HORMONAL CONTROL OF PROTEIN GLYCOSYLATION: ROLE OF STEROIDS AND RELATED LIPOPHILIC LIGANDS

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Abstract. Glycosylation represents one of the most frequent and certainly the most variable co- and post-translational modification of proteins. Carbohydrate moieties of glycoproteins are known to provide important prerequisites for various biological functions, and their structural diversity can serve as ideal candidate to carry also biological information. Production and/or function of various glycoproteins is under control of steroids and other ligands of nuclear receptors which influence synthesis, glycosylation, storage or usage of target proteins. It appears that among small lipophilic hormonal compounds the steroids are chiefly involved in regulation of protein glycosylation. There is no apparent difference between ability of these hormones to regulate *N*- or *O*-glycosylation, but majority of documented cases deals with terminal modifications involving sialylation or fucosylation of *N*-linked carbohydrate moieties. In spite of the knowledge on glycosylation in general, published results offers only a glimpse of data on the hormonal control of glycosidase activity which is equally required for carbohydrate chain elongation as is the activity of various glycosyltransferases. The significance of this research is daily growing owing to the fact that changes in glycosylation pattern of various intracellular or secretory proteins not only reflect developmental or differentiation stage but also serve as well established markers in invasiveness or regression of numerous cancers responding to hormonal stimuli. Combination of classical methods and more complex approach in genetically well defined model systems can not only increase recent surge of interest in glycosylation but also provide a formidable amount of qualitatively new type of data on the mechanism how hormones control glycosyltransferases and glycosidases and how their activity is interconnected to the synthesis of substrates, posttranslational maturation, and final destination or function of target proteins.

Key words: Hormones - Steroids - Thyroid hormones - Retinoids - *N*-glycosylation - *O*-glycosylation - Glycosidases - Glycosyltransferases

Introduction

Understanding the specific molecular interactions that govern correct functions of the cells is of pivotal scientific and clinical relevance. When considering these molecular interactions and their regulation, there is still a strong prejudice towards thinking only in terms of proteins. In recent past, however, it is starting to be appreciated that the carbohydrate moieties of glycoproteins provide important prerequisites for various biological functions. By their potential for structural diversity they can serve as ideal candidates to carry also biological information. Thus, it is not surprising to unravel programmed and strictly regulated changes in carbohydrate composition and sequence in glycoconjugates within the course of complex processes such as development and differentiation. Consequently, strong changes in presentation of cellular glycoproteins have also been detected upon malignant transformation and tumorigenesis (SCHAUER et al. 1988; SHARON 1991; HAKOMORI 1996). In addition, mevalonate-sensitive *N*-linked glycosylation of proteins is necessary for cell growth, including initiation and propagation of DNA synthesis in normal and tumor-transformed cells

(Carlberg and Larsson 1993; Wejde et al. 1993; Carlberg et al. 1996; Chan et al. 2001).

Glycoproteins as prominent and most variable group of glycoconjugates play a prominent part (*e.g.* as mucins) in the secretion. The biosynthesis of the glycan chains was demonstrated to be controlled by various exogenous (such as nutritional) or endogenous (such as developmental) factors. Even though the metabolic regulation by dietary variations seems to be less apparent as they cause mostly quantitative changes, on the other hand, modifications in glycosylation during animal development control the quality of the glycan chains of mucins and other glycoproteins (Takasaki et al. 1991; Hirayama and Wright 1992; Van Beers et al. 1995; Thorens 1996). Developmental changes including tissue differentiation are accompanied by changes in glycosylation patterns, *e.g.* like a shift from sialylation to fucosylation, depending on coordinate changes in glycosyltransferase activities, in sugar-nucleotide breakdown or synthesis and activity of regulatory proteins (Biol-N'garagba and Louisot 2003). These activities are largely sensitive to developmental stimulations involving hormonal triggers (Sommer and Cowley 2001). Small lipophilic hormones including steroids, retinoids, thyroids, vitamin D_3 as well as large variety of ligands for peroxisome proliferator activated receptors (PPARs), liver X receptor (LXR), farnesoid X receptor (FXR) and other nuclear receptors are known to affect protein synthesis at translational and transcriptional levels. However, there is very fragmentary information on the specific hormonal control of glycoprotein production with special emphasis on secretion and whole machinery involved in protein glycosylation preceding process of secretion. With the advent of completing genomes of several eukaryotes including yeasts, *Caenorhabditis*, *Drosophila*, mouse and humans it become possible to undertake comparative studies for conservation or evolutionary divergence of proteins involved in glycosylation and secretion, but the role of particular hormones in developmental and/or metabolic regulation of enzymes participating in protein glycosylation remains fragmentary and obscurely defined. In this paper we try to review our present knowledge on mechanisms how steroid hormones and other related small lipophilic ligands regulate protein glycosylation and thus influence subsequent function of these proteins.

