Encyclopedia of Stress second edition Vol. 2, Academic Press, Oxford, pp. 222 - 227

Glycobiology of stress

Gordan Lauc and Mirna Flögel
Faculty of Pharmacy and Biochemistry
University of Zagreb, Ante Kovačića 1, 10000 Zagreb, Croatia
tel +385 1 4818757; fax +385 1 4856201; email glauc@pharma.hr

Summary

Psychological stress is associated with numerous diseases, but molecular mechanisms linking stress to the development of disease are only starting to be understood. Stress alert is conveyed by hormonal signals throughout the body, yet a particular cell response to a hormonal signal is not determined by the signal itself, but by the molecular composition, energy content, and by the physiological role and current status of the target cell. Stress induced changes in glycoconjugate structures and expression of their receptors lectins appear to be an important molecular consequences of stress experience. Though at the moment only little is known about the metabolic steps leading to these changes, there is evidence that activities of glycosyltransferases are also affected by stress. Since there is no doubt that many vital physiological processes and disorders strongly depend on glycoconjugate-receptor interactions, it seems only justified to assume that glycosylation changes contribute to the development of diseases that are associated with stress.

Primary headings:

I. Glycoconjugates are important mediator of many physiological functions
II. Glycoproteins in stress
III. Lectins in stress
IV. Stress affects the activity of glycosyltransferases
V. Glycobiology of stress

Glossary

Glycoconjugate -	A compound composed of an oligosaccharide linked to a protein or a lipid.
Glycolipid -	A compound containing both lipid and oligosaccharide moieties.
Glycoprotein -	A protein molecule containing one or more oligosaccharide attachments.
Glycosylation -	Post-translational modification of a protein by the addition of a carbohydrate moiety. Glycosylation is catalyzed by specific enzymes called glycosyltransferases.
Glycosyltransferase -	A common term for a group of enzymes that transfer monosaccharides to a growing oligosaccharide chain on glycoconjugates.
Lectin -	A physiological receptor for carbohydrate structures attached to glycoconjugates.
Sialyltransferase -	An enzyme that transfers sialic acid to a glycoconjugate.

Metabolic response to psychological stress is a very complex and demanding physiological process that involves numerous organs and organ systems. Though it is highly important for survival in ever changing environment, its excessive activation is associated with various detrimental effects. A number of epidemiological and experimental studies conducted during the past years have clearly demonstrated a link between stress and the development and course of many diseases; from simple virus infections and gastric ulcers, to cardiovascular diseases and cancer. It is estimated that up to two-thirds of all visits to the physician's office are associated with stress.

Molecular mechanisms underlying the link between the response to stress and the development of disease appear to be exceedingly complex and are only partly understood. Though hormonal changes are key mediators of the physiological changes in stress, other factors appear to be decisive in the development of stress-associated disorders. Molecular response of a target cell appears to be defined, not only by the incoming hormone, but also by the current molecular situation within the cell itself. Good illustration of this principle is the way corticosteroids affect neurons in the hippocampus. If neurons are at rest, corticosteroids apparently have no effects. These effects become visible only when neurons are shifted from their basal condition by the action of neurotransmitters.

After more than fifty years of research, a lot is known about the endocrinology of the stress response, but the key molecular mechanism that could explain how and why corticosteroids and other stress hormones cease to be beneficial, and start to cause damage are still not known. One important link between stress and disease is the stress-induced decrease in the immune response (see several articles on immune system in this encyclopedia), but the decrease in the immune response cannot explain all effects of stress, and other mechanisms have to be involved. Glycosylation appears to be involved, and the modification of glycoconjugate structures and their interaction with lectin receptors modulate at least some of these processes.

I. Glycoconjugates are important mediator of many physiological functions

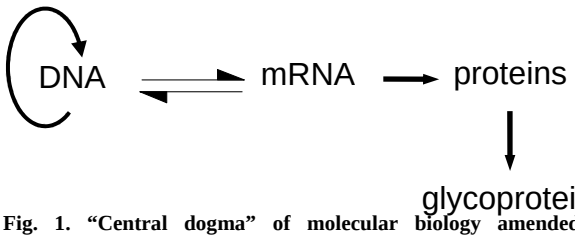

Fig. 1. "Central dogma" of molecular biology amended with biosynthesis of glycoproteins. Contrary to proteins, which all have their templates in the DNA, carbohydrate structures attached to glycoproteins are not defined by genes. Their exact structures are determined by the regulation of expression, intracellular localization and activity of various glycosyltransferases.

