REVIEW ARTICLE

Specific features of glycogen metabolism in the liver

Mathieu BOLLEN[1], Stefaan KEPPENS and Willy STALMANS

Afdeling Biochemie, Faculteit Geneeskunde, Katholieke Universiteit Leuven, Campus Gasthuisberg, Herestraat 49, B-3000 Leuven, Belgium

Although the general pathways of glycogen synthesis and glycogenolysis are identical in all tissues, the enzymes involved are uniquely adapted to the specific role of glycogen in different cell types. In liver, where glycogen is stored as a reserve of glucose for extrahepatic tissues, the glycogen-metabolizing enzymes have properties that enable the liver to act as a sensor of blood glucose and to store or mobilize glycogen according to the peripheral needs. The prime effector of hepatic glycogen deposition is glucose, which blocks glycogenolysis and promotes glycogen synthesis in various ways. Other glycogenic stimuli for the liver are insulin, glucocorticoids, parasympathetic (vagus) nerve impulses and gluconeogenic precursors such as fructose and amino acids. The phosphorolysis of glycogen is mainly mediated by glucagon and by the orthosympathetic neurotransmitters noradrenaline and ATP. Many glycogenolytic stimuli, e.g. adenosine, nucleotides and NO, also act indirectly, via secretion of eicosanoids from non-parenchymal cells. Effectors often initiate glycogenolysis cooperatively through different mechanisms.

INTRODUCTION

Most mammalian cells store glycogen as a reserve for the production of glucose 6-phosphate as a metabolic fuel for glycolysis. In liver, glycogen is mainly stored as a glucose reservoir for other tissues. As a consequence, the level of hepatic glycogen changes considerably (between 1 and 100 mg/g) with the feeding condition. The estimated contribution of hepatic glycogenolysis to the total glucose production during the first day of starvation varies from 40 to 80 %, depending on the experimental design and methodology [1,2]. At longer starvation periods, the hepatic glycogen stores become depleted and the contribution of gluconeogenesis becomes predominant. Hepatic glycogenolysis also accounts for nearly all of the initial (2 h) increase in glucose production in response to a physiological increment in plasma glucagon [3,4] or a moderate, insulin-induced hypoglycaemia [5]. That gluconeogenesis cannot completely compensate for hepatic glycogenolysis is strikingly illustrated by the ketotic hypoglycaemia induced by fasting in individuals with a genetic deficiency of glycogen synthase in the liver [6].

The general mechanism of glycogen synthesis and degradation is the same in all tissues [7–9]. The first step in the biogenesis of glycogen is the autocatalytic attachment of C-1 of glucose to a single tyrosine residue of the enzyme glycogenin, using UDP-glucose as glucosyl donor (Scheme 1). Subsequently, glycogenin autocatalytically extends the glucan chain by six to seven α-1,4-linked glucose residues. This 'primed' glycogenin is further and similarly elongated by glycogen synthase, which is initially complexed to glycogenin, but dissociates during the elongation process. Finally, branching enzyme transfers a terminal oligoglucan (at least six glucose units) from an elongated external chain and attaches its C-1 to a C-6 in a neighbouring chain. A mature glycogen particle has a bush-like structure with branches that form a left-handed helix with 6.5 glucose residues per turn. About half of the glycogen mass is attributable to the external branches. The internal branches carry side chains separated by about four glucose units. The bush-like structure of glycogen accounts for the spherical shape of the β-particles (30 nm diameter; up to 60000 glucose units) that are present in most cells. In the liver about 20–40 β-particles are associated into larger complexes known as α-rosettes.

The degradation of glycogen requires the concerted action of glycogen phosphorylase and the bifunctional debranching enzyme (Scheme 1). In the presence of P_i, phosphorylase releases the terminal glucose residue of an external chain as glucose 1-phosphate and continues to do so until the external chains have been shortened to four glucose units. Subsequently, the transferase activity of the debranching enzyme removes a maltotriose unit from the α-1,6-linked stub and attaches it through an α-1,4-glucosidic bond to the free C-4 of the main chain. The single remaining α-1,6-linked glucose unit is then liberated as glucose by the α-glucosidase activity of debranching enzyme, while additional α-1,4-linked glucose residues become available for phosphorylase. Phosphorylase and debranching enzyme can also 'unprime' glycogenin, i.e. by phosphorolysis of the last α-1,4-linked glucose residues and hydrolysis of the α-glucosidic glucose–glycogenin bond respectively. In the liver, glucose can be produced from glucose 1-phosphate by the successive actions of phosphoglucomutase and glucose-6-phosphatase.

For nearly all glycogen-metabolizing enzymes there are hepatic isoforms that are uniquely adapted to the role of the liver in the maintenance of the blood glucose level. The first part of this review focuses on the properties and regulation of these hepatic isoenzymes. The second part deals with the role and mechanism of action of glycogenic and glycogenolytic agents in the liver.

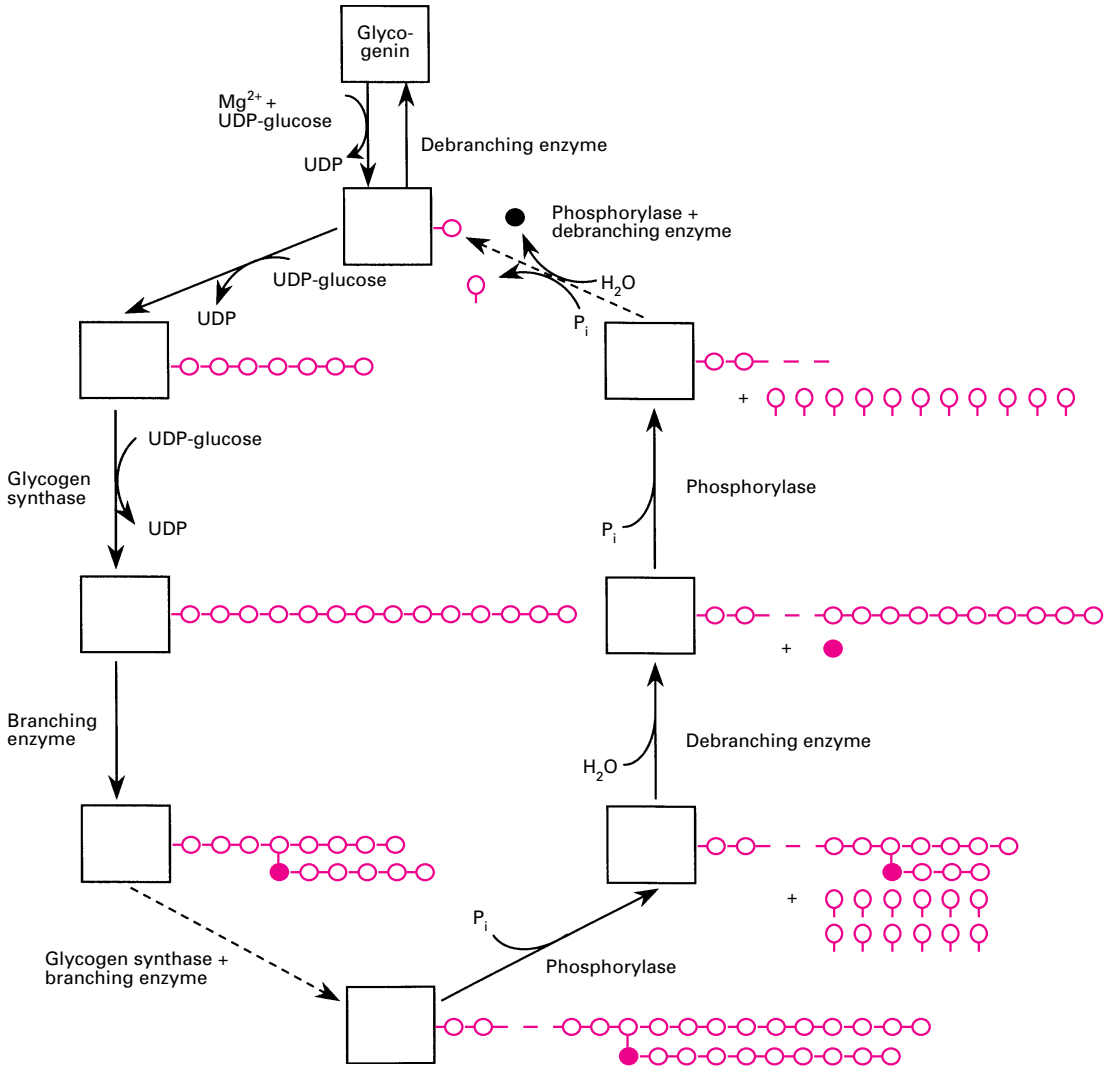
GLYCOGEN-METABOLIZING ENZYMES

Glucose transporter (GLUT)

The bidirectional flux of glucose across the plasma membrane of hepatocytes is accomplished by facilitative diffusion mediated by the GLUT-2 transporter [10]. GLUT-2 (53 kDa) is not acutely controlled by insulin, and its transport capacity is not rate-

Abbreviations used: GLUT, glucose transporter; GSK, glycogen synthase kinase; IRS1, insulin receptor substrate 1; PI3-kinase, phosphatidylinositol 3-kinase; PP-1, protein phosphatase-1; PP-1G, glycogen-associated PP-1; PP-2A, protein phosphatase-2A.

