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DIFFERENTIAL EFFECTS OF THYROTROPIN ON VARIOUS GLYCOSYLTRANSFERASES IN PORCINE THYROID CELLS

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SUMMARY. Porcine thyroid cells were cultured with or without thyrotropin for 9 days. It is known that the hormone enhances the synthesis of thyroglobulin, with an increase in the content and stability of its m-RNA. We show in the present work that thyrotropin also stimulates a number of glycosyltransferases diversely situated along the process of N-glycosylation. The most increased was oligosaccharyltransferase, responsible for attachment to nascent peptides of preformed core carbohydrate sequences. The relatively low activity level of oligosaccharyltransferase and its preferential responsiveness to thyrotropin supports the possibility of a regulation point at this enzyme.

Thyroid cells in primary cultures retain the property to respond to thyrotropin (1) several effects of which are related to the metabolism of thyroglobulin. A modulation of its synthesis occurs at the m-RNA level (2, 3). That some regulation might be exerted at post-translational steps required for glycosylation was suggested from an in-vivo study showing increased glycoprotein β-galactosyl-and Dol-P mannosyl transferases under propylthiouracil (4).

Porcine thyroglobulin the major product synthesized in thyroid cells contains some twenty or more "high mannose" and "complex" N-linked carbohydrate units (5, 6). Structural features pertaining to these units might represent signals for proper sorting and further endocytosis. Tunicamycin, an antibiotic that inhibits N-glycosylation by a block at the Irst step of the dolichol-mediated cycle, suppresses thyroglobulin secretion (7).

In this paper we have studied in porcine thyroid cells with or without TSH three enzymes that participate in the rough endoplasmic reticulum to "core-glycosylation" events (8) : GDP-Man : Dol-P mannosyltransferase, UDP-Glc : Dol-P glucosyltransferase and Dol-P-P-oligosaccharide : peptide oligosaccharyl-

ABBREVIATIONS : TSH, thyrotropin ; Dol, dolichol.

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transferase. We have also measured two Golgi-located enzymes : UDP-Gal : glycoprotein galactosyltransferase and CMP-Sial : glycoprotein sialyltransferase, responsible for peripheral additions.

MATERIALS AND METHODS

Radiochemicals were from Amersham or NEN, biochemicals from GIBCO, Calbiochem-Behring and SIGMA. Thyrotropin was from Organon.

Cell culture. Cells were isolated from porcine thyroid glands by trypsinization and cultured onto Falcon dishes in Eagle's minimum essential medium containing 5 % newborn calf serum (9). TSH when present (1 mU/ml) was added at the onset of seeding.

Cell sampling. The medium was replaced by a spinner salt solution without Mg^{2+}, made 3 mM EGTA. After 30-40 min, cells were collected by low speed centrifugation, washed once in Earle's medium and twice in 0.1 M cacodylate pH 7.0, 0.15 M NaCl. Suspended cells were stored frozen as small aliquots in liquid N_2 ($2.5\text{-}5 \times 10^6$ cells/25 µl). DNA was determined according to (10). We have assumed from previous controls that 1×10^6 cells = 10 µg DNA. Protein/DNA was close to 10.

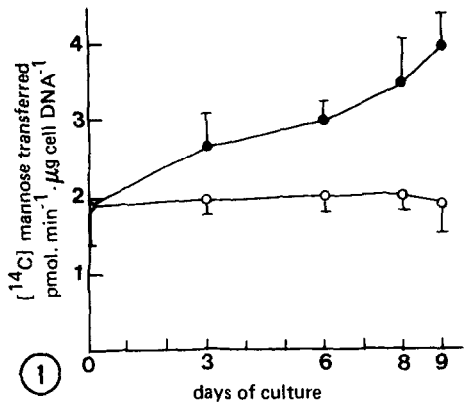
Cell homogenates. Frozen aliquots were thawed, briefly vortexed and placed in an ice box. They immediately received variable amounts (75-225 µl) of the following ice-cold media, were vortexed again and used for assays, either within minutes (glucosyl-and mannosyl transferases) or after 20-30 min (others) : for glucosyl and mannosyl transferases, 50 mM Tris-HCl pH 7.2, 0.2 % (v/v) Triton X-100, 0.15 M NaCl and 12 mM $MgCl_2$; for oligosaccharyltransferase, 50 mM Tris-HCl pH 7.4, 1 % Nonidet P-40, 0.5 M NaCl and 10 mM $MnCl_2$; for galactosyltransferase 0.1 M Na cacodylate pH 7.1, 0.2 % Triton X-100, 0.15 M NaCl and 10 mM $MnCl_2$; for sialyltransferase, the same except no $MnCl_2$.

