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MINIREVIEW

The Hepatic Glycogenoreticular System*

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One of the major liver functions is the ability of hepatocytes to store glucose in the form of glycogen for various purposes. Beside glucose production and secretion, the synthesis of glucuronides and ascorbate has been reported to be dependent on the extent of the glycogen stores and on the rate of glycogenolysis in the liver. It is common that the final steps of these pathways are catalysed by intraluminally orientated enzymes of the endoplasmic reticulum, which are supported by transporters for the permeation of substrates and products. On the basis of the close morphological and functional proximity of glycogen, glycogen-dependent pathways and the (smooth) endoplasmic reticulum we propose to use the term „glycogenoreticular system" for the description of this export-orientated hepatocyte-specific metabolic unit. (Pathology Oncology Research Vol 7, No 2, 107–110, 2001)

Keywords: glycogen; glycogenolysis; endoplasmic reticulum; glucuronidation; ascorbate; glucose-6-phosphatase

Introduction

One of the most important functions of the liver is the export of various molecules for utilisation by other organs or the whole organism. Among others, the liver is the primary source of plasma proteins, lipoproteins, glucose, ketone bodies, conjugated xeno- and endobiotics, ascorbate, bile acids and cholesterol. Till now attention has been focused mainly on the secretory mechanism of macromolecules, which is tightly linked to the endomembrane system of the hepatocyte (for reviews see 1). Recent findings suggest that the synthesis and export of smaller, glycogen-derived compounds (ascorbate and glucuronides) are also related to the endoplasmic reticulum. In the present paper we would like to outline the connections between glycogen metabolism and the synthesis of these molecules as well as the endoplasmic reticulum-dependent machinery of their export.

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Glycogen-dependent biosynthetic pathways in the liver

Hepatic glycogen is known to play a primary role in the maintenance of blood glucose level by glycogenolysis during the early phase of starvation.[2,3] Recently the glycogenolysis dependence of two other processes, glucuronidation [4,5] and ascorbate synthesis[6] has also been described in the liver. Both the inhibition of glycogenolysis by various agents (insulin, fructose, glucose) in glycogen-containing isolated hepatocytes from fed mice and glycogen depletion during starvation decreased the capacity of glucuronidation.[5,7,8] Similarly, ascorbate production (in mice, *i.e.* in a species which is able to synthesize this compound) was also dependent on the extent of hepatic carbohydrate reserves. The stimulation of glycogenolysis increased, while its inhibition decreased the rate of ascorbate synthesis.[6] The known glutathione depletion dependent stimulation of glycogenolysis[9] resulted in the increased capacity of glucuronidation[10] and ascorbate synthesis.[11] It has been concluded that UDP-glucuronic acid, the cofactor for glucuronidation and the precursor of ascorbate synthesis, is predominantly originated from glycogen breakdown.[12] In accordance with this conclusion, addition of UDP-glucose or UDP-glucuronic acid to permeabilised hepatocytes restored the capacity of both processes decreased by glycogen depletion/glycogenolysis inhibition.[10,11,13]

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Glycogen and endoplasmic reticulum: spatial and temporal coincidences

On the basis of the above mentioned observations a crucial question has emerged: why are the other pathways of carbohydrate metabolism (*i.e.* glucose uptake or gluconeogenesis) are unable to maintain the capacity of glucuronidation and ascorbate production? The underlying mechanism may be the sub-compartmentation of the cytosolic glucose-6-phosphate pool which has been described in hepatocytes; one pool is linked to glycogenolysis and the other to gluconeogenesis.[14,15] The authors suggested that this phenomenon could be caused by the organisation of multienzyme complexes of a specific pathway, *e.g.* glycogenolysis can be associated with the glucose-6-phosphatase activity of the (smooth) endoplasmic reticulum. The recent observations strengthen this view demonstrating other glycogenolysis-dependent pathways. Furthermore, the new observations can help to understand the morphological basis of the phenomenon, since UDP-glucuronosyltransferases and gulonolactone oxidase (similarly to glucose-6-phosphatase) are integral membrane proteins of the endoplasmic reticulum.[16,17]

The close association between glycogen particles and the vesicles and tubules of smooth endoplasmic reticulum was already described in an early study on the subcellular structure of hepatocytes.[18] Newly formed glycogen appears primarily in endoplasmic reticulum-rich regions of liver cells and remains associated with it during glycogen deposition and depletion[19,20] indicating that this subcellular structure is suitable for both glycogenesis or glycogenolysis, according to the actual demands.

