Differential Processing and Regulation of Thyroid-Stimulating Hormone Subunit Carbohydrate Chains in Thyrotropic Tumors and in Normal and Hypothyroid Pituitaries

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ABSTRACT: Thyroid-stimulating hormone (TSH) α- and β-subunit glycosylation was investigated in mouse thyrotropic tumor and in normal and hypothyroid pituitary cells for various periods of time in the presence of [^{3}H]mannose or [^{3}H]galactose. After sequential precipitation with anti-α and anti-β sera, subunits were treated with Pronase followed by endo-β-*N*-acetylglucosaminidase H (Endo H) and analyzed by paper chromatography. In primary cultures of thyrotropic tumor cells incubated for 60 min with [^{3}H]mannose, primarily $Man_9GlcNAc$ and $Man_8GlcNAc$ were found on TSH + α subunits, whereas $Glc_1Man_9GlcNAc$ and $Man_9GlcNAc$ were prominent on free β subunits. After preincubation of cells for 16 h in the presence or absence of glucose followed by a 60-min pulse of [^{3}H]mannose, there was an 8-fold increase in labeled TSH + α but only a minimal change in free β or total proteins. In the absence of glucose, there was a selective accumulation of $Man_8GlcNAc$ on TSH + α but not on free β or total proteins; however, there was no detectable accumulation of Endo H resistant forms during glucose starvation on TSH subunits or total proteins. Normal mouse and rat pituitary minces incubated for 60 min with either [^{3}H]mannose or [^{3}H]galactose showed no glucose-containing species on TSH subunits, but equal amounts of $Man_9GlcNAc$ and $Man_8GlcNAc$ on TSH + α, and mostly $Man_9GlcNAc$ on free β subunits. In contrast, hypothyroid mouse and rat pituitaries exhibited an increase in Glc_1Man_9NAc and $Glc_1Man_8GlcNAc$ on free β but not on TSH + α or total proteins. Thyrotropin-releasing hormone (TRH) injection of newly thyroidectomized rats also caused an increase in $Glc_1Man_9GlcNAc$ on intracellular TSH + β or free β subunits but not on free α. These data indicate that TSH and total protein glycosylation is very similar in thyrotropic tumors and nonneoplastic pituitaries. However, TSH subunit carbohydrate chains are processed at a different rate, and this processing can be independently regulated by both metabolic and endocrine factors. Nonphysiological glucose starvation affects primarily TSH α-subunit glycosylation whereas the physiologic endocrine factors, thyroid hormones and TRH, reciprocally modulate β-subunit glycosylation.

The pituitary hormones luteinizing hormone (LH),[1] follicle-stimulating hormone (FSH), and thyroid-stimulating hormone (TSH) as well as the placental hormone chorionic gonadotropin (CG) are a family of glycoproteins composed of two noncovalently linked α and β subunits. Within a species, the α-subunit polypeptide chain is virtually identical in these hormones, while the β subunit is unique and confers the hormonal specificity (Pierce & Parsons, 1981). In TSH, the α subunit contains two asparagine-linked, complex-type carbohydrate chains while β contains only one, with a total carbohydrate content of 15%.

TSH is the major regulator of thyroxine and triiodothyronine produced by the thyroid gland, and its secretion is controlled by a complex feedback mechanism. It has long been recognized that negative regulation by thyroid hormones is the primary control mechanism for TSH secretion. More recently, it has been shown that hypothalamic hormones such as thyrotropin-releasing hormone (TRH), somatostatin, or dopamine are also physiologic regulators of TSH secretion (Morley, 1981). However, the effects of these various agents on the de novo biosynthesis of the TSH polypeptide and carbohydrate chains have received little attention. Thyroid hormones have been shown to inhibit the biosynthesis of the TSH polypeptide (Cacicedo et al., 1981; Taylor & Weintraub, 1985a) and carbohydrate (Taylor & Weintraub, 1985a) chains, probably secondary to inhibition of α and β messenger RNA levels (Gurr & Kourides, 1983; Shupnik et al., 1983). In contrast, TRH causes minimal or no change in the biosynthesis of TSH polypeptide chains (Marshall et al., 1981; Wilber, 1971; Taylor & Weintraub, 1985b) but a major stimulation of glucosamine incorporation (Wilber, 1971; Taylor & Weintraub, 1985b), suggesting an effect on at least the late glycosylation of the hormone.