[1] To whom correspondence should be sent (e-mail Mathieu.Bollen@med.kuleuven.ac.be).



Scheme 1 Steps in the synthesis and degradation of glycogen

●, Glucose; ⚲, glucose 1-phosphate; ○-○, α-1,4-linked glucose units; ⚲, α-1,6-linked glucose units. In the glycogen particles at the right, broken lines represent the bulk of the glycogen structure.

Adapted from Bollen, M. and Stalmans, W. in Molecular Biology and Biotechnology: A Comprehensive Desk Reference (Meyers, R. A., ed.), pp. 385–388, copyright 1995 John Wiley & Sons, Inc. [8]. Adapted by permission of John Wiley & Sons Inc.

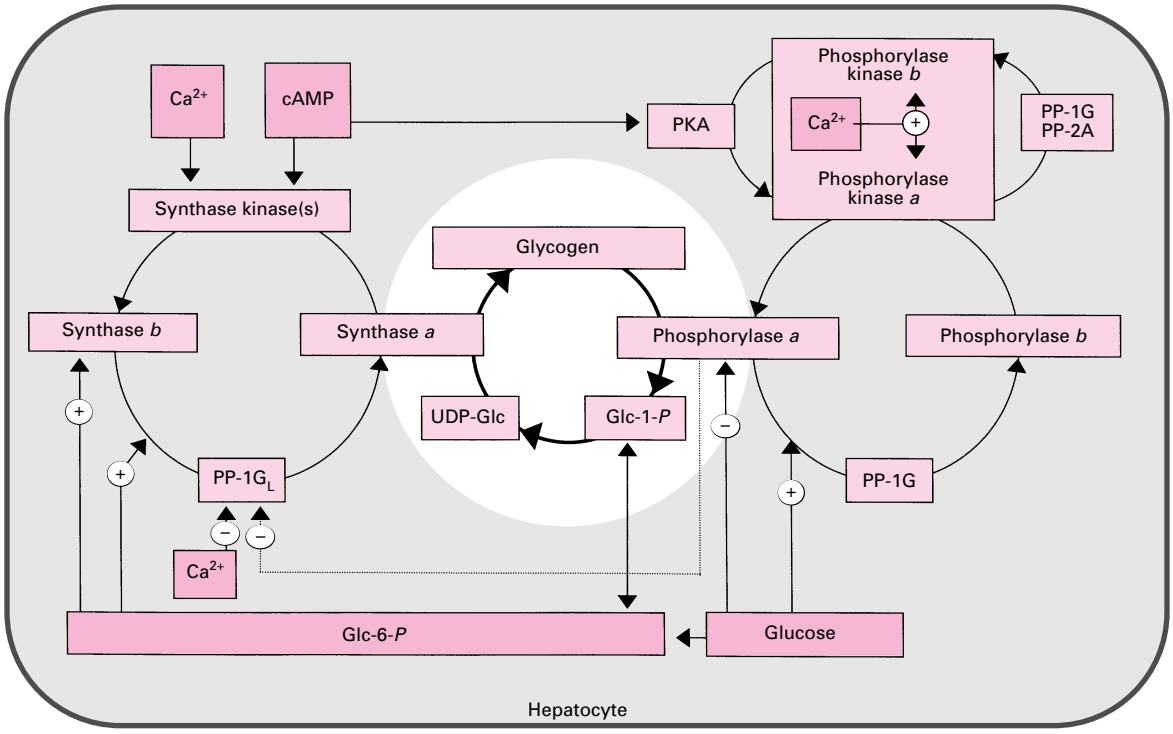
limiting for the hepatic uptake or release of glucose. This implies that the concentrations of glucose in the blood and in hepatocytes are the same, which enables the liver to function as a sensor of the blood glucose concentration.

Glucokinase

The conversion of glucose into glucose 6-phosphate is catalysed by glucokinase (also known as hexokinase IV or hexokinase D [11]). Unlike other hexokinases, glucokinase (52 kDa) has a supraphysiological K_m for glucose and is not inhibited by physiological concentrations of glucose 6-phosphate. The enzyme is acutely controlled, however, by a 'regulatory protein' (68 kDa) that inhibits the enzyme in the presence of fructose 6-phosphate [12]. The complex is dissociated by binding of fructose 1-phosphate to the regulatory protein or of glucose to glucokinase [13]. The glucokinase reaction is a limiting factor for glycogen synthesis from glucose in the liver [14–16].

Glycogenin

It has recently become apparent that there are two glycogenin genes and that from one of these genes (*glycogenin-2*), various isoforms of glycogenin can be generated by alternative splicing [17]. Glycogenin-1 (37 kDa) has a broad tissue distribution, whereas the expression of glycogenin-2 (50–55 kDa) is mainly restricted to liver, pancreas and heart. Both forms of glycogenin are Mn^{2+}/Mg^{2+}-dependent glucosyltransferases with a K_m for UDP-glucose that is two or three orders of magnitude lower than that of glycogen synthase. In the liver, the level of glycogenin-1, as detected by Western- and Northern-blot analysis, is very low [18,19]. One cannot exclude the possibility that glycogenin-1 is actually only expressed in non-parenchymal liver cells. Interestingly, in H4IIEC3 hepatoma cells, all the glycogenin-1 is covalently bound to glycogen, while more than 80% of glycogenin-2 is free [17]. However, in the liver of fed rats, essentially all the glycogenin appears to be glycogen-bound [20].



Scheme 2 Control of hepatic glycogen metabolism by enzyme phosphorylation and by metabolites

Abbreviations: PKA, protein kinase A; Glc-1-*P*, glucose 1-phosphate; Glc-6-*P*, glucose 6-phosphate; UDP-Glc, UDP-glucose.

The description of an hepatic isoform of glycogenin [17] cannot account for all the unique features in the biogenesis of glycogen in the liver. Thus it remains unclear why some glycogen molecules tend to be completed before others start to grow [21]. It is also not known how β-particles are clustered into α-particles. It has been proposed that this aggregation is mediated by a covalently bound 'backbone' protein of 60 kDa [22] which, based upon its amino acid composition, should be clearly different from glycogenin [17]. However, the putative 'backbone' protein has not been further characterized during the past decade.

Glycogen synthase

It is firmly established that liver glycogen synthase (a homodimer of 81 kDa protomers) is tightly controlled by the reversible phosphorylation of multiple serine residues near the N- and C-termini (reviewed in [23,24]). *In vivo*, liver glycogen synthase can be phosphorylated up to a stoichiometry of six phosphate groups per subunit. Generally, phosphorylation is associated with an inactivation of glycogen synthase (conversion to the *b*-form; Scheme 2), which is primarily accounted for by a decreased V_{max}. In contrast, phosphorylation of muscle glycogen synthase mainly decreases the affinity for the substrate UDP-glucose. Although several phosphorylation sites appear to be conserved, liver glycogen synthase lacks the equivalents of sites 1a and 1b [25] that are the prime targets for phosphorylation by protein kinase A in skeletal muscle [23,24]. *In vitro*, liver glycogen synthase can be phosphorylated by various protein kinases, including protein kinase A, phosphorylase kinase, protein kinase C, Ca^{2+}- and calmodulin-dependent protein kinase II, protein kinases CK1 and CK2, glycogen synthase kinase-3 (GSK-3) and the AMP-stimulated protein kinase [23,26]. However, it remains to be determined which of these protein kinases, or any other ones, phosphorylate glycogen synthase *in vivo*. Phosphopeptide mapping has revealed that glucagon and glucose, the major physiological stimuli for the inactivation and activation of glycogen synthase respectively, affect the phosphorylation level of many sites (see [27] and references cited therein), suggesting the involvement of multiple protein kinases and/or protein phosphatases.