Glycosylation is a very complex posttranslational modification that plays an important role in the integration of higher organisms. Surface of all cells is covered by oligosaccharide structures covalently attached to proteins and lipids (glycoproteins and glycolipids). Escalation in extent and diversity of protein glycosylation correlates with the appearance of multicellular life, and apparently most of interaction between cells and their surroundings include carbohydrate-protein recognition. An essential difference between proteins and oligosaccharide structures attached to them is the fact that the carbohydrate part is not encoded in the DNA (Fig. 1). While genes unequivocally define the structure of each protein, there is no template for attached oligosaccharides. This makes them inherently sensitive to all changes within the cell, and glycan structures that are being produced at any individual moment actually mirror all relevant past events in the cell. In addition, the evolution of carbohydrate structures is much faster than the evolution of proteins, and often in different species we see significant differences in carbohydrate structures attached to nearly identical protein or lipid backbones.

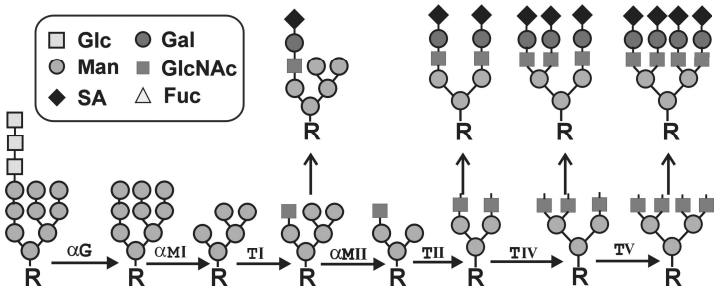

Fig. 2. Schematic representation of processing of N-linked glycans. N-linked glycans are being synthesized by sequential action of numerous glycosyltransferases and glycosidases that add or remove specific monosaccharides to a growing oligosaccharide chain.

Glycan parts of glycoconjugates are being synthesized by glycosyltransferases that act in a sequence and each enzyme transfers specific monosaccharide to a specific acceptor contributing to the final glycan structure (Fig 2). It is estimated that over 300 specific enzymes (glycosidases and glycosyltransferases) are involved in the synthesis of carbohydrate structures on glycoconjugates, and the whole process is very complex and energetically expensive for the cell. Defects that disturb early phases of glycoconjugate synthesis are incompatible with multicellular life, while mutations in the end-modifications of oligosaccharides result in specific physiological alterations. Since oligosaccharide structures are not encoded in genes, final structure of a glycan is determined by current cellular repertoire of expressed GTs (expression, intracellular localization and regulation of specific glycosyltransferases that is commonly referred to as *glycosylation phenotype*).

At the level of individual molecule, from the structural point of view, there is no significant difference between protein and carbohydrate parts. Glycoprotein is an entity composed of a protein and a carbohydrate part, and both parts have structural and functional roles that are specific to a given protein. Due to very high structural variability and the lack of adequate methods to analyze them, carbohydrate parts of glycoconjugates were ignored by the most of the scientific community until very recently. However, thanks to the development of novel methods, in the last decade there is an exponential increase in the knowledge about the physiological roles of carbohydrate structures. Now it is known that glycan part of glycoconjugates are crucial for many physiological processes from fertilization and development, to regulation of hormonal activity and the formation of memory. Recent evidence has established differential glycan processing as a novel regulator of protein function (Partridge *et al.*, 2004, Science **306**, 120-124), and the speculation that the invention of glycosylation was the key evolutionary step that enabled the development of multicellular organisms appears to be correct.

II. Glycoproteins in stress

The influence of stress on glycosylation was first studied on gastric mucousa in rats. Mucousal cells of rats stressed by immersion in water were found to incorporate up to 50% less N-acetylgalactosamine than control animals. Subsequently it was also shown that stress is associated with changes in binding of lectins to gastric mucousa. Stress-induced alterations were found in binding of several lectins, the most specific being changes detected with PNA lectin that specifically recognizes galactose β(1,3) linked to N-acetylgalactosamine. These studies were not continued, but they undoubtedly indicated that stress does have some influence on glycosylation. Helicobacter pylori (causative agent of most peptic ulcers) attaches to the gastric mucousa through specific interactions with carbohydrate structures (Ilver *et al.*, 1998, Science **279**, 373-377), and the stress-induced changes in the expression of these structures might be one of the molecular mechanisms that could explain effects of stress on the development of gastric ulcers.