Glycosyltransferase assays. They were performed in quadruplicate in conditions (Table 1) ensuring a linear dependency between the radiolabel transferred and the amount of cell homogenate or incubation length.

RESULTS AND DISCUSSION

The present work relies on 4 independent cultures. Without TSH the typical monolayers were observed till the last day examined, day 9. With TSH the folliculisation culminated around days 5-6 and was followed by a tendency to transform into monolayers. Order of magnitude of glycosyltransferase activities did not greatly vary from one preparation to another one, neither did the time courses of their changes and sensitivities to TSH (studied in at least 2 cultures for each transferase, results being means ± S.E.M.)

Fig. 1. and Fig. 2 show the evolution of Dol-P mannosyl- and glucosyl transferases. Their reaction products, Dol-P-Man and Dol-P-Glc are the donors for completion of Dol-P-P-linked oligosaccharides to high mannose units, ready for transfer. In the absence of TSH enzyme activities were more or less maintained. In its presence they progressively increased. At day 9 increments of



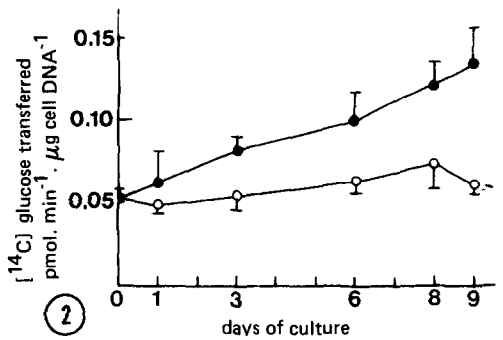


Fig. 1 (left). Time course of changes in mannosyltransferase activity in cultured porcine thyroid cells. With(●) or without (○) TSH.

Fig. 2 (right). Glucosyltransferase activity in the same porcine thyroid cells. With (●) or without (○) TSH.

Experimental conditions as described in the text and in Table 1.

activities due to TSH were + 105 % for mannosyl-and + 116 % for glucosyl transferase. As recently pinpointed by Ravoet et al (14) these two enzymes are quite unstable in the presence of the neutral detergent needed for their solubilization. We nevertheless obtained satisfactory values in the conditions used here. It is worth mentioning that the solubilized glucosyltransferase remains inhibited by UDP-Man whereas it becomes insensitive to GDP-Man (data not given) in contrast with its behavior in undispersed particles (11). Apparent Kms were similar in homogenates from TSH-treated or untreated cells : 4 µM for UDP-Glc, 1.4 µM for GDP-Man, 10 and 80 µM for Dol-P (glucosyl-and mannosyltransferases respectively).

Measurement of oligosaccharyltransferase was based upon the use of a synthetic Asn-X-Thr containing peptide as acceptor, in the presence of a previously labeled oligosaccharide-lipid as donor (Table 1). The striking finding for this enzyme was its rapid rise in TSH-treated cells as compared to controls (Fig. 3). At the period of folliculisation in hormone treated cells its specific activity was about 6-fold that in control cells. This values was more than 4-fold that of the cell suspension at day zero. Core-glycosylation is in most cases and in particular during thyroglobulin synthesis a co-translational event (15) occuring during a limited period of time when growing chains pass through

TABLE 1. CONDITIONS USED FOR GLYCOSYL TRANSFERASE (TF) ASSAYS[a]

Transferase	Donor (μM)	Acceptor (μg)	Buffer (pH)	Divalent cation (μM)	Detergent % (v/v)	Others	Tp (°C)
Man TF	GDP-Man (5.1)	Dol-P (8)	Tris-HCl (7.2)	Mg^{2+} (12)	Triton X-100 (0.2)	AMP (6 mM)	25
Glc TF	UDP-Glc (3)	Dol-P (3)	Tris-HCl (7.2)	Mg^{2+} (12)	Triton X-100 (0.2)	AMP (6 mM)	25
Oligosacch. TF	Dol-P-P-oligosacch. (0.4) [b]	heptapeptide (33) [c]	Tris-HCl (7.6)	Mn^{2+} (10)	Nonidet P-40 (0.2)	dimethyl-sulfoxide, phospho-lipids [d]	37
Gal TF	UDP-Gal (160)	ovomucoid (2 000)	cacodylate -HCl (7.0)	Mn^{2+} (10)	Triton X-100 (0.2)	AMP (6 mM)	37
NeuAc TF	CMP-NeuAc (133)	asialofetuin (1 150)	cacodylate -HCl (6.8)	no	Triton X-100 (0.2)	no	37