Studies from this laboratory and others [reviewed in Weintraub et al. (1985)] have demonstrated that TSH subunit biosynthesis occurs through separate messenger RNA species producing precursor pre-α and pre-β subunits containing amino-terminal signal peptides. Cotranslational cleavage of the signal peptide and glycosylation precede and appear necessary for the posttranslational α-β-subunit combination, which begins in the rough endoplasmic reticulum (Weintraub et al., 1980, 1983; Magner & Weintraub, 1982). We have recently demonstrated (Ronin et al., 1984) that in thyrotropic tumors, TSH subunit glycosylation, like that of other eukaryotic glycoproteins (Hubbard & Ivatt, 1981), occurs by transfer of preassembled oligosaccharides from lipid carriers. However,

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[1] Abbreviations: CHO, Chinese hamster ovary; CG, chorionic gonadotropin; Con A, concanavalin A; Endo H, endo-β-*N*-acetylglucosaminidase H; FSH, follicle-stimulating hormone; LH, luteinizing hormone; SDS, sodium dodecyl sulfate; TSH, thyroid-stimulating hormone; VSV, vesicular stomatitis virus; TRH, thyrotropin-releasing hormone; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride; EDTA, ethylenediaminetetraacetic acid.

some selectivity was found in the early steps of TSH subunit carbohydrate processing compared to that of other nonsecretory cell proteins.

In the present report, we have further compared the carbohydrate processing of both TSH subunits from thyrotropic tumors to that of nonneoplastic pituitaries from normal, hypothyroid, or TRH-stimulated animals. Our results indicate that TSH subunit carbohydrate chains are processed at a differential rate and that this processing is independently regulated in vitro by nonphysiological glucose starvation, as well as by the physiologic status of the pituitary glands.

Experimental Procedures

Materials. D-[2-^{3}H]Mannose (13.4 Ci/mmol) and D-[1-^{3}H]galactose (8.2 Ci/mmol) were from Amersham/Searle. Endoglucosaminidase H was kindly provided by Dr. F. Maley (New York State Department of Health, Albany, NY). Trypsin and Pronase were from Sigma. TRH was from Behring Diagnostics. All other chemicals were of analytical grade from various commercial suppliers.

Incubation of Mouse Tumor Cells. Dispersed cells were prepared from mouse thyrotropic tumors as previously described (Blackman et al., 1978). They were preincubated for 30 min at 37 °C in 5% CO_2–95% air in sterile tubes containing glucose-free and serum-free Dulbecco's modified Eagle's medium supplemented with 2 mM glutamine. In continuous labeling experiments, cells were incubated in glucose-free, serum-free medium containing 2 mM glutamine in the presence of 200–500 μCi/mL [^{3}H]mannose for 60 min. Incubations were terminated by washing with an excess of cold medium. At the end of the incubation, cell were centrifuged at 1000*g* for 2 min, media were removed, and the pellets were washed once, frozen, and stored at −20 °C.

Incubation of Mouse and Rat Pituitaries. Male and female LAF mice (2–6 months old), as well as Sprague-Dawley rats (150–250 g), were studied both under normal conditions and after 2–4 months of hypothyroidism, induced by either radiothyroidectomy (^{131}I, 200 μCi to mice) or surgical thyroidectomy (rats).

Groups of seven normal or hypothyroid mice and rats were killed, and their pituitaries were rapidly excised and minced. Pituitary minces were incubated, as described above, for 60 min at 37 °C in the presence of 500 μCi/mL [^{3}H]mannose or [^{3}H]galactose in glucose-free, serum-free Dulbecco's modified Eagle's medium. At the end of the incubation, minces were centrifuged at 100*g* for 2 min, media were removed, and the pellets were washed once, frozen, and stored at −20 °C.

For TRH stimulation, groups of five male rats 7 days postthyroidectomy were injected 3 times with 100 μg of TRH or saline at 27, 21, and 3 h prior to sacrifice. Pituitaries were excised and incubated, as described above for 3 h at 37 °C, in the presence of 500 μCi/mL [^{3}H]mannose with or without 1 ng/mL TRH in the media.

Immunoprecipitation Procedures. Cells or minces were thawed and homogenized in 0.15 M NaCl, 0.02 M Tris-HCl, pH 7.4, 0.01 M EDTA, 1% Triton (w/v), and 1 mM mannose and glucose. After sonication, the lysates were centrifuged at 100000*g* for 2 h.