Glucose 6-phosphate is an allosteric activator of glycogen synthase *b*. In the presence of 10 mM glucose 6-phosphate the *a* and *b* forms are equally active [26]. The effect of glucose 6-phosphate is antagonized by 10 mM Na_2SO_4 or by physiological concentrations of P_i, and hence only glycogen synthase *a* is thought to be active *in vivo*. There is indeed an excellent linear correlation between the activity of glycogen synthase *a* and the rate of glycogen synthesis [26]. Glucose 6-phosphate also promotes the dephosphorylation of glycogen synthase by glycogen-associated protein phosphatase-1, and this appears to be an essential step in the glucose-induced activation of glycogen synthase [28]. In addition, it has recently been reported that incubation of purified liver synthase with glucose 6-phosphate causes a time-dependent activation (increased V_{max}) that is not mediated by dephosphorylation, and was therefore termed 'pseudo-activation' [29]. Pseudo-activated glycogen synthase has properties that are intermediate between those of the *a* and *b* forms, i.e. unlike synthase *a* it requires glucose 6-phosphate to be fully active, but unlike synthase *b* the effect of glucose 6-phosphate is not opposed by sulphate.

On the basis of the *in vitro* activity of glycogen synthase at physiological concentrations of various known effectors, Nuttall and Gannon [30] concluded that glycogen synthase could only account for a minor fraction of the glycogen-synthetic rates

found *in vivo*. This paradoxical finding led them to invoke the existence of a still-unidentified stimulator of glycogen synthase or of an alternative pathway for glycogen synthesis. However, it is questionable whether the adopted effector concentrations are physiologically relevant, in particular in view of growing evidence for a subcellular compartmentation of glycogen synthase and its effectors (see below).

Branching enzyme

Branching (Scheme 1) is an essential, but not rate-controlling, step in the synthesis of glycogen, which increases the solubility of the polysaccharide. A deficiency of branching enzyme is associated with an accumulation of insoluble polysaccharide particles and foreign-body reaction, resulting in liver cirrhosis [31]. The cDNA cloning of human branching enzyme (80 kDa) has not revealed any tissue-specific isoforms [32].

Phosphorylase

There are three mammalian glycogen phosphorylases, designated the 'muscle', 'brain' and 'liver' isoenzymes according to the tissue in which they are preferentially expressed (reviewed in [33,34]). They are homodimers of subunits of ≈ 100 kDa and are encoded by different genes. All isoenzymes are converted from the inactive *b*-form into the active *a* form through phosphorylation of Ser^{14} by phosphorylase kinase (Scheme 2). In addition, the muscle and brain isoenzymes are also allosterically activated by AMP and inhibited by glucose 6-phosphate, which enables these isoenzymes to sense and respond to an intracellular need for energy. The liver enzyme, on the other hand, is much more tightly controlled by phosphorylation than by allosteric regulation, as is shown by the inability of the liver to break down glycogen in the absence of functional phosphorylase kinase [35]. Also, purified liver phosphorylase binds AMP and glucose 6-phosphate with much lower affinity than do the muscle or brain isoenzymes, and the binding of these effectors has relatively little effect on the activity of the liver enzyme. From a functional viewpoint the poor allosteric control of liver phosphorylase is not unexpected, since this isoenzyme is mainly designed to respond to extracellular signals that are involved in the maintenance of the blood glucose level. These extracellular signals control hepatic glycogenolysis mainly by modulating the phosphorylation state of phosphorylase (see below).

While most extracellular signals control glycogen metabolism via transmembrane signalling pathways, glucose does so by directly binding to phosphorylase *a* [26]. Since the glucose concentration in the blood and in hepatocytes is the same, phosphorylase acts as a 'sensor' of the blood glucose level. The binding of glucose to the active site of phosphorylase not only inhibits phosphorylase *a* competitively, but also makes it more susceptible to inactivation by dephosphorylation. On the basis of the crystal structure of the complex between glucose and phosphorylase, glucose analogues have been designed that inhibit phosphorylase with much higher efficiency than does glucose [36,37]. Such analogues could become useful for the control of glycaemia in diabetes.

Debranching enzyme

The two enzymic activities involved in debranching, i.e. the α-1,6 $\rightarrow$ α-1,4 glucanotransferase and α-1,6-glucosidase activities (Scheme 1), are catalysed by different sites on a single polypeptide chain [38]. The enzyme is also capable of hydrolysing *in vitro* the glucose–tyrosine bond in glycogenin [9]. Human debranching enzyme (172 kDa) is encoded by a single gene from which six different mRNA species can be generated that differ at their 5′ end [39,40]. The liver expresses only isoform-1, while the muscle expresses isoforms 1–4. This could explain why some mutations, resulting in a loss of functional isoform-1, cause a complete deficiency of debranching enzyme in the liver only.

Phosphorylase kinase

Most structural information has been gathered from the enzyme purified from rabbit skeletal muscle. The latter is a hexadecamer composed of four different subunits ($\alpha_4\beta_4\gamma_4\delta_4$), with an overall mass of 1300 kDa [41]. Microscopic images suggest that the subunits are arranged in a bilobal 'butterfly' structure, where each lobe contains two $\alpha\beta\gamma\delta$ protomers [42]. The δ-subunit (17 kDa) is identical with calmodulin and confers on phosphorylase kinase activation by Ca^{2+}. Unlike most calmodulin-regulated enzymes, phosphorylase kinase retains its δ-subunit, even in the absence of Ca^{2+}. The catalytic centre resides on the γ-subunit (45 kDa), which contains a kinase domain and a C-terminal calmodulin-binding domain [43]. The α-subunit (138 kDa) and β-subunit (125 kDa) are very similar in their primary structure, except for sequences surrounding the phosphorylation sites [44,45]. The latter subunits are inhibitory, but this inhibition is alleviated by autophosphorylation and by phosphorylation with cAMP-dependent protein kinase [46]. Both the β- and the γ-subunits contain an inhibitory sequence that seems to act as a pseudosubstrate [46–48]. Activation of phosphorylase kinase by phosphorylation or by Ca^{2+} presumably results from the release of the pseudosubstrate domains in the β- and γ-subunits respectively.

The enzymic properties of liver phosphorylase kinase are ill-understood, mainly for lack of intact, non-proteolysed enzyme [49,50]. Generally the liver enzyme looks similar to the muscle enzyme with respect to its Ca^{2+}-dependency and its activation by (auto)phosphorylation. Yet, there are likely to be specific regulatory features, since there are liver-specific isoforms of the α-, β- and γ-subunits. Two genes on the X-chromosome encode the α-subunit, but only one of these is expressed in the liver, giving rise to several alternatively spliced isoforms of the α-subunit. About 75 % of all genetic deficiencies of liver phosphorylase kinase in man are sex-linked and caused by mutations in the α-subunit [45,51]. There is only one (autosomal) gene encoding the β-subunit of phosphorylase kinase, but in the liver several splice variants are expressed [44].

The liver contains the γ_{TL} isoform of the catalytic subunit, which is also particularly abundant in testes [52,53]. The autosomal deficiency of liver phosphorylase kinase in so-called *gsd* rats [35] and in some human glycogenoses has been explained by mutations in the γ_{TL} isoform [52–54]. In the *gsd* rat, the γ_{TL}-isoform mRNA level is normal, but there is no protein, suggesting that the deficiency is due to untranslatable mRNA or unstable protein [52].

Protein phosphatase-1G

The glycogen fraction contains only Ser/Thr protein phosphatases of type-1, termed PP-1G (glycogen-associated PP-1) (Table 1). They consist of a catalytic subunit (37/38 kDa) and a glycogen-binding G-subunit. Four structurally related mammalian G-subunits have been cloned, three of which are present in the liver, although not necessarily all in the parenchymal cells. The three hepatic G-subunits are much smaller than the muscle-type G_M/R_{GL} subunit, which can be explained by their lack of a domain for interaction with the endoplasmic reticulum [58–

Table 1 Mammalian glycogen-binding subunits of protein phosphatase-1

Designation	Mass (kDa)	Tissue distribution	Identity with G_L (%)	References
G_M; R3; R_{GL}	124	Striated muscle	23*	[55,56]
G_L; R4	33	Liver	–	[57,58]
R5; PTG	36	Striated muscle and liver	42	[59–61]
R6	33	Widespread	31	[62]

* The identity only refers to the overlapping N-terminal region of the G_M-subunit.