General effects of hormones on secretory process and glycoprotein formation

The earliest studies of Spiro and Spiro (1968, 1971) have suggested that thyroid hormone has pronounced effect on thyroglobulin biosynthesis and glycosylation, as well as on the subcellular distribution of skin, thyroid, kidney and spleen glycosyltransferases in rats. Glycosyltransferase distribution and activity was also affected by pregnancy when hormonal milieu of females have drastically changed. Subcellular distribution of enzyme activities and cell fractionation provides some indication that one of the targets of the hormone action and glycosyltransferase activity might be collagen. Complex approach of Spiro and Spiro (1971) has provided also some clues that galactose, glucosamine and glucose moieties are being predominantly utilized under high glycosyltransferase activities in mentioned organs and tissues. Vonderhaar (1975) concluded that thyroxine and hydrocortisone principally affect differentiation of mouse mammary gland by influencing lactalbumin synthesis and maturation via changes in galactosyltransferase activity. Tadolini et al. (1976) have found causal relationship between rat testicular and prostate *N*-acetylglucosaminylglycopeptide-β-1,4-galactosyltransferase activity, prostate secretory capabilities and levels of plasma testosterone. Similar conclusions were reached also for human testicular and prostate glycosyltransferase with potential diagnostic significance. Both the formation and size of Golgi apparatus and the cytoplasmic dense bodies in pig female pinealocytes are dependent on continuous presence of ovarian steroid hormones and their secretory activity become strongly diminished upon castration (Przybylska et al. 1993). However, readministration of estradiol or combination of estradiol and progesterone rescues secretory features of the cells and also lead to decrease in relative volume of lysosomes whereas failure in supplementing steroids lead to enlarged lysosomes and spontaneous cessation. In this case, continuous presence of the steroid provides for antiapoptotic or survival signal which is sufficient to maintain secretory, probably main, physiological program of the tissue, and thus hormones act at very general and non-specific level.

Among the first serious indications that small lipophilic hormones acting through nuclear receptors may be involved in the control of glycoprotein synthesis and/or modification comes from the observation that thyroxine, hydrocortisone and testosterone influence the activity of the glycosyltransferases responsible for the sequential transfer of xylose and galactose from UDP-xylose and UDP-galactose, respectively, in the formation of first two monosaccharide units of the chondroitin sulfate-protein linkage region during cartilage deposition in young rats (Schiller 1976). Another important

observation was that protein galactosyltransferase of the ventral prostate of hypophysectomized rats is markedly enhanced by testosterone and potentially downregulated by androgens (Reddy et al. 1976). Subsequently it was described that cortisol affects collagen biosynthesis and the activities of collagen galactosyltransferase and collagen glucosyltransferase in chick embryo tendon cells (Oikarinen 1977). On the other hand, it was observed that testosterone administration causes rat pituitary gland cells to become ovoid and their Golgi complex enlarged, displaying dilated cisternae with increased numbers of immature secretory granules followed by increased levels of pituitary and serum prolactin (Herbert et al. 1977). The work of Ingenbleek and De Visscher (1979) on endemic goiter has indicated that thyroid hormone can be involved in the control of secretory activities and protein glycosylation in the liver. Quite interesting was study of Ip (1980) who reported that changes in sialyltransferase activity are not only related to proliferation and involution of the rat mammary gland but it was possible to upregulate this enzyme by administration of estradiol and progesterone. In addition, testosterone was found to be crucial for induction and maintenance of large areas of Golgi cisternae and production of secretory granules in mouse epididymal epithelial cells (Yamaoka et al. 1983). Very similar general effect of testosterone on enlargement of rough endoplasmic reticulum and Golgi areas has been described for rat male seminal vesicles which secrete several tissue-specific glycoproteins. The mRNA for two of these glycoproteins that have been analyzed, was 1000-fold induced during androgen treatment, suggesting relatively complex action of the steroid hormone on secretory process (Fawell and Higgins 1984). Production and secretion of rabbit uteroglobin, the primary secretory protein of uterine epithelium, appeared to be dependent on 17β-estradiol and progesterone (Shroyer et al. 1987). Progesterone appears to regulate uteroglobin synthesis at the transcriptional level and to regulate also mode of its secretion by the induction of a different pathway, compared with the one used when 17β-estradiol is administered alone, showing that two different steroid hormones acting on the same target tissue utilize particular mechanisms to provide full and complex control over its secretory process. On the contrary, Tseng et al. (1987) described synergistic action of corticosterone and thyroxine on formation of secretory granules and mucus production in rat gastric chief cells, in which thyroxine alone failed to induce any positive response and corticosterone alone was insufficient to provide full induction. Several other authors came to similar conclusion regarding overall control of secretion and secretory material production by hormones and feed-back mechanism under hormonal control including requirements of estradiol and corticosteroids for normal secretory function of exocrine pancreas (Davis et al. 1984; Tsukada et al. 1985; Beaudoin et al. 1986; Ferreira and Celis 1986; Moor and Crosby 1987). Explanted guinea pig endometrial glandular cells grown in primary culture if treated with 17β-estradiol plus progesterone, or with oestrone sulphate alone become stimulated for development of large endoplasmic reticulum and Golgi system, displaying secretory activity (Alkhalaf et al. 1989). Likely, the maturation of the secretory apparatus, including formation of granules of varying electron density, in the uterine glandular epithelium of the ovariectomized sheep continued only under estrogen treatment (Murray 1992). Similar situation has been observed in estrogen-primed and progesterone treated sheeps indicating cooperative action of both steroid hormones on the same process.