Aliquots of lysates were precipitated with specific rabbit anti-bovine LH-α or anti-bovine TSH-β, as indicated, by using a staphylococcal protein A method described previously (Weintraub et al., 1980). Each antisubunit antibody has been shown to precipitate both the respectively uncombined (free) subunit as well as the combined subunit in TSH. The antibodies against both TSH subunits were employed, as indicated, in two different immunoprecipitation procedures: (i) lysates were first precipitated with anti-α (TSH + α) and the supernatant reprecipitated with anti-β (free β); (ii) lysates were also first precipitated with anti-β (TSH + β) and the supernatants precipitated with anti-α (free α). These methods have been validated previously (Weintraub et al., 1980) and were useful in determining the subunit specificity of carbohydrate processing. However, because of limitations in material, it was not possible to analyze separately the carbohydrate structures of each free and combined subunit of TSH.

Immune complexes were eluted from protein A by boiling in 0.06 M ammonium bicarbonate for 5 min. Trypsin (5% w/v) was subsequently added, and the samples were digested for 4 h at 37 °C, lyophlized, and further redissolved in 75 μL of 0.1% SDS, 1% mercaptoethanol, and 0.01 M sodium citrate, pH 5.5. After being boiled, samples were treated with Endo H (5–10 mIU) for 16 h at 37 °C.

Total Proteins. Aliquots of lysates were precipitated by 10% trichloroacetic acid–1% phosphotungstic acid (w/v) washed twice, extracted twice by acetone–ether (1:1 v/v), and redissolved in 200 μL of 0.06 M ammonium bicarbonate. Trypsin was subsequently added, and the samples were incubated for 16 h at 37 °C, lyophilized, and further incubated with SDS, mercaptoethanol, and Endo H as described above.

Paper Chromatography. The size of Endo H released oligosaccharides was estimated by chromatography on Whatman 1 paper using 1-propanol–nitromethane–water (5:2:4) as the developing solvent (Staneloni et al., 1980) for 48 h. Each ^{3}H-labeled sample was run with selected ^{14}C-labeled internal standards as described elsewhere (Ronin et al., 1984).

Radioactivity Measurements. Paper strips (1 × 3 cm) were eluted for 2 h with 1 mL of water, and liquid scintillation counting was performed by using 10 mL of a detergent-containing solution (Ultrafluor, National Diagnostics) as previously described (Ronin et al., 1984).

Results

Processing of TSH Subunit Oligosaccharides in Thyrotropic Tumors. In primary cultures of thyrotropic tumor cells incubated for 60 min with [^{3}H]mannose, three distinct oligosaccharides were found on both α and β subunits (Figure 1), comigrating respectively with $Glc_1Man_9GlcNAc$, $Man_9GlcNAc$, and $Man_8GlcNAc$ standards. TSH + α subunits contained primarily $Man_9GlcNAc$ and $Man_8GlcNAc$, whereas free β subunits contained primarily $Glc_1Man_9GlcNAc$ and $Man_9GlcNAc$.

Effect of Glucose Deprivation on TSH Glycosylation in Thyrotropic Tumors. Omitting glucose in cell culture medium has been shown to result in the transfer of truncated Endo H resistant oligosaccharides to proteins in CHO cells (Turco, 1980; Rearick, et al., 1981), NIL fibroblasts, and 3T3 cells (Gershman & Robbins, 1981). To determine whether TSH glycosylation could also be modified by the composition of cell incubation medium during in vitro labeling experiments, we incubated mouse thyrotropic tumor cells immediately or after 16 h of preincubation in the presence or absence of glucose in a medium containing 10% dialyzed hypothyroid calf serum or serum dialyzed vs. glucose-free media, respectively. The cells were then labeled for 60 min in the presence of [^{3}H]-mannose. Glycopeptides from TSH were prepared from sequential immunoprecipitation by using first anti-α followed by anti-β and compared to those of total proteins in order to detect any possible modification of glycosylation.