An indirect confirmation of the hypothesis that structures of glycoconjugates might change in stress came from the studies of acute-phase proteins in depression. For some time it is known that depression can significantly influence immune system, especially some positive acute-phase proteins like haptoglobin or α_1-acid glycoprotein. Similar changes were detected in association with surgical stress, and recently it was shown that even a single episode of uncontrollable stress can activate acute phase response in the experimental animals. Nearly all acute-phase proteins are glycosylated, and in addition to changes in the concentration of the whole proteins, depression is also associated with changes in the individual carbohydrate structures attached to acute-phase proteins. Specific changes in carbohydrate structures on glycoproteins also occur during inflammation and some diseases that are associated with stress. In their recent work, W. van Dijk and colleagues proposed that these changes might be associated with the regulation of the immune response (van Dijk *et al*., 1998, Adv. Exp. Med. Biol. **435**:145-150).

First indications that psychological stress can influence human glycoconjugates came from the studies of M. Flögel and colleagues in early nineties. They used lectin-Western blot to analyze changes in glycosylation of proteins in sera of prisoners released from the Serbian concentration camps during the war in Croatia and Bosnia. Significant changes in glycosylation patterns were established with several different lectins, each recognizing specific segments of the oligosaccharide structure (Table 1).

Table 1. Specificity of lectins. Lectins are physiological receptors for carbohydrate structures in animals and plants. Plant lectins are easy to isolate and they are commercially available as tools for specific recognition of the exactly defined segments of carbohydrate structures.

Lectin	Source	Specificity
GNA	Galanthus nivalis	Man-α(1,3), Man-α(1,6); Man-α(1,2)-Man
SNA	Sambucus nigra	Sia-α(2,6)-Gal
MAA	Maackia amurensis	Sia-α(2,3)-Gal
PNA	Peanut	Gal-β(1,3)-GalNAc
DSA	Datura stramonium	Gal-β(1,4)-GlcNAc

Corticotropin-releasing factor (CRF) plays a crucial role in integrating the body's overall response to stress including integration of the response of the immune system to physiological, psychological and immunological stressors. In addition to hypothalamus, where it was initially identified, CRF, its receptors and related ligands are present throughout the brain and periphery. By activating the glucocorticoid and catecholamine secretion, CRF from the central nervous system mediates the suppressive effects of stress on the immune system. The action of CRF is exhibited through CRF receptors CRF_1 and CRF_2, that are differentially expressed on brain neurons located in neocortical, limbic and brainstem regions of the CNS and on pituitary corticotropes. An intriguing aspect of both CRF receptor and CRF binding protein is their glycosylation pattern. CRF_1 contains five potential N-glycosylation sites. Early studies have shown that its glycosylation differs in different regions of the central nervous system, indicating potential functional roles for different glycoforms. Conversion of oligosaccharide chains to oligomannose structures by kifunensine (inhibitor of mannosidase I that prevents conversion of oligomannose to complex glycans) was reported not to change ligand-binding properties of individual receptors, but mutation experiments have shown that the presence of at least three (out of five) oligosaccharide chains is required for normal CRF_1 function.

Another system where glycosylation might play an important role in the stress response is colinergic activation of the brain. It is generally accepted that in the CNS, stress induces primarily cholinergic hyperactivation. Within several hours, the excess acetylcholinesterase (AChE) exerts a protective effect by retrieving cholinergic balance through enzymatic acetylcholine (ACh) hydrolysis. In addition to hydrolysis of ACh at brain cholinergic synapses and neuromuscular junctions, AChE also affects cell proliferation, differentiation and responses to various insults. Through alternative splicing of its 3'-end, AChE is considered to be one of the general stress-responding proteins, both in the brain and in the periphery. AChE is GPI-anchored protein and efficient glypiation is necessary for proper localization and secretion. For more than 10 years it is known that differences in glycosylation affect stability of the enzyme (Velan *et al.*, 1993, Biochem. J. **296**:649-656), but functional consequences of altered glycosylation are still not understood. Pharmacokinetic profiling of the AChE glycoforms demonstrated correlation between circulatory longevity and the number of attached N-glycans. Glycosylation of AChE was shown to be altered in breast cancer, but this was not studied into details. Abnormally glycosylated forms of the enzyme also accumulate in the cerebrospinal fluid in some neurological disorders, and changes in AChE glycosylation were reported to be a potential diagnostic marker for Alzheimer's disease.