Nevertheless, study of Capony et al. (1987) was one of the first which indicated for very complex role of a steroid hormone in the control of secretory process in mammalian cells. An estrogen-induced 52 kD autocrine mitogen of MCF7 human breast cancer cell line requires proteolysis, glycosylation and phosphorylation for its maturation, secretion and function. These authors provided evidence that at least synthesis, high mannose *N*-glycosylation and proteolytic cleavage of the 52 kD mitogenic protein, which very probably is cathepsin-like lysosomal protease, depend on the estrogen action. They also concluded that mature form of this 52 kD protease facilitates proliferation, migration and metastatic activity of mammary cancer cells, which therefore may be controlled by manipulating estrogen levels. Natural and synthetic glucocorticoids are known to affect collagen metabolism at the level of its production and also posttranslational modifications including hydroxylation of proline and lysine residues followed by glycosylation of hydroxylysine residues on procollagen. Particularly, hydroxylation of lysine and glycosylation on hydroxylysine are depressed by glucocorticoids which is caused via downregulation of the activity of specific enzymes involved in these posttranslational modifications (Kucharz 1988). These effects of glucocorticoids has important pathophysiological aspect due to the role of collagen metabolism in hepatic and pulmonary fibrosis as well as in keloids.

Hormonal regulation of the secretion or of the activity of secretory tissue may take place at another and rather

unusual level. Widespread secretory components, chromogranin B and secretogranin II, which are normally present in almost every type of mammalian secretory vesicle are considered to be also responsible for aggregation of regular secretory proteins in trans-Golgi network (TGN). Several hormones, including corticosteroids, increase formation of pituitary secretory granules via upregulation of granins synthesis rather than affecting synthesis of own secretory proteins (CHANAT and HUTTNER 1991). The granins preferentially exclude glycosaminoglycan chains containing polypeptides from TGN towards constitutive secretory bulk and concentrate regular secretory proteins into vesicles of regulated pathway; hormonally upregulated pool of granins then increases the rate of aggregation of regular secretory proteins and formation of vesicles. Mineralocorticoids are thought to decrease intracellular pH and mobilize TGN pools of calcium, two other factors facilitating aggregation of granins and formation of secretory vesicles.

Good and rather interesting example of steroid hormone effects on glycoprotein formation and secretion is in glucocorticoid regulation of the transport and processing of mouse mammary tumor virus (MMTV) glycoproteins in viral-infected HTC rat hepatoma cells. GOODMAN and FIRESTONE (1993) have documented that administration of synthetic glucocorticoid, dexamethasone, resulted in a 5-fold increase in the steady state level of the intracellular and cell surface MMTV glycoproteins. Regardless of the fact that terminal ends of MMTV DNA contain well defined glucocorticoid responsive elements, under these conditions, dexamethasone did not alter the level of MMTV glycoprotein transcripts or translatability of the messages as incorporation of ^{35}S-labelled methionine has not been increased. Data these authors provided strongly suggest that glucocorticoids are able increase the stability of MMTV glycoproteins by a posttranslational mechanism and that this effect is occurring relatively early within the exocytotic pathway.

Great chapter of the hormone involvement in glycoprotein production deals with regulation of mRNA synthesis at transcriptional level. For example, Muc-1, a major mucin glycoprotein expressed on the surface of mammary epithelial cells, has attracted considerable attention as it is expressed in an aberrant form on many breast tumor cells. Under continuous supply of insulin mouse Muc-1 mRNA levels are increased by both hydrocortisone and prolactin or their combination leading thus to increased rate of Muc-1 protein maturation and its secretion (PARRY et al. 1992). Additionally, Muc-1 displays developmentally-linked variations in its glycosylation as it become increasingly sialylated during the course of pregnancy and lactation indicating role of estrogens in this specific step just preceding secretion. Subsequently, level of Muc-1 glycosylation affect its intracellular localization being tightly polarized to the apical surface of the epithelium of lactating and pregnant mice whereas showing non-polarized distribution in ductal cells of virgin mice. Similar transcriptional mechanism for the control of secretory protein production has been observed also in amphibians. In *Xenopus laevis* liver, two out of four major estrogen-regulated (inducible) proteins are secreted vitellogenin and serpin Ep45 (HOLLAND et al. 1992). The mRNA levels of both of them are almost undetectable in absence of estrogen, and become more than 100-fold increased upon its administration, and encoded proteins secreted shortly thereafter. An opposite effect the steroids can be seen on corticosteroid-binding globulin (CBG), an acidic glycoprotein produced primarily by the liver which is the major glucocorticoid transport protein in mammalian blood. While estrone, prednisolone or corticosterone produced no significant response of this protein and its mRNA, both protein and RNA were significantly reduced (14-fold) after administration of dexamethasone or estradiol (SMITH and HAMMOND 1992). More complex level of the protein glycosylation has been found in the regulation of 2,6-sialyltransferase mRNA by thyroid hormone in the rat thyroid cell line FRTL-5. GROLLMAN et al. (1993) have demonstrated that both the cell surface membrane and the thyroglobulin secreted by cells grown in the presence of the hormone exhibit a marked decrease in the level of α-2,6-bound sialic acid with little or no change in the number of α-2,3-sialic acid residues. Steady-state levels of the thyroid hormone tend to decrease 2,6-sialyltransferase mRNA. An additional, and unexpected, sequel is the finding of a coordinated decrease in all of the core monosaccharide constituents of the secreted thyroglobulin. This shows invariable and tight feed-back mechanism for the control of thyroglobulin secretion under particular hormonal milieu. Merely different but to mammals may be a common way of thyroglobulin synthesis and secretion operates in porcine thyroid cells. Steroid hormone hydrocortisone alone did not modify total RNA or thyroglobulin mRNA content but the hormone amplified total RNA and thyroglobulin mRNA when insulin and pituitary thyroid stimulating hormone (TSH) were present together. The basal level of thyroglobulin secreted into the apical medium was increased threefold by insulin and fourfold by TSH. However, when the three hormones were added together, the hor-