After as long as 16 h in the absence of glucose, tumor cells were still able to synthesize high mannose type oligosaccharides (Figure 2, left panels compared to right panels). Over 80%

Table I: Relative Amounts of Endo H Released Oligosaccharides from Total Proteins, TSH + α, and Free β after Glucose Starvation[a]

	total proteins		TSH + α		free β		
conditions	dpm × 10^{-3}	M_8:G_1 + M_9 ratio	dpm × 10^{-3}	M_8:G_1 + M_9 ratio	dpm × 10^{-3}	M_8:G_1 + M_9 ratio	TSH + α:free β ratio
no preincubation							
−Glc	2582	0.32	4684	0.39	2707	0.38	1.73
+Glc	1368	0.29	2854	0.65	2841	0.35	1.01
after 16 h of preincubation							
−Glc	2276	0.36	38359	1.34	2158	0.43	17.78
+Glc	2110	0.27	18620	0.83	2254	0.28	8.26

[a] Thyrotropic tumor cells were studied immediately after collagenase dispersion (no preincubation) or after 16-h preincubation in the presence or absence of glucose (see Experimental Procedures). Cells were studied after 60-min continuous labeling with [^{3}H]mannose as described in the legend to Figure 4.

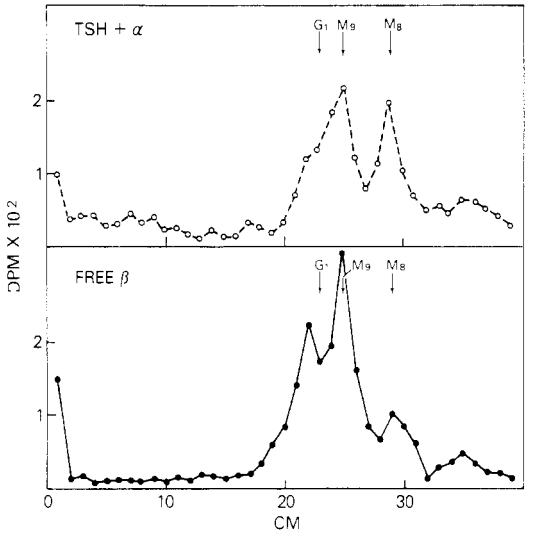

FIGURE 1: Paper chromatography of Endo H treated TSH subunit carbohydrate chains from tumor cells incubated for 60 min in the presence of [^{3}H]mannose. The immunoprecipitation was performed sequentially, first with anti-α [(top panel) TSH + free α] followed by anti-β [(bottom panel) free β]. The markers are $Glc_1Man_9GlcNAc$ (G_1), $Man_9GlcNAc$ (M_9), and $Man_8GlcNAc$ (M_8).

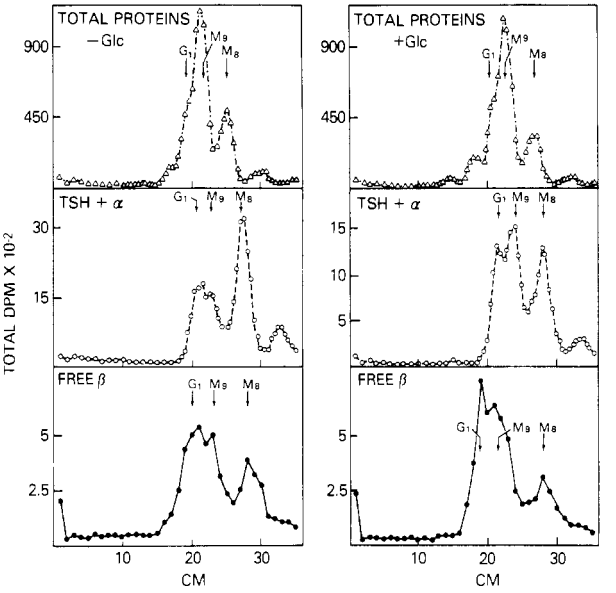

FIGURE 2: Paper chromatography of Endo H treated oligosaccharides from tumor cells preincubated for 16 h in the absence (left panels) or presence (right panels) of glucose with 10% hypothyroid serum dialyzed against glucose-free media or nondialyzed, respectively, followed by 60-min continuous labeling with [^{3}H]mannose. (Top panels) Total protein; (middle panels) TSH + free α; (bottom panels) free β. Immunoprecipitation and markers are as in Figure 1.

of the labeled total proteins was released by Endo H. Various sizes of oligosaccharides could be identified ranging from $Man_9GlcNAc$ to $Man_6GlcNAc$, the major species being $Man_9GlcNAc$ in the absence or presence of glucose (Figure 2, top panels). On TSH subunits, the labeled oligosaccharides remained sensitive to Endo H when the cell incubation was derived of glucose. On TSH + α, the amount of $Glc_1Man_9GlcNAc$ and $Man_9GlcNAc$ clearly decreased in the absence of glucose with a concomitant increase in the $Man_8GlcNAc$ intermediate (Figure 2, middle panels). In contrast, the free β-oligosaccharides were only slightly affected by glucose starvation, showing only a very small increase in $Man_8GlcNAc$ (Figure 2, bottom panels).