In addition to colocalisation, a temporal arrangement of glycogen synthesis/breakdown and endoplasmic reticulum proliferation/degradation can also be observed. In the fetal life the activity of glucose-6-phosphatase,[20] UDP-glu-

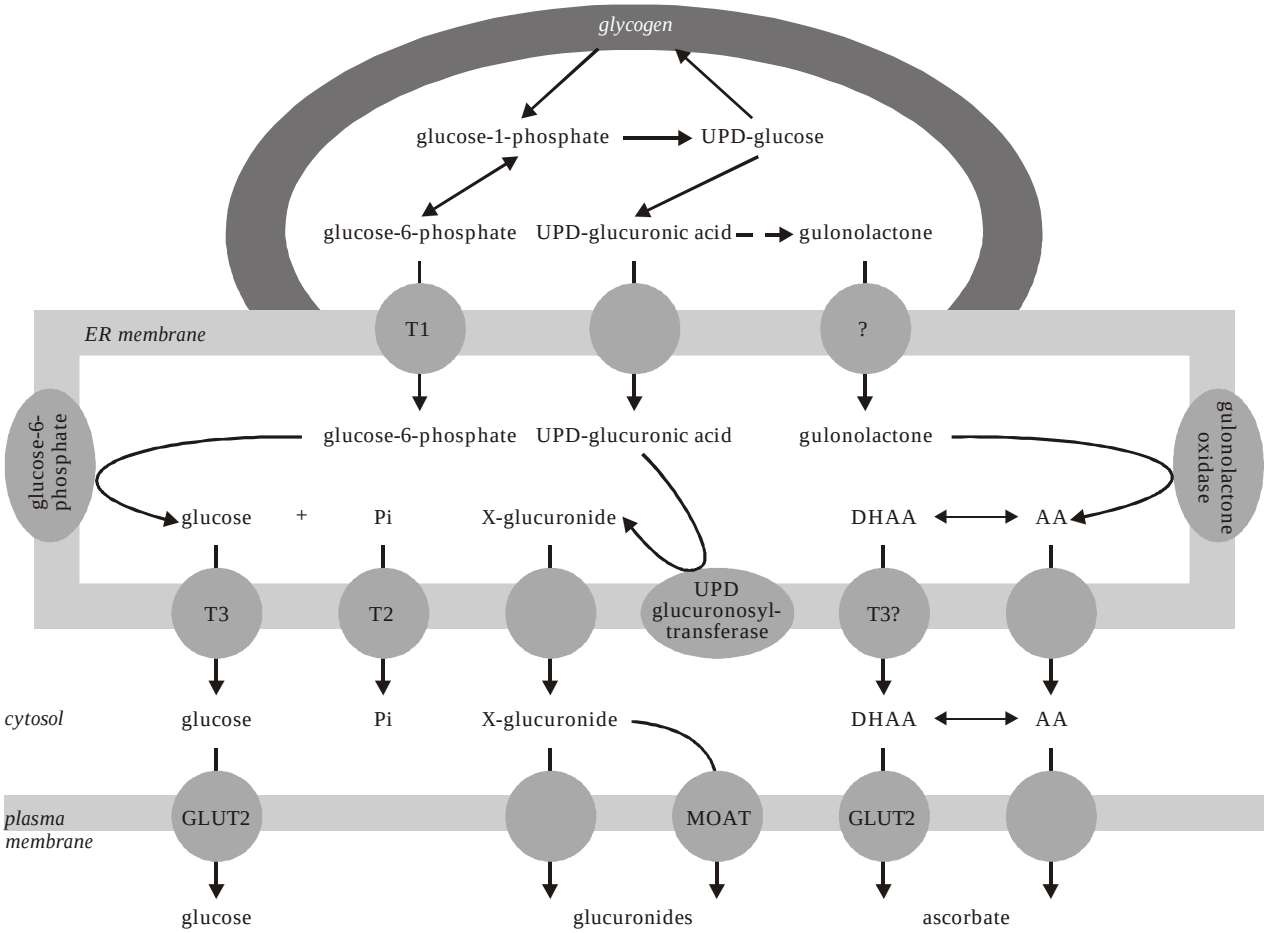

Figure 1. Scheme of the glycogenoreticular system of hepatocytes. The enzymes of the endoplasmic reticulum with intraluminal active site are indicated with oval symbols, the transporters are shown as circles. For the sake of clarity the antiport mechanisms in case of transport processes in glucuronidation are not represented. Abbreviations: T1, T2 and T3 are the glucose-6-phosphate, phosphate and glucose transporters of the glucose-6-phosphatase system, respectively. AA, ascorbic acid, DHAA, dehydroascorbic acid, Pi, inorganic phosphate.

curonosyltransferase[22] and gulonolactone oxidase (unpublished observation) is very low in the liver. Glycogenolysis and the glycogenolysis-dependent pathways seem to be activated together in the early postnatal period.[23] During pregnancy large glycogen stores accumulate in the fetal liver, which is rapidly mobilised and exhausted after the birth. At the same time, the smooth endoplasmic reticulum proliferates[24] and its enzymes (glucose-6-phosphatase, the majority of UDP-glucuronosyltransferase isoenzymes, gulonolactone oxidase) are induced. A similar phenomenon appears during the fasting-refeeding cycle: at maximal glycogen accumulation the proportion of the smooth endoplasmic reticulum is very low in the hepatocyte, while intensive glycogenolysis is associated with the proliferation of the smooth fraction of endoplasmic reticulum.[19,25]