Contraception, which is a form of hormonal stress for the body, also affects glycosylation. Oral estrogen treatment induces an increase in the degree of branching, and a decrease in fucosylation and sialyl Lewis x expression on α_1-acid glycoprotein compared to individuals receiving no estrogens or transdermal estrogen treatment. The effects of oral estrogens are identical in both males and females, and they can be reduced by administration of progestagen. Effects of oral estrogens on glycosylation of α_1-acid glycoprotein are opposite to those induced by inflammation, indicating that estrogens can modulate the glycosylation-dependent inflammatory actions of α_1-acid glycoprotein. Interestingly, estrogens that are applied transdermally do not exert this effect on hepatic glycosylation (Brinkman-Van der Linden *et al*., 1996, Glycobiology **6**:407-412).

III. Lectins in stress

Some glycans on glycoconjugates have only structural roles, but some also take part in specific recognition processes. One of the major mechanisms how glycoconjugates perform their molecular functions is the interaction with their specific molecular receptors - lectins. Hundreds of endogenous lectins function as physiological receptors for oligosaccharides that interpret molecular information encoded in glycans. They take part in numerous physiological processes including folding, intracellular transport, fertilization, regulation of the inflammatory response, and brain plasticity.

The best known example of lectin function is inflammation, where the interaction between lectins called “selectins”, and carbohydrate structures on glycoproteins represent the first decisive step leading to adhesion of circulating lymphocytes to endothelium at the site of inflammation. Carbohydrate-lectin interactions are very interesting because they can be inhibited with small oligosaccharides that are generally non-toxic, and using modern carbohydrate cycling technologies relatively inexpensive to prepare for therapeutic purposes.

First indications that lectins might change in stress came from a study of a phenomenon only remotely associated with stress. C. Bardosi and colleagues were analyzing influence of prolonged anesthesia on mannose-receptor and some other lectins on murine peripheral blood polymorphonuclear leukocytes. They have demonstrated reduced expression of mannose-receptor and changes in the expression of receptors for several other sugars, indicating that prolonged anesthesia affects regulation of lectin expression.

Evidence that psychological stress can influence lectins came from studies of G. Lauc and colleagues on lectins in livers of rats exposed to immobilization stress. Immobilization stress was found to influence galectin-3, a galactose-specific lectin, so that it binds to another nuclear lectin CBP67 (67 kDa Carbohydrate Binding Protein). Formation of the complex between CBP67 and galectin-3 resulted in binding of the galactose-specific galectin-3 to glucose-affinity column for which it shows no affinity under normal conditions. This is a very interesting effect because galectin-3 is involved in mRNA splicing, and changes in its functions might have profound effects on the whole cell. However, whether this binding was mediated by protein-protein interactions, or through lectin-like binding of galectin-3 to galactose residues on carbohydrate structures attached to CBP67, as well as other details of this interaction are not known.

Galectin-3 is a versatile galacotoside-binding lectin that has been implicated in numerous cellular functions. It also appears to be affected by stress, and interestingly, different types of stress have exactly opposite effects on its expression. As shown by Dumić and colleagues in 2000, while exposure to UV light or transfer to *in vitro* conditions induces galectin-3 in cultured cells, immobilization stress *in vivo* results in a decrease in galectin-3 in mouse spleen and liver. Regulation of galectin-3 expression involves transcription factor NF-κB, what connects galectin-3 with corticosteroids (and CRF) and places its expression downstream from hormonal signals in the stress response pathway.