These data indicate that thyrotropic tumor cells are resistant to previously described effects of glucose starvation in certain cell lines. Oligosaccharide lipids remained unaffected (data not shown) as did total protein and β-linked oligosaccharides (Table I), suggesting that the specific activity of these compounds was not greatly modified in the presence or absence of glucose. Interestingly, compared to cells studied immediately after enzymatic dispersion, there was a specific 9-fold stimulation of the TSH + α to free β ratio in cells studied after 16-h preincubation, in both the absence and presence of glucose (Table I). However, after 16 h of preincubation, the labeling of TSH + α oligosaccharides and the TSH + α to free β ratio was increased 2-fold in the presence of glucose.

Processing of TSH Subunit Oligosaccharides in Rat Pituitaries. Since thyrotropic tumors synthesize primarily TSH and its α subunit but no other glycoprotein hormones, they provide a convenient system to study the biosynthesis of α and β subunits. However, thyrotropic tumors produce an excess of free α subunits, and the α to TSH ratio proved to be more elevated for the tumor than for plasma or pituitary extracts of thyroidectomized mice and rats (Blackman et al, 1978). To determine whether the processing of TSH subunit carbohydrate chains involves the same intermediates in the tumor compared to nonneoplastic pituitary glands, pituitaries from normal or hypothyroid rats and mice were incubated for 60 min in the presence of [^{3}H]mannose or [^{3}H]galactose in glucose-free, serum-free medium (Figure 3). Galactose was previously shown to be a specific precursor for glucose labeling of high-mannose oligosaccharide (Rearick et al., 1981). Due to the limited amount of material, α and β subunits were isolated by sequential immunoprecipitation using first anti-α followed by anti-β serum. As found in the tumor, virtually all the mannose label associated with TSH subunits or total proteins at 60 min was released by Endo H treatment. The mannose-labeled TSH + α-linked oligosaccharides appeared to be composed of equal amounts of $Man_9GlcNAc$ and $Man_8GlcNAc$ species (Figure 3, top panels) in both normal and hypothyroid rats. No glucose was detected in these species. On free β subunits from normal rats, $Man_9GlcNAc$ was the major species with minor amounts of $Glc_1Man_9GlcNAc$ and

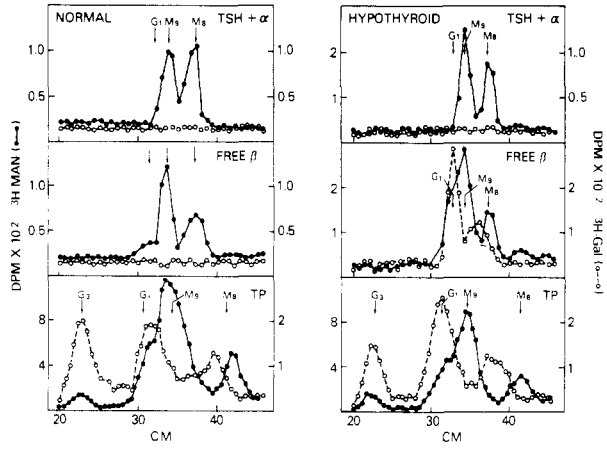

FIGURE 3: Paper chromatography of Endo H treated oligosaccharides from normal (left panels) or hypothyroid (right panels) rat pituitaries incubated for 60 min in the presence of [^{3}H]mannose (●) and [^{3}H]galactose (○). (Top panels) TSH + α; (middle panels) free β; (bottom panels) total proteins (TP). Immunoprecipitation and markers are as in Figure 1.