60,62]. Also, while the G_M-subunit is controlled by reversible phosphorylation, no evidence has been obtained for a similar regulation of the other G-subunits [58,59,61–63].

The G-subunits promote the dephosphorylation of glycogen-associated substrates in three different ways. First, they anchor protein phosphatase-1 to the glycogen particles that also bind the substrates glycogen synthase, phosphorylase and phosphorylase kinase [57,60,62,64]. The R5/PTG-subunit has also been shown to act as a molecular scaffold by directly binding the phosphatase as well as its substrates [60,61]. Secondly, the G-subunits alter the specific activity towards the substrates of glycogen metabolism [56,61,64,65]. Thirdly, the G-subunits decrease the sensitivity to inhibition by cytoplasmic regulators like inhibitor-1/DARPP-32 and inhibitor-2 [61,64].

The liver-specific PP-1G_L holoenzyme seems to be the major, if not the only, glycogen-associated synthase phosphatase in the liver. Indeed, phosphorylase *a* blocks all detectable glycogen-synthase phosphatase activity in a crude glycogen fraction, and the G_L-subunit is the only isoform that contains an inhibitory allosteric binding site for phosphorylase *a* [58]. Actually, the affinity of the G_L-subunit for phosphorylase *a* is about 1000-fold better than the K_m of PP-1G_L for phosphorylase *a* as a substrate, as well as the affinity of R5/PTG for phosphorylase *a*. An essential role for PP-1G_L in the activation of glycogen synthase is also suggested by a recent report showing that the deficient glycogen-associated synthase phosphatase activity in the liver of insulin-dependent diabetic or adrenalectomized starved rats is associated with a loss of G_L protein and mRNA [66]. Unexpectedly, the hepatic glycogen fraction from these same animals still contains about half of the normal phosphorylase phosphatase activity [66], suggesting that PP-1G_{R5}, PP-1G_{R6}, or a holoenzyme with a putative G-subunit of 160 kDa [67] also represent major phosphorylase phosphatases. The existence of different phosphatases acting on glycogen synthase and phosphorylase could also explain why phosphorylase *a* does not allosterically inhibit its own dephosphorylation.

While PP-1G clearly plays an essential role in the dephosphorylation of the substrates of glycogen metabolism, a role for other protein phosphatases cannot be excluded either. For example, it has been reported that the dephosphorylation of glycogen synthase by PP-1G is synergistically increased by an ill-characterized cytosolic species of PP-1 [64]. It should also be noted that the α-subunit of phosphorylase kinase can *in vitro* only be dephosphorylated by PP-2A (cf. Scheme 2).

Glucose 6-phosphatase

This enzymic system is located in the endoplasmic reticulum (reviewed in [68]). It comprises a hydrolase (35 kDa) the catalytic site of which faces the lumen of the endoplasmic reticulum, and translocases that mediate the transmembrane transport of the substrate glucose 6-phosphate and probably of the products glucose and P_i. The hydrolase and the glucose 6-phosphate transporter (46 kDa) have been cloned [68,69]; mutations in these polypeptides are responsible for the glycogen-storage diseases of type Ia and type Ib respectively. However, it has recently been admitted [70] that the identification of a microsomal glucose transporter, previously termed GLUT-7, should be considered as a cloning artefact. Except for an inhibition by unsaturated fatty acids and fatty acyl-CoA esters [68] and by phosphatidylinositides, especially phosphatidylinositol trisphosphate and bisphosphates [71], the acute regulation of glucose 6-phosphatase remains poorly understood.

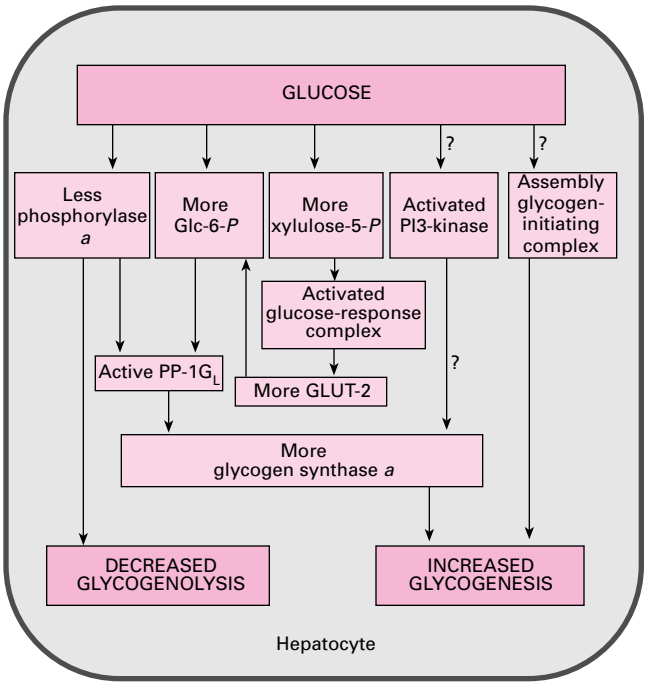
GLYCOGENIC AGENTS

The main postprandial glycogenic stimuli for the liver are glucose, insulin and parasympathetic (vagus) nerve impulses [72,73]. The relative contribution of these stimuli is still a matter of debate and is species-dependent. Gluconeogenic precursors such as fructose and amino acids also activate glycogen synthesis. Glucocorticoids, which provide long-term protection against stress, promote glycogen synthesis and thus prime the liver for acute glycogenolytic stress signals.

Glucose

The inactivation of phosphorylase precedes the activation of glycogen synthase

In the liver, phosphorylase *a* functions as a glucose receptor. The binding of glucose competitively inhibits the enzyme and induces conformational changes that make PSer[14] more accessible to protein phosphatases (reviewed in [26,64,74]). The resulting inactivation of phosphorylase causes the arrest of glycogenolysis and, at the same time, the removal of phosphorylase *a* relieves PP-1G_L from an allosteric inhibitor (Schemes 2 and 3). This



Scheme 3 Mechanisms of the glycogenic action of glucose

Abbreviation: Glc-6-*P*, glucose 6-phosphate. Questions marks indicate incompletely established pathways.

mechanism prevents the simultaneous synthesis and degradation of glycogen and explains why the glucose-induced activation of glycogen synthase *in vivo* and in isolated hepatocytes only occurs after a latency, which represents the time required to inactivate phosphorylase *a* below the inhibitory threshold level.

The glycogen-synthase phosphatase activity of purified PP-$1G_L$ is already completely inhibited by less than 50 nM phosphorylase *a*, whereas the threshold to phosphorylase *a* is 20–60 times higher *in vivo* and in isolated hepatocytes (reviewed in [64]). This indicates that the inhibitory potency of phosphorylase *a* is restrained *in vivo* and may be subject to regulation. AMP, a well-known ligand of phosphorylase, decreases the inhibitory effect of phosphorylase *a*, and we suggest that AMP is largely responsible for the lesser inhibitory potency of phosphorylase *a in vivo*. Another regulatory component is a glycogen-associated 'de-inhibiting' protein that is induced by glucocorticoids and abolishes the inhibition of PP-$1G_L$ by phosphorylase *a* (see below). On the other hand, some glycogen is needed for the allosteric inhibition to be effective, apparently because both PP-$1G_L$ and phosphorylase *a* need to be bound to glycogen. This explains why phosphorylase *a* and glycogen synthase *a* co-exist at fairly high levels in the liver of fasted rats [75]. It should also be borne in mind that the allosteric control by phosphorylase *a* occurs only in the liver and is there restricted to PP-$1G_L$. Glycogenic agents that act via a glycogen-synthase phosphatase other than PP-$1G_L$ could thus activate glycogen synthase independently of the concentration of phosphorylase *a*.