Major problem in studying changes of lectin activity in different physiological processes is the lack of adequate methods to measure lectin activity in complex biological samples. A new method (Fig 3) using photoaffinity glycoprobes labeled with digoxin was recently developed, and hopefully it would enable easier identification of changes in lectins in different diseases (Lauc *et al.*, 2000, Glycobiology **10**, 357-364). Until now, only one new lectin was found to appear in stress. It was found to bind to glucose-containing glycoprobes, and according to the electrophoretic mobility of the protein isolated from rat liver named CBP33 (Lauc et al., 1994, Glycoconjugate J. **11**:541-549.).

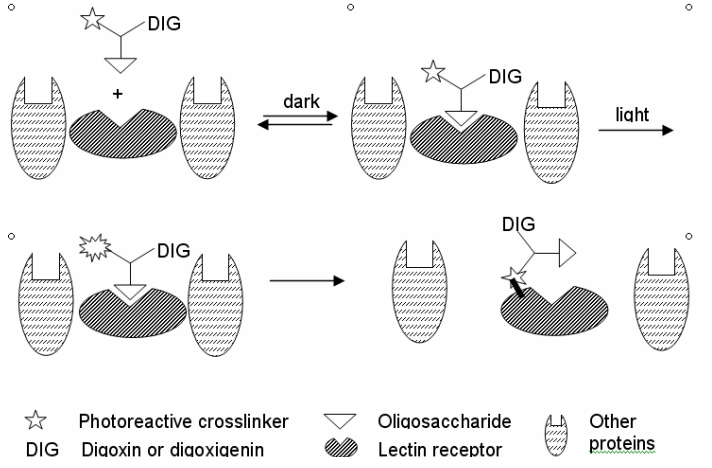

Fig. 3. Photoaffinity method for the detection of lectins. Photoaffinity glycoprobes containing target carbohydrate structures are incubated with biological samples to allow formation of non-covalent complexes between the probe and lectin receptors in the sample. Illumination activates the photoreactive crosslinker and it forms covalent bonds with neighboring molecules, mostly lectin receptors. The result is a lectin with covalently incorporated digoxin tag that can be easily identified with labeled antibodies against digoxin.

IV. Stress affects the activity of glycosyltransferases

Carbohydrate parts of glycoconjugates are synthesized by sequential action of numerous glycosyltransferases, enzymes that are specific for both structure of the glycoconjugate acceptor, and for the monosaccharide that is being added. Appearance of novel or altered glycoconjugate structures in stress indicates that stress should somehow affect the activity of glycosyltransferases, but only glycosyltransferases whose relation with stress have been studied by now are sialyltransferases (enzymes that transfer sialic acids to glycoproteins).

For some time it is known that corticosteroids can change the activity of sialyltransferases in vitro. K. Breen and colleagues have shown that corticosteroids and other hormones from the adrenal gland significantly influence the activity sialyltransferases in vivo (Coughlan et al. 1996, Glycobiology **6**:15-22). Adrenalectomy and subsequent administration of corticosterone and/or aldosterone significantly influence the activity of sialylransferases in various rat tissues. While sialyltransferases in some tissues like e.g. kidney are apparently not influenced by adrenalectomy or by the addition of steroid hormones, sialyltransferases in liver are under negative control of corticosteroids. Adrenalectomy results in the increased activity of sialyltransferases in the liver that can not be reverted to normal values by the administration of dexamethasone.

Enzymes that perform the same function in the brain react to same hormonal signals on the exactly opposite way. Adrenalectomy and the consequential lack of circulating corticosteroids leads to the decrease of total sialyltransferase activity in the brain. The subsequent administration of exogenous corticosteroids exhibits regional specificity with the enzyme activities in the cortex, cerebellum, and brainstem being stimulated by both dexamethasone and aldosterone, and enzyme activity in the hippocampus being stimulated only by aldosterone. Total sialyltransferase activity in some tissue does not represent activity of a single enzyme, but the sum of the activities of all enzymes that transfer sialic acids. K. Breen and colleagues also studied effects of corticosteroids on two individual enzymes, α(2,3)-sialyltransferase and α(2,6)-sialyltransferase. Both enzymes transfer sialic acids, but they link them to different carbon atoms on the preceding sugar in the carbohydrate structure.