(a) 100 μl assays containing 1-4 μg cell DNA. Duration 15 or 30 min. Stopped by adding 3 vol $CHCl_3$, 2 vol CH_3OH followed by 4 washings of the lower phase (14) for Man TF and Glc TF ; by adding 1.5 vol C_2H_5OH followed by paper electrophoresis for oligosacch. TF (13) ; by precipitation with 10 % trichloracetic acid, 5 % phosphotungstic acid, and 3 washings, for Gal TF and NeuAc TF. Endogenous labelings, very small, were eventually substracted with background counts.

(b) the donor, prepared as in (11), was a ^3H-Man, ^{14}C-Glc-labeled Dol-P-P-$GlcNAc_2Man_9Glc_{2-3}$. Its specific radioactivity, 580 ^3H-Man dpm/pmol was determined from a treatment with $[^3H]NaBH_4$ of the oligosaccharide released by mild acid hydrolysis (12).

(c) synthetic heptapeptide corresponding to residues 73-79 of porcine ribonuclease (13).

(d) heat inactivated thyroid rough microsomes, corresponding to approx. 100 μg microsomal protein (Franc and Bouchilloux, submitted for publication).

rough endoplasmic reticulum or just emerge into cisternae. It is conceivable that when peptide synthesis is enhanced oligosaccharyltransferase must be adjusted. Especially as its activity level appears low. In a numbers of cells including thyroid it seems that the available pool of more or less completed Dol-P-P-oli-

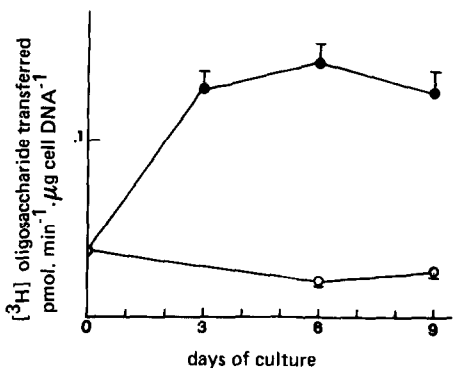
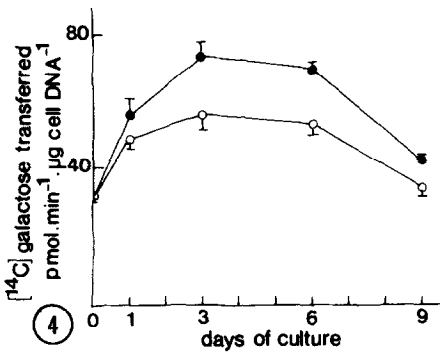


Fig. 3. Time course of changes in oligosaccharyltransferase activity in porcine thyroid cells. Cultured with (●) or without (○) TSH.

Experimental conditions in the text and Table 1.



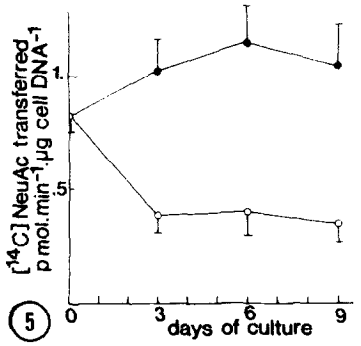


Fig. 4 (left). Time course of changes in galactosyltransferase activity in porcine thyroid cells. Cultured with (●) or without (○) TSH.

Fig. 5 (right). Sialyltransferase in the same porcine thyroid cells. With (●) or without (○) TSH.

Experimental conditions as described in the text and in Table 1.

gosaccharides normally exceeds the requirement for protein glycosylation (16). This was taken as suggestive of a rate-determining step at the transferase itself (17, 18) : our data give a direct support to this presumption, as does another report that oestrogen treatment of immature chicks enhances oligosaccharyl-transferase in oviduct microsomes (19).