monal response was amplified even more. These hormones were able also to increase both incorporation of ^{3}H-mannose into thyroglobulin and the content of the anionic residues in the thyroglobulin molecule. Thus, the effects of the hydrocortisone and two hormones supplemented together appeared to be additive (Desruisseau et al. 1994). In accordance with above mentioned data of Grollman et al. (1993) in the rat, Helton and Magner (1994a, 1995) have found that α-2,6-sialyltransferase mRNA increases in thyrotrophs of hypothyroid mice, as a consequence of thyroid hormone withdrawal indicating that this glycosyltransferase is under negative thyroid control at transcriptional level, and may partially explain the increased sialylation of TSH during hypothyroidism. Thyroid hormone, in cooperation with TSH, pro-opiomelanocortin (POMC) and lutropin (LH), another pituitary hormones, can control glycosylation process also in nonendocrine target tissue. Their action on single product, carbonic anhydrase VI, in parotid and submaxillary glands results in distinct glycoforms of the enzyme (Hooper et al. 1995). These authors have shown that while the glycoprotein:GalNAc-transferase and the GalNAc-4-sulfotransferase are coordinately expressed in bovine submaxillary gland, the GalNAc-transferase is expressed in the parotid gland in the absence of the GalNAc-4-sulfotransferase. The relative expression of these two transferases in submaxillary and parotid glands correlates with the presence of unique Asn-linked oligosaccharides on carbonic anhydrase VI synthesized in each of these tissues, and reflects tissue-specific response to the hormones. The majority of Asn-linked oligosaccharides on carbonic anhydrase VI synthesized in submaxillary gland terminate with GalNAc-4-SO_4. In contrast, carbonic anhydrase VI which is synthesized in bovine parotid gland bears oligosaccharides which terminate predominantly with β-1,4-linked GalNAc which is not sulfated. These results document that hormones can mediate tissue-specific differential expression of glycosyltransferases which has the potential to generate functionally distinct glycoforms of otherwise identical proteins.

One of the most abundant protein products of human secretory endometrium is glycodelin, which is believed to affect immunomodulatory activity during human embryonic implantation and inhibition of sperm-egg binding after ovulation. Glycodelin protein production *in vivo* by endometrial epithelial cells is directly up-regulated 4- to 9-fold by progesterone. Transcriptional regulation of the glycodelin gene promoter expressed in HeLa cells is progesterone receptor-dependent. As it was observed in the primary endometrial cells, progestins and antiprogestins both act as agonists on the *in vitro* expression of the glycodelin gene at the mRNA level (Taylor et al. 1998).

In spite of the wealth of data which provide evidence that steroid hormones and sibling lipophilic hormonal ligands control production of glycoproteins and in several cases also their secretion, among vertebrate models there is a general lack of information on exact molecular mechanism by which these effects are implemented. Nevertheless, it should be stressed that very important notion about complex control of glycoprotein production and secretion by steroid hormone comes from an invertebrate model, *Drosophila melanogaster*, and its initial insights are dated as early as mid 1970s. Larval salivary glands of *Drosophila melanogaster* are unique in their production of small group of secretory glycoproteins, referred to as SGS (**s**alivary **g**lue **s**ecretion), which are synthesized only once a life time during short period between mid and late 3rd instar. After the finding that insect steroid hormone ecdysone induces in salivary gland nuclei a set of specific chromosomal puffs representing transcriptionally active genetic loci (Clever and Karlson 1960; Ashburner 1971, 1972a,b), it was soon found that specific portion of puffing active during interecdysial period, *i.e.* when endogenous levels of ecdysone are constantly low, is tightly related to the synthesis of SGS proteins which are subsequently utilized in the production of electron-dense granules (for review see Berendes and Ashburner 1978). At the time of metamorphosis upon the burst of high ecdysone levels the granules are secreted into the lumen and the secretion is expectorated from the gland to be used as a glue to affix the newly formed puparium to the substrate (Fraenkel and Brooks 1953; Boyd and Ashburner 1977). The huge amount of SGS made an isolation of discharged secretion from the salivary gland lumen easy and facilitated their initial electrophoretic characterization which revealed presence of 6 to 7 proteins (Korge 1975; Beckendorf and Kafatos 1976). The variable size of these proteins as well as incorporation of radioactive glucose (Kress 1979; Enghofer and Kress 1980) indicated that some of them, if not all, are glycosylated. Activity of a small group of chromosomal puffs at the time of SGS synthesis gave an opportunity to link some SGS proteins to corresponding genetic loci (Korge 1977a,b; Berendes and Ashburner 1978; Velissariou and Ashburner 1980, 1981). These studies were shortly followed by cloning of 68C chromosomal cluster encoding *Sgs-3*, *Sgs-7* and *Sgs-8* genes (Meyerowitz and Hogness 1982; Meyerowitz et

al. 1985, 1987; Crowley et al. 1983, 1984; Crowley and Meyerowitz 1984; Crosby and Meyerowitz 1986), of 3C locus responsible for transcription of *Sgs-4* (Muskavitch and Hogness 1980, 1982; Chen et al. 1987), cloning of 95B locus harboring *Sgs-5* gene (Guild 1984; Guild and Shore 1984), and cloning of *Sgs-1* from 25B puff (Roth et al. 1999).