The activation of glycogen synthase requires glucose 6-phosphate

Some non-metabolizable glucose analogues are able to inactivate phosphorylase in isolated hepatocytes, but they do not activate glycogen synthase (reviewed in [76–78]). Yet an activation of glycogen synthase is obtained when glucose is added in addition [28,78]. While these data do not argue against the sequential mechanism described above, they do suggest that, in addition, a metabolite of glucose is required to activate glycogen synthase. This metabolite is probably glucose 6-phosphate, since an activation of glycogen synthase was also obtained with 2-deoxyglucose, which is not metabolized after phosphorylation [79]. Moreover, the glucose-induced activation of glycogen synthase is linearly correlated with the concentration of accumulating glucose 6-phosphate, while inhibitors of glucokinase inhibit the rise in glucose 6-phosphate and cause a corresponding inhibition in the activation of glycogen synthase [28,80]. Also, the overexpression of glucokinase in hepatocytes was reported to be associated with an increased glucose-induced activation of glycogen synthase [81]. Conversely, the activation state of glycogen synthase was also closely correlated with the level of glucose 6-phosphate when the latter was decreased by overexpression of the catalytic subunit of glucose 6-phosphatase [82]. Glucose 6-phosphate seems to act primarily via stimulation of the dephosphorylation of glycogen synthase, since its effect in hepatocytes was cancelled by microcystin-LR, an inhibitor of Ser/Thr protein phosphatases-1 and -2A, but was not affected by the inhibition of protein kinases with 5-iodotubercidin [28]. In further agreement with this interpretation, it was found that the glycogen-associated synthase phosphatase activity is virtually entirely glucose 6-phosphate-dependent at physiological ionic strength. Importantly, the glucose 6-phosphate-dependent synthase phosphatase activity of PP-$1G_L$ was completely inhibited by phosphorylase *a*. This implies that the inactivation of phosphorylase is also a prerequisite for the glucose 6-phosphate-mediated activation of glycogen synthase. It thus appears that the glucose-induced activation of glycogen synthase by PP-$1G_L$ represents a two-step mechanism; it requires both the removal of the allosteric inhibitor phosphorylase *a* and the generation of the activator glucose 6-phosphate (Scheme 3).

The above data cannot explain observations by Krause et al. [83] showing that the glucose-induced activation of glycogen synthase in isolated hepatocytes is partially blocked by wortmannin, an inhibitor of phosphatidylinositol 3-kinase (PI3-kinase) (Scheme 3). The latter kinase also mediates insulin signalling to glycogen synthase via an inactivation of GSK-3 (Scheme 4).

Xylulose 5-phosphate mediates glucose-induced transcription

In addition to its metabolic effects, glucose also enhances the expression of genes encoding for example GLUT-2 and the catalytic subunit of glucose 6-phosphatase [84–86]. The increased expression of glucose 6-phosphatase is seen as a feedback system aimed at limiting the size of the glucose 6-phosphate pool in response to sustained increases in glucose production [86]. The transcriptional effects of glucose appear to be mediated by xylulose 5-phosphate (Scheme 3), an intermediate of the pentose phosphate pathway, which activates the 'glucose-response complex' via an increased DNA-binding of the transcription factor SP1 through dephosphorylation by protein phosphatase-1 [87–89].

Does glucose promote the assembly of a glycogen-initiation complex?

An exciting recent development has been the demonstration that the subcellular localization of some glycogenic enzymes is controlled by glucose. Agius (see [90]) discovered that glucokinase was not cytoplasmic in hepatocytes incubated with a low glucose concentration (< 5 mM); however, the enzyme could be released by high concentrations (30 mM) of glucose or by less than 1 mM fructose. It is now clear that, in glucose-deprived hepatocytes, glucokinase and its regulatory protein are present in the nucleus and are retained there by a Mg^{2+}-dependent mechanism [90]. Glucose and, more generally, agents that weaken the binding between glucokinase and the regulatory protein, allow glucokinase to migrate to the cytosol [13,90–93], whereas the regulatory protein remains sequestered in the nucleus [92,93]. In the cytosol the free glucokinase is active, as shown, for example, by the excellent correlation between the amount of free glucokinase and the rate of glycogen synthesis from glucose in hepatocytes [94]. Seoane et al. [81] made the surprising observation that glucose 6-phosphate produced by the overexpression of glucokinase, but not by the muscle-type hexokinase, promotes the activation of glycogen synthase in hepatocytes. Since only glucokinase was translocated in response to glucose, it was proposed that the failure of hexokinase-derived glucose 6-phosphate to activate glycogen synthase was due to compartmentation of glucose 6-phosphate. This proposal agrees with other studies concluding that glucose 6-phosphate does not exist as a homogeneous pool in hepatocytes [95,96].

Upon expression in hepatocytes, muscle-type glycogenin-1 was present in both the nucleus and the cytoplasm, but its distribution did not change upon the addition of glucose [97]. The cytoplasmic pool of glycogenin seemed to be associated with the actin microfilaments, since its specific localization disappeared after disruption of the actin cytoskeleton or after introduction of a point mutation in a C-terminal heptapeptide with a consensus sequence for association with actin. Interestingly, immunofluorescence studies have recently shown that glycogen synthase also moves to the actin-rich cortex of hepatocytes upon

the addition of glucose [98]. The translocated enzyme was found to sediment at 10000 ***g***, which is compatible with an association of glycogen synthase with the cytoskeleton [99]. However, direct experimental evidence for such an association is currently lacking.

Collectively the above data suggest that the glucose-induced initiation of glycogen synthesis not only requires activation of enzymes, but also involves the translocation of several enzymes to the actin microfilaments near the cell cortex, where, indeed, the initial glycogen synthesis appears to take place [98]. It is tempting to speculate that the initiation of glycogen synthesis depends upon the glucose-induced assembly of an initiation complex consisting of glucokinase, glycogenin and glycogen synthase (Scheme 3). It remains to be seen whether the other enzymes involved in glycogen synthesis, i.e. PP-1G_L and branching enzyme, are also part of this putative glycogen-initiation complex.

The glycogenic action of glucose is potentiated by a 'portal' factor

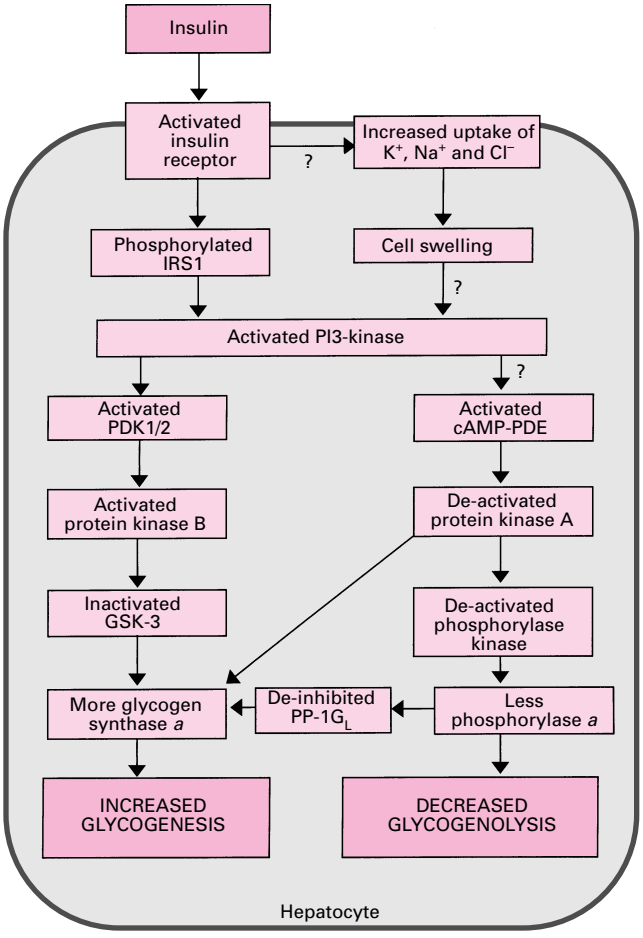
In perfused liver and isolated hepatocytes a considerable activation of glycogen synthase requires glucose concentrations above 20 mM [28]. This low sensitivity to glucose is not caused by an increased allosteric inhibition of PP-1G_L by phosphorylase *a*, since prior and complete inactivation of phosphorylase does not reduce the concentration of glucose that is required for activation of glycogen synthase. The lower *in vivo* threshold to glucose may in part be explained by the presence of insulin and glucocorticoids, which are both glycogenic and have also been shown to decrease the threshold to glucose in isolated hepatocytes [73,100]. In addition, it has been demonstrated that hepatic glycogenesis and activation of glycogen synthase is much greater after an oral or intraportal glucose load than after administration of the same amount of glucose by peripheral intravenous infusion, despite controlled insulin and glucagon levels [72]. This has led to the postulate that enteral or intraportal glucose delivery evokes a 'portal signal' that enhances glucose uptake by the liver. This portal signal probably involves the autonomous nervous system. It has indeed been shown that afferent fibres in the hepatic branch of the parasympathetic (vagus) nerve can detect the glucose concentration in the portal vein and that electrical stimulation of the efferent limb of the vagus nerve can activate liver glycogen synthase [72,101,102]. Also, acute vagotomy reduces net glycogen deposition in rats given an oral glucose load.