Finally we examined two Golgi-located monosaccharyltransferases which contribute to completion of complex type units. Fig. 4 shows the pattern of activity for galactosyltransferase (essentially glucosaminide β1→4 galactosyltransferase in our assay with ovomucoid as acceptor). Even without TSH there is a substantial activity rise during the first 3-4 days of culture, which was enhanced in hormone treated cultures (+ 30 % at day 4). Activities then progressively reverted to their day zero level. It can be noticed that thyroid contains a relatively high level of galactosyltransferase compared to the others examined here.

Fig. 5. presents the effect of TSH addition on sialyltransferase, in fact asialofetuin sialyltransferase. In the absence of TSH enzyme activity rapidly leveled down thus giving an almost 3-fold relative increase in hormone-treated cells. Our measurement might recover several enzymic entities even though β-galactoside α2→6 sialyltransferase is probably the major one. We have controlled from the percentages of $[^{14}C]$ NeuAc releasable by specific neuraminidases that the major sialylation was α2→6, with some α2→3 sialylation occuring too (data not shown).

In conclusion TSH stimulated to various extents all the glycosyltransferases we examined. The highest increments were for oligosaccharyltransferase an enzyme postulated to be limiting in the whole process of N-glycosylation and then in decreasing order sialyltransferase, mannosyl-and glucosyl transferases, and finally galactosyltransferase. It is highly probable that these effects are related at least in part to the well known increase in thyroglobulin synthesis, even though for another part they might reflect an enhanced synthesis of other glycoproteins. The fact that when examined affinities of the transferases for their substrates were found unmodified with TSH favors the possibility of higher enzyme levels, but further work would be necessary to ascertain this presumption.

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REFERENCES

1. Fayet, G., Hovsepian, S., Dickson, J.G. and Lissitzky, S. (1982) J. Cell. Biol., 93, 479-488.
2. Chabaud, O., Chebath, J., Giraud, A. and Mauchamp, J. (1980) Biochem. Biophys. Res. Commun., 93, 118-126.
3. Tosta, Z., Chabaud, O. and Chebath, J. (1983) Biochem. Biophys. Res. Commun., 116, 54-61.
4. Spiro, M.J. (1980) Archiv. Biochem. Biophys., 202, 35-42.
5. Tsuji, T., Yamamoto, K., Irimura, T. and Osawa, T. (1981) Biochem. J., 195, 691-699.
6. Yamamoto, K., Tsuji, T., Irimura, T. and Osawa, T. (1981) Biochem. J., 195, 701-713.
7. Björkman, U. and Ekholm, R. (1982) Europ. J. Biochem. 125, 585-591.
8. Hubbard, S.C. and Ivatt, R.J. (1981) Ann. Rev. Biochem., 50, 555-583.
9. Hovsepian, S., Feracci, H., Maroux, S. and Fayet, G. (1982), Cell Tissue Res., 224, 601-611.
10. Mc Intyre, F.C. and Sproull, M.F. (1957) Proc. Soc. Exptl. Biol., 95, 485-462.
11. Ronin, C., Caseti, C. and Bouchilloux, S. (1981) Biochim. Biophys. Acta, 674, 48-57.
12. Spiro, R.G., Spiro, M.J. and Bhoyroo, V.D. (1976) J. Biol. Chem., 251, 6409-6419.
13. Ronin, C., Bouchilloux, S., Granier, C. and Van Rietschoten, J. (1978) FEBS Lett., 96, 179-182.
14. Ravoet, A.M., Amar-Costesec, A., Godelaine, D. and Beaufay, H. (1982) J. Cell. Biol., 91, 679-688.
15. Alvino, C.G., Tassi, V., Polistina, C., Di Lauro, R. and Bonatti, S. (1982) Europ. J. Biochem. 125, 15-19.
16. Murphy, L.H. and Spiro, R.G. (1981) J. Biol. Chem. 256, 7487-7494.
17. Hubbard, S.C. and Robbins, P.W. (1980) J. Biol. Chem., 255, 11782-11793.
18. Tenner, A.J. and Scheffler, J.E. (1979) J. Cell. Physiol. 98, 251-266.
19. Singh, B.N. and Lucas, J.J. (1981) J. Biol. Chem., 256, 12018-12022.