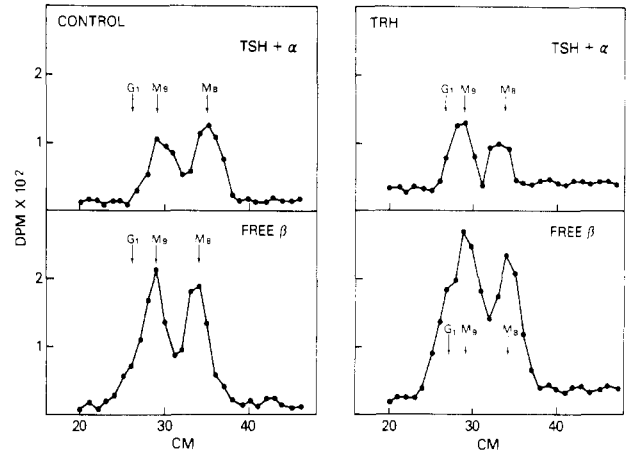

FIGURE 4: Paper chromatography of Endo H treated TSH carbohydrate chains from pituitaries of rats 7-days postthyroidectomy in the absence (control, left panels) or presence of TRH treatment (right panels, see Experimental Procedures) incubated for 180 min in the presence of [^{3}H]mannose. (Top panels) TSH + α; (bottom panels) free β. Immunoprecipitation and markers are as in Figure 1.

$Man_8GlcNAc$ (Figure 3, middle left panel), a pattern very similar to that identified in the tumor. Again, minimal labeling of glucose-containing species was noted with the galactose precursor. In contrast, hypothyroid pituitaries exhibited a striking enhancement of glucose-labeled $Glc_1Man_9GlcNAc$ on free β subunits together with the appearance of a new intermediate migrating between $Man_9GlcNAc$ and $Man_8GlcNAc$, probably $Glc_1Man_8GlcNAc$ (Figure 3, middle right panel). This modification appeared restricted to free β subunits, total protein and α-subunit carbohydrate chains remaining identical in normal and hypothyroid pituitaries (Figure 3, top and bottom panels). Similar results were obtained with normal and hypothyroid mice (data not shown). These data demonstrate that TSH processing in nonneoplastic pituitary glands is very similar to if not identical with that previously noted in the thyrotropic tumor. In addition, glucose-containing oligosaccharides are clearly detectable on β subunits rather than on α and are stimulated by thyroid hormone deficiency.

In Vivo TRH Stimulation of TRH Glycosylation. Pituitaries from rats 7-days postthyroidectomy were incubated for 180 min with [^{3}H]mannose after both in vivo and in vitro TRH stimulation (see Experimental Procedures). Intracellular TSH subunits were examined after two different immunoprecipitation procedures: (i) anti-α followed by anti-β (to identify TSH + α and free β, respectively); (ii) anti-β followed by anti-α (to identify TSH + β and free α, respectively). As previously noted after stimulation with prolonged thyroid hormone deficiency, oligosaccharides from free β derived from TRH-stimulated rats exhibited a higher amount of $Glc_1Man_9GlcNAc$ than those of control rats (Figure 4). $Man_9GlcNAc$ and $Man_8GlcNAc$ were also present in similar amounts on both conditions. In contrast, minimal modification of the glucose-containing species was noted in oligosaccharides linked to TSH + α subunits after TRH stimulation.

When the immunoprecipitation sequence was reversed, the TRH stimulation of the $Glc_1Man_9GlcNAc$ species was even more clearly obtained in the TSH + β compared to free α subunits, confirming that this effect was specific for β subunits (data not shown).

DISCUSSION

Work from several laboratories has previously shown modifications of glycosylation in virus-infected cells (Kornfeld et al., 1978), glucose-starved cells (Turco, 1980; Rearick et al., 1981; Gershman & Robbins, 1981), lectin-resistant cells (Krag & Robbins, 1982), or cells incubated with drugs that inhibit oligosaccharide processing (Tulsiani & Touster, 1983; Arumugham & Tanzer, 1983), energy metabolism (Godelaine et al., 1981), or cation transport (Peters et al., 1983). However, these conditions were highly unphysiological, and although useful in identifying key intermediates in glycoprotein biosynthesis, they did not directly elucidate the physiological regulation of this complicated process. In the present studies, we have examined both in vivo and in vitro selective regulation of TSH subunit glycosylation by comparing the processing of the hormone carbohydrate chains in thyrotropic tumors and normal, hypothyroid, and TRH-stimulated pituitaries.