The direct versus the indirect pathway of glycogen synthesis

After a mixed meal, about 25% of the ingested glucose is converted into liver glycogen [103]. In overnight (16 h)-fasted human subjects, only about half of this glycogen was directly synthesized from glucose [104]. The other half was synthesized from glucose that had first been glycolytically degraded to lactate before being converted into glucose 6-phosphate via gluconeogenesis. The occurrence of both a direct and an indirect pathway of glycogen synthesis from glucose has been linked to the metabolic zonation in the liver acinus (reviewed in [103]). This zonation stems from differences in the concentration of oxygen and nutrients in the blood that reaches periportal and perivenous hepatocytes, resulting in a differential gene expression and metabolism. Periportal cells receive more oxygen and nutrients than the perivenous cells and have a more aerobic metabolism. After a meal, glucose is mainly taken up by perivenous cells, initially to synthesize glycogen and, when the glycogen stores become filled, glucose is degraded to lactate. Lactate arrives via the systemic circulation at the periportal cells, where it is converted by gluconeogenesis into glycogen. This zonation of glycogen synthesis is in accordance with the finding that the expression of glucokinase is mainly restricted to the perivenous cells, which limits the periportal cells to glycogen synthesis from gluconeogenic precursors.

Insulin (Scheme 4)

The binding of insulin to the α-subunit of its receptor activates the Tyr-protein kinase associated with the β-subunit, which then phosphorylates itself as well as exogenous proteins such as the insulin receptor substrate 1 (IRS1). Tyrosine phosphorylation creates in IRS1 recognition sites for binding of proteins with SH2 domains [105]. One of the latter proteins is PI3-kinase, which is thereby activated and converts PtdIns(4,5)P_2 into PtdIns(3,4,5)P_3. The latter binds to the pleckstrin homology domain of protein kinase B and recruits the kinase to the plasma membrane, where it is phosphorylated and activated by the Ser/Thr-protein kinases PDK1 and PDK2 [106,107]. One of the substrates of protein kinase B is GSK-3, which is inactivated by phosphorylation, resulting in a lesser phosphorylation of glycogen synthase [106]. The PI3-kinase also activates other signalling pathways, leading, for example, to activation of protein kinases p90^{rsk} and p70^{rsk}, but these kinases do not appear to be involved in the insulin-mediated control of glycogen synthesis in the liver [108,109].



Scheme 4 Some pathways by which insulin affects glycogen metabolism in the liver

Abbreviations: PDK, phosphoinositol-dependent protein kinase; PDE, phosphodiesterase. Questions marks indicate incompletely established pathways.

Insulin also stimulates $Na^+/K^+/2Cl^-$ co-transport, Na^+/H^+ exchange and the Na^+/K^+-ATPase in hepatocytes [110–112]. The resulting intracellular accumulation of Na^+, K^+ and Cl^- causes cell swelling, which induces an activation of glycogen synthase via a PI3-kinase-dependent mechanism (Scheme 4; [83]). Cell swelling is further promoted by the insulin-stimulated Na^+-dependent uptake of amino acids [112]. The PI3-kinase is also upstream of a mechanism that results in an activation of a cAMP phosphodiesterase (Scheme 4), leading to a lower concentration of the glycogenolytic agent cAMP [113]. The removal of phosphorylase *a* not only decreases the rate of glycogenolysis, but also attenuates the allosteric inhibition of PP-1G_L, thus contributing to an enhanced glycogen synthesis.

Other insulin effects that decrease glycogenolysis and promote glycogen synthesis in the liver are not yet understood at the molecular level [64,74,114,115]. These include an inhibition of adenylate cyclase, apparently mediated by protein kinase C, an inactivation of phosphorylase kinase that is independent of changes in the activity of protein kinase A, an increase of the phosphorylase phosphatase activity and an inhibition of α-adrenergic signalling. Insulin is also required for the expression of glucokinase [116] and of the G_L-subunit of PP-1G in the liver [66]. This explains why insulin-dependent diabetic animals lose their ability to synthesize hepatic glycogen [64,66].

Glycogenic amino acids

Na^+-co-transported amino acids such as glutamine, alanine, asparagine and proline promote hepatic glycogen synthesis (reviewed in [110,117,118]). They do so by activating glycogen synthase without affecting the level of phosphorylase *a*. The glycogenic action of these amino acids is mediated by the cell swelling that is a consequence of the intracellular accumulation of Na^+ and metabolites such as glutamate and aspartate. Accordingly, amino acids are no longer glycogenic when cell swelling is prevented by incubation in hyperosmotic media, and a hypoosmotic shock *per se* is sufficient to cause an activation of glycogen synthase.

The activation of glycogen synthase by cell swelling and amino acids is blocked by the inhibition of the PI3-kinase with wortmannin [83]. However, this inhibition only occurs after a delay, and is incomplete, suggesting the involvement of additional pathways in the activation of glycogen synthase. Also, glycogenic amino acids do not cause an inactivation of phosphorylase, which would have been expected from an activation of PI3-kinase (Scheme 4). Thus the activation of glycogen synthase by amino acids may be partially mediated by the direct stimulation of the glycogen synthase phosphatase activity by metabolites such as glutamate and aspartate [119]. Cl^- has been shown to inhibit the glycogen synthase phosphatase activity in liver fractions, and the extrusion of Cl^-, which is a compensatory response aiming at restoring the initial cell volume, is therefore also expected to promote the dephosphorylation of glycogen synthase. The observation that proline stimulates glycogen synthesis more than can be accounted for by the increased cell volume has been correlated with a rise in the concentration of glucose 6-phosphate, which is not seen with other glycogenic amino acids [120].

Fructose

This sugar activates glycogen synthase, and at supraphysiological concentrations (> 2 mM) also causes paradoxically an activation of phosphorylase [121–123]. Fructose is rapidly converted into fructose 1-phosphate, which activates glucokinase by its dissociation from the inhibitory polypeptide and its translocation to the cytoplasm [13]. The glycogenic action of fructose may be partially accounted for by an increase in the concentration of glucose 6-phosphate [124], an activator of PP-1G_L. However, this is at most a partial explanation, since fructose is also able to activate glycogen synthase in hepatocytes from insulin-dependent diabetic rats [125] that are deficient in PP-1G_L [64,66]. In hepatocytes from diabetic rats, fructose does not cause a larger accumulation of glucose 6-phosphate than that which is obtained with glucose alone, which in itself is no longer glycogenic [64,125]. Also at variance with a mediatory role of PP-1G_L are observations that, under some experimental conditions, fructose can activate glycogen synthase in the presence of phosphorylase *a* [121], an allosteric inhibitor of PP-1G_L [55]. Probably fructose or a metabolite promotes glycogen synthesis by stimulation of another glycogen-synthase phosphatase or by the inhibition of GSK(s).

The fructose-induced activation of phosphorylase has been explained by the accumulation of free Mg^{2+} [123], which stimulates non-phosphorylated phosphorylase kinase from rat liver [123,126]. On the other hand, fructose 1-phosphate is a competitive inhibitor of phosphorylase *a* and, furthermore, the accumulation of this metabolite causes a trapping of P_i [121], which limits the phosphorolysis of glycogen in the liver [127]. This may explain why the fructose-induced phosphorylase *a* is not operative *in vivo* [121].

Glucocorticoids

Administration of glucocorticoids *in vivo* causes an inactivation of phosphorylase and an activation of glycogen synthase [128,129]. These effects are maximal after 3–4 h and are dependent on protein synthesis. The glycogenic action of glucocorticoids is accounted for by a 1.5–2-fold increase in the phosphorylase phosphatase activity and by the synthesis of a glycogen-associated 'de-inhibiting' protein that abolishes the allosteric inhibition of PP-1G_L by phosphorylase *a*. Moreover, glucocorticoids have a role in the maintenance of the G_L-subunit, which is completely lost during starvation of adrenalectomized rats [64,66]. Glucocorticoids also initiate hepatic glycogen deposition during fetal development by inducing the synthesis of glycogen synthase and of the glycogen-associated synthase phosphatase activity [130], which is now attributed to PP-1G_L [66].