As shown by Dabelić and colleagues in 2004, immobilization stress affects sialyltransferase activity in different rat tissues. Acute and chronic stress have different effects, but what is even more interesting same type of stress have opposite effects on sialyltransferase activity in different tissues. In general, stress induces sialyltransferase activity in extraneural tissues, and suppress the activity of same enzymes in most brain tissues. The fact that same enzymes respond differently to same hormonal signals depending on their cellular environment, exemplifies the fact that the molecular setup of the targeted cell, and not hormonal signal by itself, is the decisive player in determining the direction and consequences of the stress response.

V. Glycobiology of stress

At the moment only several fragments of the glycobiological mechanisms involved in the physiological response to psychological stress are known, but the complete picture is slowly emerging. Stress causes numerous changes in the circulating hormones, and many molecular details of this process are known. It is also known that corticosteroids affect activity of at least one glycosyltransferase both in vitro and in vivo. Altered activity of glycosyltransferases results in different carbohydrate structures attached to glycoproteins, and these changes have been demonstrated both in humans and in experimental animals. A change in the carbohydrate structures attached to a glycoprotein is a well-established way to change its structural and functional properties, and recently this was shown to be one of the mechanisms that control activity of membrane receptors. Although this type of glycosylation-mediated receptor modulation in stress still has to be proven, it is a very interesting hypothesis. On the other hand, new glycoconjugate structures could also represent novel signals on the cell surface that could alter interaction of the cell with neighboring cells in a process analogous to selectin-mediated adhesion of lymphocytes. Stress is also known to be associated with the appearance of novel lectins, but the exact mechanism of this process is not known. These lectins could be receptors for either novel, or also “normal” glycoconjugate structures, translating their structures into molecular functions. Although most of this is still speculative, hopefully more will be known soon about the molecular role of glycoconjugates, their lectin receptors, and glycosyltransferases in the physiological response to psychological stress.

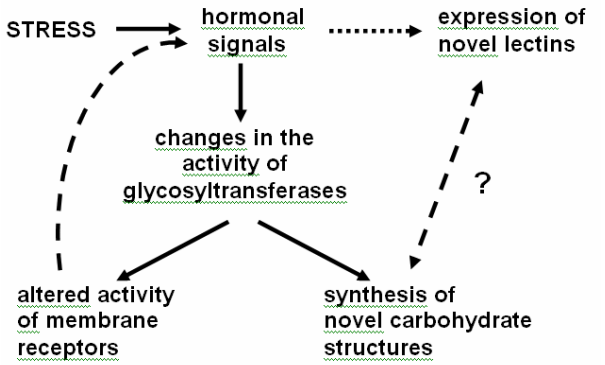

Fig. 4. Hypothetical model of the glycobiology of stress. Hormones mediate numerous stress-associated changes, and among them alter activity of different glycosyltransferases. Changed activity of glycosyltransferases results in the appearance of different carbohydrate structures on glycoproteins, which could either present a novel structures with potential to interact with specific endogenous lectins, or modify the activity of various membrane receptors. Although this has yet to be proven, it could be one of mechanisms explaining why prolonged stress has different effects than acute stress. In the same time it is known that stress is associated with changes in composition and activity of endogenous lectins, but the exact mechanisms linking stress and changes in lectin activity are still not known.

Further reading

Assil, I.Q. and Abou-Samra, A.B. (2001) N-glycosylation of CRF receptor type 1 is important for its ligand-specific interaction. American Journal of Physiology Endocrinology and Metabolism, 281, E1015-1021.

Axford, J. (2001) The impact of glycobiology on medicine. Trends in Immunology, 22, 237-239.

Chitlaru, T., Kronman, C., et al. (2002) Overloading and removal of N-glycosylation targets on human acetylcholinesterase: effects on glycan composition and circulatory residence time. Biochemical Journal, 363, 619-631.

Dabelic, S., Flogel, M., et al. (2004) Stress causes tissue-specific changes in the sialyltransferase activity. Zeitschrift fur Naturforschung, 59, 276-280.

Lauc, G., Supraha, S., et al. (2003) Digoxin derivatives as tools for glycobiology. Methods in Enzymology, 362, 29-37.

Nalivaeva, N.N. and Turner, A.J. (2001) Post-translational modifications of proteins: acetylcholinesterase as a model system. Proteomics, 1, 735-747.

Partridge, E.A., Le Roy, C., et al. (2004) Regulation of cytokine receptors by Golgi N-glycan processing and endocytosis. Science, 306, 120-124.