Recent studies from our laboratory (Ronin et al., 1984) have shown differences in early glycosylation and carbohydrate processing between TSH subunits and other nonsecretory cell glycoproteins of thyrotropic tumor cells, suggesting that the biosynthesis of the carbohydrate chains is carefully modulated and closely dependent on the nature of the polypeptide backbone. While cell glycoprotein glycosylation occurs through a $Glc_3Man_9GlcNAc_2$ precursor[2] as in fibroblasts (Hubbard & Ivatt, 1981), no such intermediate was detected on either TSH subunit. Instead, $Glc_1Man_9GlcNAc_2$, $Man_9GlcNAc_2$, and $Man_8GlcNAc_2$ were the largest oligosaccharides detected on both α and β subunits. This finding is compatible either with a selective transfer of a glucose-free high-mannose-type precursor to TSH subunits followed by posttranslational addition of glucose, as already found in the thyroid (Ronin & Caseti, 1981; Parodi et al., 1983), or with a preferential rapid removal of glucose residues on TSH subunits (Ronin et al., 1984). The present study presents evidence that the carbohydrate processing of both TSH subunits was very similar in the thyrotropic tumors and normal and hypothyroid pituitaries: similar high-mannose intermediates were noted in the tumor and in the two nonneoplastic conditions, although the amount of Glc_1-containing species was highest in hypothyroid rat pituitaries. Interestingly, the TSH subunit carbohydrate chains are not processed at the same rate: the β-linked carbohydrate chains are converted to Endo H resistant, presumably complex-type forms more rapidly than α-linked chains. The differences are similar to those noted

[2] Oligosaccharides released by Endo H contain one fewer GlcNAc residue than present on the glycoprotein.

in the processing of other glycoproteins, such as VSV G protein (Kornfeld et al., 1978), thyroglobulin (Godelaine et al., 1981), fibronectin (Choi & Hynes, 1979), and transferrin (Strous & Lodish, 1980), although no structural characterization other than Endo H resistance was reported for the latter two proteins. On TSH, the same prominent intermediates $Glc_1Man_9GlcNAc_2$, $Man_9GlcNAc_2$, and $Man_8GlcNAc_2$ were observed on both subunits, suggesting that the trimming of each subunit carbohydrate chain follows a similar if not identical pathway. Since β-subunit synthesis has been suggested to be a rate-limiting step in hormone synthesis (Weintraub et al., 1980; Magner & Weintraub, 1982), it is likely that the fate of the β subunit chiefly reflects that of the combined hormone. Therefore, different rates of carbohydrate processing between immunoprecipitated α and β subunits may in fact reflect differences in the fate of combined vs. uncombined subunits. More detailed studies of carbohydrate processing of combined vs. uncombined subunits will be necessary to clarify this point.

After submission of the manuscript, detailed studies of the carbohydrate processing and secretion of CG subunits in choriocarcinoma cells were reported (Peters et al., 1984). Certain aspects of CG and TSH subunit processing appear similar, including the predominance of $Man_8GlcNAc$ as an intermediate in α compared to β subunits. However, there are also major differences between the two systems, such as the presence of significant amounts of intracellular and extracellular free CG-β subunits. Compared to TSH subunits, CG subunits appear to have a somewhat different distribution of high-mannose intermediates and show minimal accumulation of mature, complex oligosaccharides intracellularly. Finally, the secretion and processing of CG subunits in choriocarcinoma cells have not been demonstrated to be influenced by physiological endocrine factors, as described herein for TSH subunits.