Furthermore, regulation of expression of *Sgs-3* and *Sgs-4* has been extensively studied as synthesis SGS proteins was considered to be a "textbook" example of tissue-specific gene expression. These studies revealed valuable information on redundant tissue-specific enhancers upstream of 5' transcription start site of both *Sgs-3* (Giangrande et al. 1987, 1989) and *Sgs-4* (Krumm et al. 1985; Hofmann et al. 1987; Jongens et al. 1988). Besides specific binding sites, these two promoters are commonly bound by several transcription factors including ecdysone receptor (*EcR/USP* complex), broad-complex factors (*BR-C*) and fork head (*fkh*) (Lehmann 1996; Lehmann et al. 1997). In addition, the *Sgs-4* promoter was found to bind also secretion enhancer binding proteins SEBP2 and SEBP3 factors the binding sites of which are in the vicinity or overlap with those for EcR/USP and BR-C within so-called ecdysone response unit (Lehmann and Korge 1995, 1996). Taken together, levels of the regulation of *Sgs* system in *Drosophila* reveal tremendous complex of functions related to steroid signalling which encompass positive as well as negative control of *Sgs* transcription, possibly control of SGS protein glycosylation and granule formation, and definite control of exocytosis in which ecdysone plays the role of ultimate trigger.

Small lipophilic hormones can control both *N*- and *O*-glycosylation

An important insight in specific hormonal control of glycosylation has been brought about by paper of Philipp and Shapiro (1981) who demonstrated that estrogen regulates hepatic 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGcR) in *Xenopus* frogs. In addition to its main function being a major rate-limiting enzyme in the cholesterol biosynthesis, the HMGcR is also a key regulator of all other isoprenoids synthesis including dolichol phosphate, a hydrophobic lipid carrier, required for *N*-glycosylation of proteins by attaching core oligosaccharide to asparagine residues on target polypeptide skeleton. Besides general effects of estrogen on enlargement of endoplasmic reticulum and the Golgi apparatus, it controls also synthesis of the egg yolk precursor, vitellogenin, which is highly *N*-glycosylated. Another and very interesting level of the control of protein *N*-glycosylation by steroid hormone testosterone has been presented by Iusem et al. (1984) studying secretory glycoproteins of rat epididymis which are known to interact with spermatozoa in order to facilitate their maturation. In this case dolichol phosphate pathway was not affected, excluding HMGcR from the androgen action, but rather glucosyl and mannosyl transferase activities in rat epididymal microsomes were drastically decreased upon castration, and restored by testosterone readministration. The restoration of glucosyl and mannosyl transferase activities was blocked by simultaneous application of cyproterone, indicating involvement of androgen receptor. An analogical case was soon observed in rabbit mammary gland where cortisol along with insulin and prolactin control mannosyl transferase activity associated with transferrin secretion (Bradshaw et al. 1985). Thyroid hormones were shown to be involved in the control of *N*-glycosylation of thyroid gland proteins by regulating utilization of UDP-glucosyl, -galactosyl and -mannosyl precursors for synthesis of dolichol-P-P-sugar moieties (Spiro and Spiro 1985) which may represent tissue-specific mechanism depending also on feed back action of the hormone. Human chorionic gonadotropin of normal pregnant women contains four Asn-linked sugar chains and four mucin-type sugar chains. The structures of asparagine-linked sugar chains of this hormone are constant and site-specific. Chorionic gonadotropins obtained from the patients with invasive mole or choriocarcinoma, which show significantly lowered progesterone and estrogen levels, have quite different sets of oligosaccharides although the primary structures of the polypeptides and the numbers of the sugar chains are the same as those of normal gonadotropin. The extent of sialylation of oligosaccharides in the this gonadotropin reflects its hormonal activity where complete desialylation induced remarkable loss of full activity (Amano et al. 1990). Results of these authors indicated not only that the structures of the neutral oligosaccharide portion are important for full hormonal activity of gonadotropins, but also that level of this *N*-linked glycosylation can be under progesterone and/or estrogen control. As discussed in more detail below, one aspect of hormonal modulation of Asn-linked glycoprotein biosynthesis is to regulate the synthesis of glucosidase I, endoplasmic reticulum enzyme involved in glucose trimming from *N*-linked glucomoieties, by coordinate action of hydrocortisone, insulin and prolactin on rat mammary gland, where they all have lactogenic effect (Shailubhai et al. 1990a,b). In

addition, thyroid hormone has been shown to control degree of sialylation of Asn-linked carbohydrate chains in the circulating thyrotropin (PERSANI et al. 1998). These modifications have a major impact on TSH biological properties. In particular, highly sialylated TSH is characterized by impaired intrinsic bioactivity and prolonged half-life, whereas lower degree of TSH sialylation is associated with higher biological activity and shorter half-life. The impaired thyroid hormone action induces an expression of α-2,6-sialyltransferase activity and results in the secretion of high amounts of sialylated TSH, while normally acting thyroid hormone downregulates α-2,6-sialyltransferase expression.