Other glycogenic compounds

It has been reported that 5-iodotubercidin, an inhibitor of adenosine kinase, activates glycogen synthase and inactivates phosphorylase when added to isolated hepatocytes [131]. Subsequent investigations have revealed that 5-iodotubercidin causes the dephosphorylation of many proteins, simply because it is an inhibitor of a broad range of Ser/Thr- as well as Tyr-protein kinases [132].

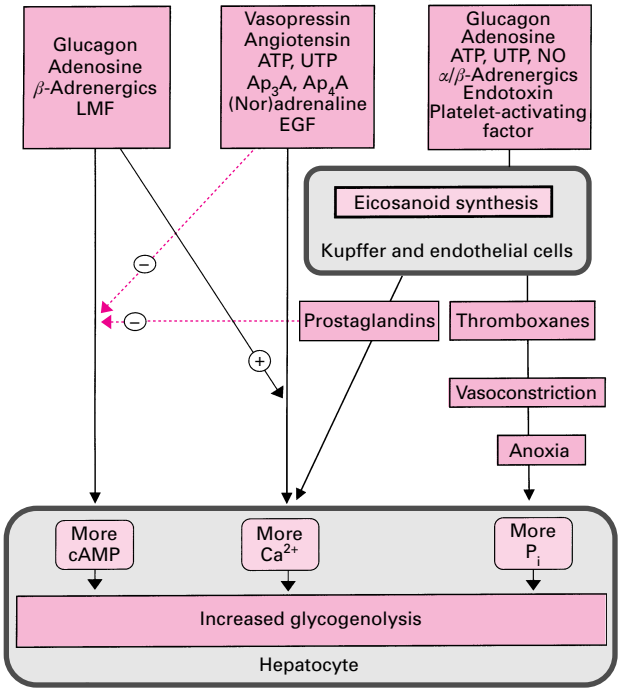
The phenacyl imidazolium compound proglycosin is a hypoglycaemic agent that causes the sequential inactivation of phosphorylase and activation of glycogen synthase [133]. This compound does not cause cell swelling and, if anything, decreases the concentration of glucose 6-phosphate. Van Schaftingen [134] presented evidence that the glycogenic action of proglycosin and derivatives such as resorcinol or phenol is mediated by glucuronidated metabolites that inhibit phosphorylase kinase. The ensuing removal of the allosteric inhibitor, phosphorylase *a*, would then enable PP-1G_L to activate glycogen synthase. However, it seems unlikely that this accounts fully for the glycogenic action of proglycosin, since the mere removal of phosphorylase *a* does not appear to be sufficient for the activation of glycogen

synthase (see above). Perhaps the glucuronidated metabolites also inhibit GSK(s), or they may stimulate a glycogen-synthase phosphatase.

GLYCOGENOLYTIC AGENTS

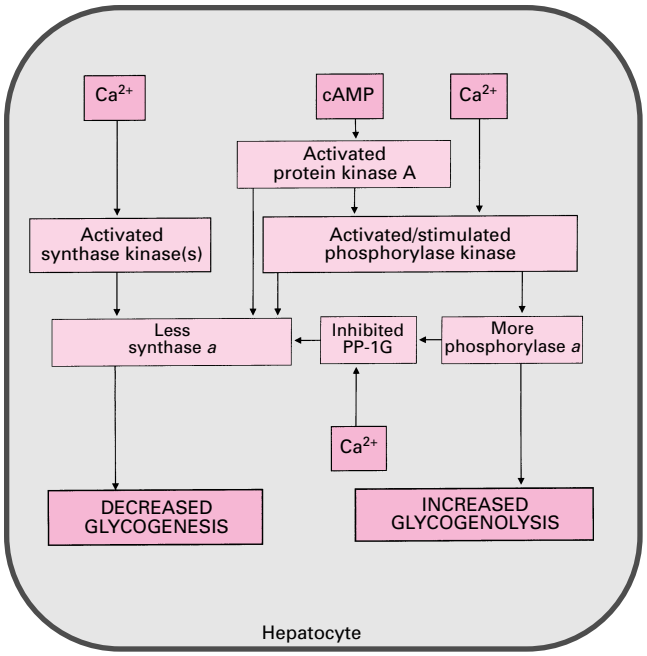
In the liver, glycogen can be degraded by a hydrolytic as well as a phosphorolytic pathway [135]. However, the hydrolysis of glycogen by α-glucosidase in the lysosomes is only a manifestation of autophagocytosis and is quantitatively unimportant in the overall process of glycogen mobilization. The regular phosphorolytic pathway of glycogenolysis is catalysed by phosphorylase *a* and results in the release of glucose 1-phosphate, which is in equilibrium with glucose 6-phosphate by the action of phosphoglucomutase. In the periportal zone, which is rich in glucose 6-phosphatase, glucose 6-phosphate is mainly converted into glucose when the circulating glucose concentrations are falling [103]. In the perivenous zone, on the other hand, glucose 6-phosphate is mainly converted into lactate in the post-absorptive phase.

The phosphorolysis of glycogen is induced by agents that (i) increase the concentration of phosphorylase *a* via pathways dependent on cAMP or Ca^{2+} and/or (ii) change the concentration of metabolites (in particular P_i) that affect the catalytic efficiency of phosphorylase (Scheme 5). The hepatic glycogenolysis during fasting or moderate exercise is mainly brought about by glucagon [136–138]. An additional level of control in these conditions is exerted by noradrenaline and ATP, which are released as neurotransmitters by the hepatic branch of the orthosympathetic (splanchnic) nerve [101,102]. During extreme stress situations or hard labour, the circulating concentration of adrenaline may increase sufficiently to stimulate hepatic glycogenolysis further [138,139]. Various glycogenolytic stimuli also instigate non-parenchymal liver cells to secrete eicosanoids (prostaglandins and thromboxanes), which themselves promote glycogenolysis in hepatocytes [140]. While numerous glycogenolytic agents have been described, we have limited the present overview to those with an established or probable physiological function.



Scheme 5 Mechanisms and interactions of glycogenolytic agents in the liver

Abbreviations: Ap_3A and AP_4A, diadenosine tri- and tetra-phosphate respectively; EGF, epidermal growth factor; LMF, lipid-mobilizing factor.



Scheme 6 Effects of cAMP and Ca^{2+} on hepatic glycogen metabolism

Glucagon

The glucagon receptor is coupled to adenylate cyclase via a stimulatory G_s-protein and also to a cAMP phosphodiesterase via an inhibitory G_i-protein [141,142]. The binding of glucagon will thus generate cAMP (Scheme 5), which initiates a signalling cascade leading consecutively to the activation of protein kinase A, phosphorylase kinase and phosphorylase (Scheme 6). Phosphorylase *a* not only increases the rate of glycogenolysis, but also antagonizes glycogen synthesis by inhibiting PP-$1G_L$. Furthermore, both protein kinase A and phosphorylase kinase phosphorylate and inactivate glycogen synthase. Glucagon also causes cell shrinkage [110], which does not seem to be involved in the glycogenolytic action [143]. Observations that glucagon causes the phosphorylation of glycogen synthase in hepatocytes on multiple sites, including those that are not directly phosphorylated by protein kinase A [23,24], are in agreement with effects of this hormone on both GSKs and phosphatases.

Glucagon (and other cAMP-mediated agonists) act synergistically with Ca^{2+}-mobilizing agents (Scheme 5). This synergism is explained by an increased affinity of α-adrenergic receptors for their agonists [144], by an increased sensitivity of intracellular Ca^{2+} stores to Ins(1,4,5)P_3 [145] and by an increased Ca^{2+} influx in response to limiting concentrations of the Ca^{2+}-mobilizing agents [146]. Actually, supraphysiological concentrations of glucagon *per se* are capable of promoting the influx of Ca^{2+} via Ca^{2+} channel(s) in the plasma membrane [74].

Prolonged exposure of hepatocytes to cAMP-mediated agonists results in a desensitization that is due to phosphorylation

of the receptors, reducing their affinity for the agonists [147]. Glucagon also decreases the expression of GLUT-2 and glucokinase [85,116].

Adrenaline

Adrenaline interacts with both α- and β-adrenergic receptors (Scheme 5). The relative contribution of these receptors depends on age, sex and species [74,137]. Binding to the β-adrenergic receptors results in a cAMP-mediated increase in glycogenolysis (Schemes 5 and 6).

