ± 13% TSH (hyperthyroid) was present compared with 46 ± 5% (hypothyroid) and 45 ± 5% (euthyroid). Below pH 5.5, the per cent distribution of TSH was 19 ± 5 (hyperthyroid), 37 ± 5 (hypothyroid) and 35 ± 3 (euthyroid).

DISCUSSION

Treatment of adult rats with PTU and T4 induced biochemical hypo- and hyperthyroidism respectively. Low doses of thyroid hormones have been reported to stimulate growth in the adult rat (Argov et al. 1981), although in some studies in which hyperthyroidism was induced by large doses of thyroid hormones, a fall in body weight occurred (Ianuzzo et al. 1977). In this work, the increase in body weight during the treatment period was lower (P < 0.05) in the hyperthyroid relative to the euthyroid state. In the hypothyroid group, body weight at death was markedly (P < 0.001) reduced, as has been demonstrated previously for the rat (Ladenson et al. 1986).

In this study, the heterogeneous nature of pituitary TSH in the euthyroid and hypothyroid rat has been demonstrated. However, the most striking observation was the relatively homogeneous nature
of the pituitary TSH found in the hyperthyroid group of animals. To our knowledge, this is the first demonstration of this marked and selective loss of isoforms of TSH under conditions of thyroid hormone excess and presumably suppressed secretion of thyroid-releasing hormone (TRH), the primary hypothalamic regulator of TSH secretion.

The distribution of TSH isoforms in the euthyroid, hypothyroid and hyperthyroid rats described in this work is clearly dependent on the specificity of the antiserum used in the TSH RIA, and could change if different antibodies directed against different antigenic determinants were used. Nevertheless, we were able to detect gross differences in the pattern of TSH heterogeneity in different thyroid states, presumably reflecting altered glycosylation. The nature of this altered glycosylation is unknown.

The effect of thyroid status on the isohormone profile of rat TSH has been studied previously using the technique of isoelectrofocusing. Mori et al. (1984) showed that after thyroidectomy, TSH became more heterogeneous and TSH components with more acidic pI values became evident. In contrast, Yora et al. (1979) demonstrated that hypothyroidism in the rat was characterized by a decrease in the more acidic forms of TSH to a greater extent than the other forms. In the present study, we observed the loss of one acidic form of TSH (pI 4.8) in the hypothyroid group, but the clearest new result was the homogeneous nature of TSH in the hyperthyroid state.

Administration of TRH in vivo and in vitro has been reported to alter carbohydrate structure (Mori et al. 1984; Gesundheit et al. 1987), as well as to increase secreted TSH bioactivity (Beck-Peccoz et al. 1985; Menezes-Ferreira et al. 1986). However, Menezes-Ferreira et al. (1986) have also demonstrated that thyroidectomy decreases the bioactivity of TSH stored in the pituitary without altering the biological activity of secreted TSH, leading these authors to conclude that TSH bioactivity is regulated differently by thyroid hormone deficiency and

\[ \text{Journal of Molecular Endocrinology (1992) 9, 245-250} \]
Influence of thyroid status on TSH heterogeneity  
A. J. Pickles and others

**TABLE 1.** The pI values of the major peaks seen in the euthyroid, hypothyroid and hyperthyroid groups. Results are presented as means±s.e.m., n=6

<table>
<thead>
<tr>
<th>Peak pI</th>
<th>Euthyroid</th>
<th>Hypothyroid</th>
<th>Hyperthyroid</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.9±0.1</td>
<td>6.8±0.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.6±0.1</td>
<td>6.5±0.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.2±0.1</td>
<td>6.2±0.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.8±0.1</td>
<td>5.8±0.1</td>
<td>5.8±0.1</td>
<td></td>
</tr>
<tr>
<td>5.5±0.1</td>
<td>5.4±0.1</td>
<td>5.2±0.1</td>
<td></td>
</tr>
<tr>
<td>5.2±0.1</td>
<td>5.2±0.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.8±0.1</td>
<td>5.8±0.1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The amounts of TSH with a pI of <4 were 7±3% for the euthyroid group, 11±4% for the hypothyroid group and 13±5% for the hyperthyroid group.

that hypothalamic hypothyroidism alters TSH carbohydrate structures to less complex forms and in-vivo TRH administration normalizes these structures in parallel with the return of serum free T₃ to the normal range (Taylor & Weintraub, 1989). The results of the above studies suggest that glycosylation of TSH is regulated by TRH and possibly also by thyroid hormones. In contrast to the gonadotrophins, little information is available concerning the relationship between TSH polymorphism and biological activity, although some studies suggest a relationship between TSH isohormones, carbohydrate composition and bioactivity. For example, heterogeneous forms of TSH, observed in mouse thyrotrophic tumour and serum, and which differed only in carbohydrate composition, were found to have different biological activities (Pekonen et al. 1981). In a recent report, glycosylation of the α subunit but not that of the β subunit was shown to be essential for expression of the domains involved in TSH immunoreactivity, as well as those controlling the bioactivity of the hormone (Papandreou et al. 1991). In the case of the gonadotrophins, the more basic forms exhibit the greatest degree of in-vitro bioactivity (Wilson et al. 1990; Ding & Huhtaniemi, 1991). The relationship between the pI of TSH isofoms and bioactivity has been studied by the present authors (Pickles et al. 1992) and has been examined in a recent report (Sergi et al. 1991) in which neutral forms of pituitary TSH were found to be less potent than more acidic or basic forms in inducing cyclic AMP production and in stimulating the growth of FRTL-5 cells.

In summary, in this study we have shown that both hyper- and hypothyroidism are associated with changes in the composition of pituitary TSH. This change was most marked in the hyperthyroid group, where there was a selective loss of several isofoms of TSH, leaving only one major peak with a pI of 5.8. Interestingly, the pI of this isofom corresponds to the pI of one of only two isofoms conserved
throughout three human pituitary standards (68/38 (1st IRP), 80/558 (2nd IRP) and 63/14 (MRC Research Standard A)) (Pickles et al. 1992), and may represent a basic isoform which may then be the subject of further processing, depending on thyroid status and in particular on the level of TRH.

ACKNOWLEDGEMENTS

This investigation was supported by the North Western Regional Health Authority. The supply of hormone reagents by the National Hormone and Pituitary Program of the National Institute of Diabetes, Digestive and Kidney Diseases is gratefully acknowledged.

REFERENCES


Journal of Molecular Endocrinology (1992) 9, 245–250