In addition to steroid and thyroid hormones, also vitamin A derivatives, retinoids, can influence *N*-glycosylation of proteins. *All-trans* retinoic acid (atRA) applied to mouse P19 teratocarcinoma cells caused a dose-dependent and protein synthesis-dependent induction of UDP-*N*-acetylglucosamine:dolichyl-phosphate *N*-acetylglucosamine-1-phosphate transferase (GPT) enzyme activity, and an increased transcription rate of the *GPT* gene. GPT is the first enzyme in the dolichol pathway of protein *N*-glycosylation, and is implicated in the developmental programs of a variety of eukaryotes (MEISSNER et al. 1999). The atRA was found to be capable affecting protein *N*-glycosylation also by altering ratio of the utilization between mannose and glucosamine in Vero cells and thus causing extremely potent antiviral effect against herpes simplex virus infection (ISAACS et al. 2000). Decreasing levels of viral envelope protein *N*-glycosylation was sufficient to inhibit virus propagation 10,000-fold.

On the other hand, BOLANDER and TOPPER (1981) presented evidence about complex hormonal control of mouse mammary lactalbumin, lactose synthetase and galactosyltransferase by insulin, prolactin, cortisol and triiodothyronine. Both, cortisol and triiodothyronine are required for maintaining production and secretion of maturated *O*-glycosylated lactalbumin. Role of steroid hormones in the control of specific protein *O*-glycosylation was further substantiated by DEIS and DELOUIS (1983) who observed significant downregulation of rat lactalbumin galactosyl transferase upon progesterone administration, while no apparent effect was obtained by estrogen. The ontogenic profile of sialyltransferase activity in mouse brains and its positive response to triiodothyronine in myelogenic embryonic brain cells indicated that it is at least under partial thyroid hormone control which may be shared with some other humoral factors required for brain differentiation (SHANKER and PIERINGER 1983). Mouse uterus endometrium responds to high estrogen levels or during pregnancy by increased activity of α-(1-2)-fucosyltransferase (FUT1) and upregulation of FUT1 mRNA upregulation, and this is accompanied by concomitant upregulation of polypeptide-Galβ-(1-3)-*N*-acetyl-galactosyltransferase (SIDHU and KIMBER 1999). Although, targets of these *O*-glycosylation enzymes remain unknown so far, there is a good reason to believe that those may be proteins co-regulated by estrogen at the same time. Control of *O*-glycosylation machinery by estrogens may have also another, feed-back regulatory function. Significant population of mouse estrogen receptors-α (ERα) is modified by *O*-GlcNAc moiety at C-terminal Thr575 and Ser576 as well as N-terminal Ser10 and Thr50 residues, modulating thus ERα turnover (JIANG and HART 1997; CHENG and HART 2000). In amphibian testis, androgens seem to support presentation of GalNAc and Neu5Ac(a2,6)Gal/GalNAc *O*-glycosylated moieties on the interstitial tissue, and suppress synthesis of *N*-glycosylation machinery, while do not affect significantly ratio of *N*- and *O*-linked oligosaccharides in the surrounding lobules with spermatozoa bundles (SAEZ et al. 2001).

Control of specific glycosyltransferases

Protein glycosylation is a multistep modification process which, in general, requires several sequential reactions, some of which consist of adding and some of trimming glycomoieties. Addition of mono- or oligosaccharide residues to amino acids is performed by glycosyltransferases, whereas trimming off glycomoieties is performed by glycosidases. One of the earliest reports on hormonal control of glycosyltransferases comes from the work of LEDINKO and FAZELY (1989) who found that retinoic acid and retinol acetate downregulate sialyltransferase activity in human lung carcinoma cells, A549, suppressing their invasiveness by reducing cell surface contents of sialylated glycoproteins. BIOL et al. (1992) reported interesting case of dual control of fucosyltransferase activity in suckling rats. While the enzyme did not respond to thyroxine or insulin, its expression was strongly induced by glucocorticoids as a function of the duration of treatment. This responsiveness of suckling rats to hydrocortisone or cortisone disappeared at the time of weaning. The effects of glucocorticoids were prevented by RU-38486 antagonist, showing that hormonal responsiveness is mediated by glucocorticoid receptor. However, RU-38486 did not prevent the developmental rise of the fucosyltransferase activity when administered in the