The machinery of export: intraluminal enzyme activities supported by transporters for the permeation of substrates and products

One of the most striking features of several endoplasmic reticulum enzymes is their latency: their activity is low in intact microsomal vesicles and the elimination of the membrane barrier (by detergents, pore-forming agents etc.) increases the activity. The compartmentational or substrate-transport model of latency[3,16,26] suggests that the active site of these enzymes is intraluminal and the permeation of hydrophilic substrate(s) and product(s) is mediated by specific transporters. The velocity of the transport is rate limiting in the overall enzymatic process. Recent models for the membrane topology of glucose-6-phosphatase[27,28] and UDP-glucuronosyltransferases[16,22] verify the intraluminal positioning of the catalytic sites. The corresponding transport activities have been less explored, however, their existence has been proved. The demonstration of a metabolically active, intraluminal glucose-6-phosphate pool in microsomal vesicles gives an evidence for the transport of glucose-6-phosphate.[29] The sequence of a glucose 6-phosphate translocase, mutated in glycogen storage disease type Ib, has also been described.[30] For the inward transport of UDP-glucuronic acid various antiport mechanisms have been suggested with the participation of UMP, UDP-N-acetyl-glucosamine or phenol glucuronides as counteranions.[26,31,32] The rapid permeation of gulonolactone through the microsomal membrane and the intraluminal accumulation of the products (ascorbate, hydrogen peroxide) of gulonolactone oxidase[33] suggests that this enzyme shares the orientation of glucose-6-phosphatase and UDP-glucuronosyltransferases. Therefore, the final products of these glycogen-dependent pathways are formed in the lumen of endoplasmic reticulum, which is continuous in time with the extracellular environment. There are two possibilities for the export of these compounds: they can reach the plasma membrane by vesicular transport or can be secreted after two consecutive transport steps through the endoplasmic reticulum and plasma membranes. These molecules of smaller molecular mass seem to follow the second path, however, especially in the case of more charged and/or bulky compounds the participation of the vesicular transport cannot be excluded. Recent observations indicate that glucose[34] and ascorbate[35] exit from the hepatocyte, at least partially, by this mechanism.

The existence of endoplasmic reticulum transporters for the exit of products has been demonstrated. The permeation of the products of glucose-6-phosphatase, phosphate and glucose is mediated by T2 and T3 (transporter 2 and 3), components of the glucose-6-phosphatase system.[3] Glucuronides and UMP, the end products of glucuronidation, exit by means of the above mentioned antiports. The product of gulonolactone oxidase can leave as ascorbate or as its oxidised derivative dehydroascorbate by newly described distinct transport mechanisms.[36] (The transport of dehydroascorbate is preferred, which is presumably mediated by the glucose transporter T3). Finally, the products intended for being used by other cells/organs should leave the hepatocyte by means of various transporters (multispecific organic anion transporter, GLUT2, organic anion transporting polypeptide 1, novel liver-secific transport protein etc.) on the canalicular or sinusoidal surface of the cell. *(Figure 1.)*

What is the reason for the intraluminal organisation of these enzyme activities in the endoplasmic reticulum? A plausible explanation can be that the compartmentation of the hepatocellular substrate pool into a cytosolic and an intraluminal sub-pool (the latter is tightly connected to the pool in the glycogen particle by transporters) allows their independent regulation.

Summary

Glucuronidation, ascorbate synthesis and glucose production are dependent on hepatic glycogen reserves and are connected to the endoplasmic reticulum of hepatocytes. They have similar features: an intraluminal enzymatic activity (glucose-6-phosphatase, UDP-glucuronosyltransferases and gulonolactone oxidase) is supported by transporters for the membrane permeation of substrates (glucose-6-phosphate, UDP-glucuronate, gulonolactone) and products (glucose, glucuronides, ascorbate/dehydroascorbate). The final intended purpose of these liver-specific pathways is the export of the end products. In this context the glycogen particle can be regarded as the 'ribosome' of the smooth endoplasmic reticulum. On the basis of morphological and functional connections between hepatic glycogen and the (smooth) endoplasmic reticulum we propose to use the term „glycogenoreticular system" for the description of this export-orientated metabolic unit.

The actual function of this "unit" is related to the current physiological/pathological state of the organism. The normal balance between these metabolic pathways is determined

mainly by the complex regulation of glycogen metabolism, which ensures the priority of glycemic control and prevents those processes, which occur only under pathological circumstances and in diseases. Well known phenomena, such as the depletion of glycogen stores caused by xenobiotics or increased glycogenolysis due to the altered reduced/oxidised glutathione ratio changed by oxidant drugs, can be explained and interpreted this way. Defective expression of the components of the "unit" - subunits of the glucose-6-phosphatase system in the various subtypes of the von Gierke's disease, bilirubin UDP-glucuronosyl transferase in Gilbert syndrome - alters the balance and coordination of glycogen metabolism and the connected pathways. The complex approach of the hepatic glycogenoreticular system may promote the better understanding of pathophysiological states in which redox homeostasis, glucose metabolism and biotransformation are equally involved.

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