The α-adrenergic receptor is coupled to phospholipase C via an activating G_h-protein [148], leading to the production of Ins(1,4,5)P_3 and diacylglycerol. Diacylglycerol activates protein kinase C, which seems to have an essential, but ill-understood, role in the glycogenolysis induced by α-adrenergic agonists [149]. Binding of InsP_3 to its receptor in the endoplasmic reticulum results in a rapid, but transient, Ca^{2+}-release from intracellular stores. A slower, but more sustained, Ca^{2+} influx occurs via channels in the plasma membrane that are controlled by the Ca^{2+} content of the intracellular stores [150] and/or by pertussis-toxin-sensitive G-proteins [151]. Additional data point to a role for the cytoskeleton in these Ca^{2+} fluxes [152,153]. Ca^{2+}-mobilizing agents stimulate phosphorylase kinase, and this results in an increase in the concentration of phosphorylase *a* (Scheme 6).

The inactivation of glycogen synthase by these agents is more difficult to understand, since it results at least in part from the phosphorylation of sites that are not substrates for known Ca^{2+}-dependent protein kinases [23,24]. This suggests that Ca^{2+}-mobilizing agents also activate other GSK(s) or inhibit glycogen-synthase phosphatase(s). It has indeed been reported that the glycogen-associated synthase phosphatase is inhibited by physiological concentrations of Ca^{2+} [154]. Also, it has been demonstrated that the inactivation of glycogen synthase by Ca^{2+}-mediated agonists is only seen after a short latency, corresponding to the time to activate phosphorylase [155], and that the Ca^{2+}-dependent control of glycogen synthase in liver extracts is mediated by phosphorylase kinase [156]. This has led to the proposal [156] that the inactivation of glycogen synthase is indirect and is mediated by the allosteric inhibition of PP-1G_L by phosphorylase *a* (Scheme 6).

Although Ca^{2+}-mobilizing agents act synergistically with cAMP-mediated agonists (see above), they also antagonize cAMP increases (Scheme 5), both by an inhibition of adenylate cyclase [157] and an activation of a cAMP phosphodiesterase [158].

Neural regulation

The sympathetic tone decreases during the postprandial hyperglycaemia and increases with falling glucose concentrations during starvation and in stress conditions [101,102]. Stimulation of the sympathetic (splanchnic) nerve causes the synaptic release of the Ca^{2+}-mediated agonists ATP and noradrenaline [101,102,159] (Scheme 5). ATP increases cytosolic Ca^{2+} following binding to different subtypes of P_2-receptors [160]. However, ATP differs from other Ca^{2+}-mediated agonists by its ability to activate phospholipase D, which catalyses the cleavage of phosphatidylcholine into choline and phosphatidic acid [161]. This could enhance hepatic glycogenolysis, since phosphatidic acid has been shown to stimulate the autophosphorylation and activation of (muscle) phosphorylase kinase *in vitro* [162].

By the interplay of various ectoenzymes (ATPases, apyrases, phosphodiesterase-I, 5′-nucleotidase) ATP is rapidly degraded to adenosine, which binds to the purinergic P_1-receptors, causing both a cAMP- and Ca^{2+}-mediated activation of phosphorylase (Scheme 5). Oetjen et al. [163] observed that, although adenosine and glucagon caused an equally complete activation of glycogen phosphorylase in hepatocytes, adenosine was much inferior in increasing the glucose production. Moreover, adenosine (and ATP) antagonize the stimulation of glycogenolysis induced by glucagon or cAMP [163,164]. These observations are explained by the uptake and avid phosphorylation of adenosine, which results in a substantial decrease in the cytosolic concentration of P_i, the co-substrate of phosphorylase [164].

Eicosanoids

Various glycogenolytic stimuli also promote the secretion of prostaglandins and thromboxanes from non-parenchymal Kupffer cells and endothelial cells, albeit to different extents [140,165] (Scheme 5). Prostaglandins activate phosphorylase in the hepatocytes via a Ca^{2+}-dependent mechanism and, like other Ca^{2+}-mediated agonists, also antagonize increases in cAMP [140]. The latter effect may contribute to the smaller glucagon-induced stimulation of glycogenolysis in perfused livers as compared with isolated hepatocytes [136,166]. Thromboxanes are strong vasoconstrictors, causing hypoxia, which leads to glycogenolysis at a rate higher than expected from the actual concentration of phosphorylase *a*. This has been linked to an increased catalytic efficiency of phosphorylase *a*, due to an increased concentration of the substrate, P_i [127,167]. Eicosanoids also mediate the increased glucose output from the liver in response to inflammatory agents such as endotoxins, platelet activating factor and anaphylatoxins [140].

Other glycogenolytic agents (see Scheme 5)

At circulating concentrations that probably only occur during a severe haemorrhagic shock, vasopressin and angiotensin II mobilize hepatic glycogen via a Ca^{2+}-dependent mechanism [74]. Vasopressin and angiotensin II also inhibit the accumulation of cAMP via stimulation of a cAMP phosphodiesterase [158], but at the same time they act also synergically with cAMP-mediated agonists [168]; it is difficult to assess the physiological relevance of the latter, antagonistic, events. Epidermal growth factor, an essential hepatic mitogen, is another hormone that causes glycogenolysis [169] and stimulates a cAMP phosphodiesterase [170] via a Ca^{2+}-dependent mechanism.

Nucleotides are not only secreted as neurotransmitters (see above), but they are also released from hepatocytes as a result of mechanical stimulation or cell lysis [171]. In addition, ATP, UTP and diadenosine polyphosphates are secreted by activated platelets [172]. The released nucleotides may induce a Ca^{2+}-dependent glycogenolysis in the surrounding hepatocytes [160]. The diadenosine polyphosphates are more stable than ATP and may therefore have longer-lasting effects [173]. In perfused livers, UTP is a more potent glycogenolytic agent than ATP, probably because UTP is a better stimulator of thromboxane secretion from non-parenchymal cells [174].

The signalling molecule NO, which can be released from endothelial cells, Kupffer cells as well as hepatocytes, has rather complex effects on hepatic glycogenolysis (for references see [175,176]). On the one hand, NO antagonizes some phosphorylase-activating hormones by its well-known vasodilation effect that counteracts hypoxia, and by hydrolysis of cAMP via activation of a cGMP-activated cAMP phosphodiesterase. On the other hand, NO stimulates basal glycogenolysis, and this effect appears to be mediated by eicosanoids released from non-parenchymal liver cells [175]. NO also

promotes the inactivation of glycogen synthase by inhibiting the dephosphorylation of the enzyme [176].

Cancer cachexia is associated with a progressive decrease in liver glycogen. This loss of glycogen is mediated by the 'lipid mobilizing factor' which promotes hepatic glycogenolysis via an increase in cAMP [177].

Rats that have been treated with tri-iodothyronine maintain a lower level of hepatic glycogen [178,179]. This may be due to an increased glycogenolysis and/or a decreased postprandial glycogen synthesis. Thyroid hormones affect the concentration or activity of various glycogen-metabolizing enzymes, and some of these effects are opposite to what might be expected from a glycogenolytic hormone. In addition, thyroid hormones may act indirectly by enhancing the cytosolic Ca^{2+} rise in response to, for example, α-adrenergic agents [180].

PERSPECTIVES

Research on glycogen metabolism has yielded a fair number of prime discoveries, such as the regulation of enzyme activity by reversible phosphorylation; the discovery of adenylate cyclase and of enzyme cascades; the concepts of second messengers and of lysosomal storage diseases. One may wonder, of course, whether new far-ranging discoveries should still be expected. However, even if they are not, there are still major problems awaiting a satisfactory explanation. Indeed, several proteins involved in hepatic glycogen metabolism remain to be identified (e.g. the glycogen 'backbone' protein and in general the assembly of α-particulate glycogen, the nature of the phosphorylase phosphatase activity, and of the glucocorticoid-induced protein that de-inhibits PP-$1G_L$). Also, the mechanisms by which fructose and cell-swelling agents cause activation of glycogen synthase remain incompletely solved. Furthermore, many aspects of the structure and regulation of the known hepatic enzymes (e.g. glycogenin, glycogen synthase, phosphorylase kinase and glucose-6-phosphatase) are still a mystery. Another essential issue that is only now beginning to be addressed is the compartmentation of enzymes and metabolites and the role of (transient) enzyme complexes involved in the metabolism of glycogen. Finally, the relative importance and the interaction of the various agents that contribute to glycogenolysis as well as glycogen synthesis under physiological conditions remain largely to be explored.

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