time of weaning, suggesting that the normal developmental rise of the fucosyltransferase activity is independent of glucocorticoids and must be under control of other factors which are capable of preventing glucocorticoid inducibility. As already mentioned under general effects above, GROLLMAN et al. (1993) have demonstrated that steady-state levels of the thyroid hormone tend to decrease β-galactoside-α-2,6-sialyltransferase mRNA in rat thyroid cell line FRTL-5 secreting thyroglobulin, and thus affecting degree of its glycosylation. Furthermore, the work of THOTAKURA et al. (1994) indicates that regulated levels of 2,6-sialyltransferase mRNA and protein have strong impact on thyrotropin (TSH) sialylation and subsequently on TSH biological activity. It seems that continuous 2,6-sialyltransferase activity is required for maintaining majority of TSH molecules to be sialylated and thus prevented from sequential deglycosylation, mainly removal of galactose or *N*-acetylglucosamine, the process which transform stored form of TSH into biologically highly active hormone with short half-life. Under reinitiation of 2,6-sialyltransferase expression, the deglycosylated unreleased TSH can be resialylated. From the point of view of glycosylation process it is necessary to note that sialic acid most probably, even in the case of TSH, represents terminal glycosidic residues and thus sialylation is the last glycosylation step. Sialic acid is added upon galactosyl or glucosyl residues which must be attached first due to activity of galactosyltransferase or glucosyltransferase. HELTON and MAGNER (1994b) used experimentally-induced hypothyroid mice as a model to study TSH glycosylation further, and thus found that low or undetectable levels of thyroid hormone lead to rapid 5-fold increase in pituitary β-1,4-galactosyltransferase mRNA expression and higher rate of galactosyl residues in secreted TSH molecules. Interestingly, HELTON and MAGNER (1994a, 1995) also found that this physiological conditions subsequently resulted in more highly sialylated form of TSH than in their euthyroid counterparts, also due to 1.5-fold increased β-galactoside-α-2,6-sialyltransferase and almost 3-fold increased β-galactoside-α-2,3-sialyltransferase mRNA expression. Thus, final oligosaccharide modification of TSH is achieved by coordinate, albeit not the same upregulation in expression of three key glycosylating enzymes. Both discussed enzymes, β-galactoside-α-2,3-sialyltransferase and β-galactoside-α-2,6-sialyltransferase, were found to be upregulated not by thyroid hormones but by corticosteroid in several other target tissues like rat jejunum (KOLINSKA et al. 1996) and brain, liver and kidney (COUGHLAN et al. 1996). Each enzyme responded to dexamethasone differentially and also various portions of the brain including cortex, cerebellum, and brainstem have shown variable response. Unfortunately, we lack data on target proteins of these two sialyltransferases, and therefore, we cannot clearly relate their upregulation to specific function. Such an objection is reflected in the fact that activity of neither 2,3-sialyltransferase nor 2,6-sialyltransferase must necessarily be associated with concomitant presentation of sialyl residues in intracellular or extracellular proteins as is the case of rat and human hepatocytes, neurons and gastrointestinal epithelia in which despite relatively high enzyme activities authors failed to detect 2,3- or 2,6-linked sialoglycoconjugates (KANEKO et al. 1995). In contrast to so far described stimulating effects of steroids on sialyltransferase activity and expression, HAMR et al. (1997) have studied developmentally-linked loss of sialic acid presentation on the brush-border membrane glycoproteins of the rat small intestine during the transition from suckling to weaning. This loss of sialic residues is one of the major biochemical changes which occur during mentioned postembryonic phase of rat development, and this process is speeded up by an injection of exogenous glucocorticoids. As authors found, loss of detectable sialic acid residues on the brush-border membrane is due to hydrocortisone-driven downregulation of β-galactoside-α-2,6-sialyltransferase activity and mRNA expression, and mediated by glucocorticoid receptor because hydrocortisone effects are preventable by mifepristone, a glucocorticoid receptor antagonist. Further studies of BIOL-N'GARAGBA et al. (2003) on this model revealed that hydrocortisone also acts on increasing activity of *O*-glycan:galacto-sialyltransferase and of an α-1,2-fucosyltransferase, through transcriptional regulation of the *FTB* gene, thus substantiating developmental shift from sialylation towards fucosylation. Moreover, ANIC and MESARIC (1998) obtained evidence that sex steroid hormones can regulate activity of both sialyltransferases in the rat kidney. Castration of male rats resulted in significant increase of kidney 2,3-sialyltransferase and 2,6-sialyltransferase activities, but this effect was reversed by subsequent administration of testosterone. While testosterone was able to downregulate both enzymes, estradiol and progesterone decreased only activity of 2,6-sialyltransferase but not 2,3-sialyltransferase. In the hormono-dependent MCF-7 human breast cancer cell line estradiol induces a statistically significant increase in expression of CMP-Neu5Ac:Galβ1-3(4)GlcNAc-α-2,3-sialyltransferase and a decrease in CMP-Neu5Ac:Galβ1-4GlcNAc-α-2,6-sialyltransferase,

whereas the three other enzymes, CMP-Neu5Ac:Galβ1-4GlcNAc-α-2,3-sialyltransferase, CMP-Neu5Ac:Galβ1-3GalNAc-α-2,3-sialyltransferase, and CMP-Neu5Ac:Galβ1-3GalNAc-α-2,3-sialyltransferase are not modified in their expression (PEYRAT et al. 2000). Estradiol effects were dose dependent and completely antagonized by tamoxifen, and did not show any direct relation between sialyltransferase expression and proliferation of breast cancer cells, although in some cancer types was established apparent connection between proliferation-related invasiveness and deregulated sialyltransferase activity. Potentially more widespread but so far only little studied system of complex control of protein glycosylation can be found in estrogen-induced phasic remodelling of synapses in the adult rat female arcuate nucleus. HOYK et al. (2001) by studying synaptic plasticity came to the conclusion that estradiol reduces GABAergic axo-somatic synapses by decreasing polysialylation of neural adhesion molecule (NCAM) via reduced sialyltransferase activity/expression as well as NCAM expression. In addition, active desialylation of existing NCAM molecules could play an important role in this synaptic plasticity process.