Earlier work from our laboratory demonstrated that in thyrotropic tumors labeled for 10 min virtually all the limiting β subunits were still uncombined, whereas after 60 min of chase, more than 60% were combined (Weintraub et al., 1980, 1983; Magner & Weintraub, 1982). Further, we observed that α–β-subunit combination began in the rough endoplasmic reticulum while both subunits were Endo H sensitive (Magner & Weintraub, 1982). In the present report, primarily high-mannose-type oligosaccharides were observed on TSH subunits during the pulse period, whereas mostly complex forms were observed during the chase, suggesting that α–β combination is rapidly followed by translocation to the Golgi and mannose removal. In contrast, uncombined α subunits were processed more slowly, their oligosaccharides remaining for a longer time in a mannose-rich form. It is conceivable that glucose and possibly one mannose residue can be excised on uncombined α and β subunits, but as soon as combination occurs, the inner mannose residues are very rapidly processed. The mannosidases responsible for the late processing have been shown to be located in Golgi membranes (Tabas & Kornfeld, 1978; Tulsiani et al., 1982), further supporting our view that TSH carbohydrate chains are trimmed after the subunits have combined and probably migrated to the Golgi area (Magner & Weintraub, 1982). In fact that free α-subunit processing appears delayed compared to TSH raises the possibility that uncombined α subunits may not follow the same intracellular fate as combined α subunits. Recent data from subcellular fractionation showed that processing to complex forms primarily occurs in the distal smooth endoplasmic reticulum Golgi and is more rapid for TSH than for free α subunits (Magner et al., 1984). Furthermore, it has been reported that uncombined but not combined forms of LH-α subunit are O-glycosylated (Parsons et al., 1983). If also true for TSH-α subunits, this additional posttranslational modification may account for the differential rate of processing between TSH and free α subunits as well as a possible different route within the Golgi apparatus. Alternatively, such O-glycosylation may solely be the result of a threonine residue that is conformationally accessible in free α and not in combined α subunit (Strickland & Pierce, 1983) and may be a late event unrelated to differential transport.

This study presents evidence that carbohydrate trimming is probably a regulated process. Glucose starvation of tumor cells selectively stimulated the mannose incorporation of α-linked carbohydrate chains and increased the amount of $Man_8GlcNAc_2$ intermediates on this subunit. This species was also found to accumulate in previous pulse–chase experiments (Weintraub et al., 1983), as well as on human CG-α subunits in choriocarcinoma cells (Ruddon et al., 1981) and on thyroglobulin (Godelain et al., 1981), supporting the concept that it probably is an important limiting step in the processing. The effect of glucose starvation reported here is not comparable to that previously reported for CHO cells (Turco, 1980; Rearick et al., 1981) or NIL fibroblasts (Turco, 1980): no alteration of lipid-linked oligosaccharides and a very small increase of shorter protein-linked oligosaccharides have been detected upon starvation of the tumor cells (data not shown), a situation partially resembling that of BHK cells in which some $Man_8GlcNAc_2$ was still detectable on lipids after 22 h of starvation (Turco, 1980). Such glucose concentrations are far below those encountered in severe hypoglycemic conditions in vivo and thus can be regarded as essentially nonphysiological. However, the mechanism by which this treatment induced a specific accumulation of unprocessed α subunits is unclear.

Thyroid hormone deficiency in rodents appeared to result in a striking increase in the Glc_1-containing species on free TSH β subunits. Such oligosaccharides could be detected on uncombined β subunits from hypothyroid pituitaries but not on those derived from Tsh + α. In vivo and in vitro TRH stimulation resulted in a similar modification, suggesting that hypothalamic stimulation and feedback regulation by thyroid hormones of TSH secretion may follow a very similar pathway. Recent studies from our laboratory (Taylor & Weintraub, 1985a,b) have shown that both hypothyroidism and TRH treatment increase the ratio of glucosamine to alanine in secreted TSH. Since glucosamine incorporation into secreted TSH can be regarded as a marker of terminal glycosylation, this suggests that endocrine factors may cause specific structural changes in the TSH carbohydrate moiety. Whether the late maturation of TSH carbohydrate chains is influenced by their early processing is still speculative, but this is possible since TSH assembly selectively proceeds on mannose-rich α and β subunits (Weintraub et al., 1983). Specifically, the increase of Glc_1-containing intermediates caused by hypothyroidism or TRH may result from posttranslational addition of glucose which could alter the later action of α-mannosidases and glycosyltransferases (Ronin & Caseti, 1981; Parodi et al., 1983).

Endocrine regulation of TSH assembly and glycosylation may have important physiologic implications. Differential carbohydrate processing of both combined and uncombined subunits could modulate the rate of hormone assembly and intracellular translocation as well as the biologic activity of the secreted hormone. In fact, we have demonstrated that

various forms of TSH differing in carbohydrate content have different bioactivity (Joshi & Weintraub, 1983) and that the TSH secreted after TRH stimulation has enhanced bioactivity.[3] Thus, the regulation of TSH carbohydrate processing may provide the basis for modulation of hormone action in various physiologic states.

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Registry No. TSH, 9002-71-5; $Man_9GlcNAc$, 70158-33-7; $Man_8GlcNAc$, 97372-60-6; $Glc_1Man_9GlcNAc$, 71939-21-4; TRH, 24305-27-9; Glc, 50-99-7.

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