Hormonal control of glycosidases

Regular part of glycosylation modification is also programmed removal of mono- or oligosaccharide moieties in the process of building the specific glycomoiety on the protein. Trimming step is performed by variety of glycosidases the action of which is required many times before some glycosyltransferases can add new sugars, and thus it is anticipated that synthesis of complex glycomoieties on hormonally-controlled proteins may involve also hormonal regulation of glycosidase enzymes. For example, glucosidase I, a key trimming enzyme in *N*-glycosylation, was found to be under positive control of hydrocortisone and two peptides, insulin and prolactin, in the mammary gland where they all act as lactogenic hormones (SHAILUBHAI et al. 1990a,b), although it has not been established whether this control takes place at transcriptional, translational or other level, or whether upregulation of enzyme activity is achieved by another mechanism. On the other side, there is good reason to expect that hormonal control of glucosidase I reflects coordinate action of mentioned three hormones as they also stimulate synthesis of α-lactalbumin, a major mammary protein product displaying high degree of glycosylation. Nevertheless, in one of the earliest papers on TSH glycosylation RONIN et al. (1984) suggested that thyroid hormone regulation of this process can involve not only galactosyltransfering activity, but also mannosidase activity, and ratio between these activities determine degree of TSH glycosylation and hormone half-life. In above chapter mentioned work of THOTAKURA et al. (1994) on hormonal control of 2,6-sialyltransferase activity related to TSH glycosylation shows that simultaneous glycosidase activity is necessary to ensure proper sialylation of galactose residues. Similar case, when co-deglycosylation is required for programmed glycosyl transfer, was described by HELTON and MAGNER (1994b) for thyroid hormone regulated production of TSH in hypothyroid mice. Along with β-1,4-galactosyltransferase mRNA expression, drop in thyroid hormone levels upregulates also increase in pituitary α-mannosidase-II mRNA, though not to the same extend and with different kinetics which might reflect temporal-dependent needs for degree or type of TSH glycosylation. Cell-specific and developmentally-dependent distribution of Golgi mannosidases I and II in various rat tissues strongly indicates that glycosidases might be under steroid hormone control, including glucocorticoids and sex steroids (DONG et al. 2000). And indeed, testosterone was recently found to inhibit activity of β-glucuronidase and β-*N*-acetylglucosaminidase in catfish seminal vesicles in concentration-dependent manner (CHOWDHURY and JOY 2001). Very special case of the hormonal control of monoglycosylation process has been analyzed by SLAWSON et al. (2002) by studying the activity of *N*-acetylglucosaminidase (GlcNAcase), the streptozotocin-inhibitable neutral hexosaminidase, involved in removing GlcNAc monoglycosyls from proteins during *Xenopus* oocyte maturation. Progesterone is capable of stimulating oocyte maturation by gradual downregulation of GlcNAcase activity in early stages of oocytes which are characterised by higher levels of GlcNAc monoglycosyls than older and mature stages.

Conclusions

Altogether above discussed results clearly show that steroid hormones and some other members of small lipophilic ligand family are involved in the control of protein glycosylation which in most cases is related to the process of secretion. Based on the number of reports in the literature it seems that there was no specific selection pressure during evolution to favor or eliminate hormonal control of *N*-glycosylation *versus* *O*-glycosylation, and both types of modifications are more-less equally occurring among hormonally-controlled modifications. However, there is very little evidence that there

was any co-evolution in the systems utilizing both types of glycosylation at the same time and during the same response. This is somewhat surprising if one consider the fact that numerous proteins which are glycosylated and the process of particular glycomodification is under hormonal control, contains multiple and different glycosylation sites. This view is acceptable if hormonally-controlled step in glycosylation machinery may function as rate-limiting or check-point, and therefore its regulation is sufficient to maintain control over the whole process. However, this might be true only for very simple systems in which ligand produces the same response every time and single glycosylated product is used *e.g*. for secretion. Such a simple system if exemplified by a number of tissues or cell types in which hormone controls either sialylation or fucosylation, the last glycosylation step, via upregulation or downregulation of sialyltransferase or fucosyltransferase activity/expression. On the other hand, in more complex systems in which hormone fluctuations can provoke series of different responses reflected in vast panoply of glycosylations of very different proteins with diverse functions, we can hypothesize or even expect that one ligand will regulate given process in various steps and at multiple levels. In spite of this logical assumption the most of the information we have today, in fact, will appear fragmentary. For example, there is striking contrast between the high number of systems in which steroid hormones control glycosyltransferase activity, and low number of systems where steroid hormones control glycosidases, although both types of enzymes are known to be equally required for glycosylation. Nevertheless looking at this problem from opposite point of view, these fragmentary data originating from various systems when combined can suggest that effects of small lipophilic hormones on protein glycosylation are rather complicated. Addressing this problem by means of more complex methodological approach and in well-defined model systems potentially with known genomes, can complement present and extend our knowledge in future concerning coordinate action of hormones on processes associated with or tightly linked to protein glycosylation. It is our firm belief that by adhering to such a complex methodology which in addition can utilize set of mutations or transgenes, we can obtain substantial information on the mechanism how hormonal control of glycosyltransferases and glycosidases is interconnected to the synthesis of substrates, posttranslational maturation, and final destination/function of target proteins which may not necessarily be granule formation and secretion.

Acknowledgements

This work was supported, in part, by the Research Grant 2/999533 from GAV and by Grants No. 2/7194/20 and 2/3025/23 from VEGA Slovakia, and US-Slovak collaborative grant 029/2001 from APVT to R.F. International collaborative grants from the NATO (CRG-972173 and LST.CLG-977559) are also acknowledged.

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