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REVIEW

Brain glycogen re-awakened

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Abstract

The mammalian brain contains glycogen, which is located predominantly in astrocytes, but its function is unclear. A principal role for brain glycogen as an energy reserve, analogous to its role in the periphery, had been universally dismissed based on its relatively low concentration, an assumption apparently reinforced by the limited duration that the brain can function in the absence of glucose. However, during insulin-induced hypoglycaemia, where brain glucose availability is limited, glycogen content falls first in areas with the highest metabolic rate, suggesting that glycogen provides fuel to support brain function during pathological hypoglycaemia. General anaesthesia results in elevated brain glycogen suggesting quiescent neurones allow glycogen accumulation, and as long ago as the 1950s it was shown that brain glycogen accumulates during sleep, is mobilized upon waking, and that sleep deprivation results in region-specific decreases in brain glycogen, implying a supportive functional role for brain glycogen in the conscious, awake brain. Interest in brain glycogen has recently been re-awakened by the first continuous *in vivo* measurements using NMR spectroscopy, by the general acceptance of metabolic coupling between glia and neurones involving intercellular transfer of energy substrate, and by studies supporting a prominent physiological role for brain glycogen as a provider of supplemental energy substrate during periods of increased tissue energy demand, when ambient normoglycaemic glucose is unable to meet immediate energy requirements.

Keywords: aglycaemia, glucose, glycogen, insulin, lactate.
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Glycogen, the main carbohydrate storage component in the human body, is a highly branched polysaccharide of D-glucose, which acts as a depot into which glucose, excess to immediate requirements, can be stored, and from which glucose can be rapidly released upon demand. Glycogen is an advantageous form in which to store glucose as it is trapped intracellularly, reduces the osmotic forces acting on the cell, can be rapidly metabolized, requires no ATP for initiation of metabolism, and unlike fatty acids can yield ATP under anaerobic conditions. The largest reserves of glycogen are found in the liver and skeletal muscle. There is about 100 g of glycogen in the well-fed liver and about 400 g of glycogen in resting skeletal muscle (Champe and Harvey 1994), thus glycogen accounts for 1–2% of the weight of skeletal muscle (30–100 μmol/g) and 6–8% of the weight of liver (100–500 μmol/g, Shulman *et al*. 1995). Liver glycogen is released into the systemic circulation as glucose in response to falling blood glucose levels, thereby maintaining normoglycaemia. Muscle glycogen however, is used as a localized energy source solely by skeletal muscle cells during muscular contraction.

Glycogen is also found in the brain with current estimates of human brain glycogen content between 0.5 and 1.5 g, about 0.1% of the total brain weight, thus it is no surprise that a role for brain glycogen has been neglected. It is the central dogma of brain energy metabolism that the brain is entirely dependent on glucose as an energy substrate as it has no energy reserves, and in the long term is at the mercy of the systemic circulation for a constant, uninterrupted delivery of glucose in excess of demand (Stryer 1995; Frier and Fisher 1999; Garrett and Grisham 1999). The selective permeability of the adult blood–brain barrier (BBB) limits a variety of potential metabolites present in the blood, e.g. lactate, as

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Abbreviations used: aCSF, artificial cerebrospinal fluid; BBB, blood–brain barrier; PAS, periodic acid–Schiff's base.

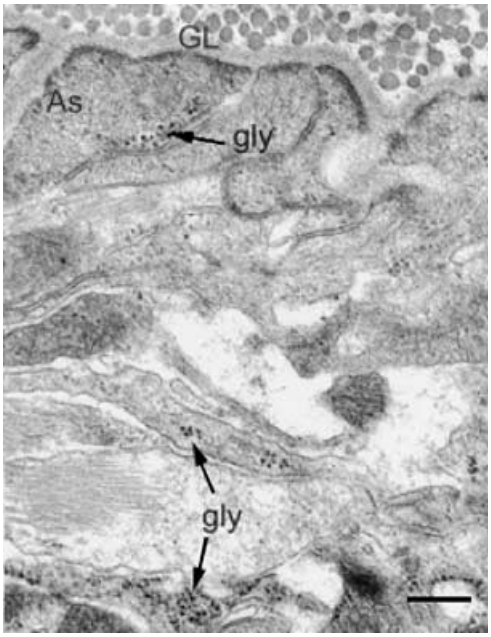

Fig. 1 Electron micrograph shows glycogen in astrocytes of rat optic nerve. Glycogen granules (gly) were 20–25 nm in diameter and located in the processes of the astrocytes (AS) at the glia limitans (GL). Scale bar 0.25 μm (from Wender *et al.* 2000).

energy sources from the brain. While there is no dispute that glucose is the main energy support of the brain it has become apparent that under *in vitro* conditions brain tissue can survive for extended periods on non-glucose substrates, including the monocarboxylates lactate, pyruvate, the sugars mannose and fructose, and the ketone bodies β-hydroxybutyrate and acetoacetate, implying that under *in vivo* conditions brain tissue could survive on glucose-derived substrates generated within the brain parenchyma. Given its astrocytic location glycogen is ideally positioned to provide energy substrate to neurones, and a prominent role for glycogen in supporting brain function is highly likely.

Location of CNS glycogen

Cellular

Glycogen is present in the central nervous system (CNS) although at much lower concentrations than liver or skeletal muscle, with the commonly accepted ratio of liver/skeletal muscle/brain 100 : 10 : 1 (Nelson *et al.* 1968). Glycogen molecules in the CNS are recognizable as electron dense particles between 10 and 30 nm in diameter; the glycogen molecule itself can be up to 10^8 Daltons in weight (Champe and Harvey 1994). Glycogen is expressed in both neuronal and glial cells, but expression varies with maturity. Glycogen is expressed in embryonic neural and glial tissue (Bloom and Fawcett 1968), but maturity coincides with decreased neuronal expression, with only certain neurones in the brain stem and ependymal and choroid plexus cells retaining glycogen (Magistretti *et al.* 1993). The majority of CNS glycogen is located in astrocytes (Cataldo and Broadwell 1986). Glycogen is excluded from the nucleus and is located primarily in the cytoplasm, although it is also present in lysozomes (Geddes *et al.* 1977). As astrocytes are expressed in all brain regions and outnumber neurones 10 : 1, this exclusive cellular localization does not dictate regional expression. The greatest accumulation of astrocytic glycogen has been reported in areas of high synaptic density (Koizumi and Shiraishi 1970a; Koizumi and Shiraishi 1970b; Phelps 1972), suggesting that astrocytic glycogen may be involved in neuronal activity (see later), although more recent data reports the highest glycogen levels in white matter (Kong *et al.* 2002). The cellular localization of the enzymes that synthesize (glycogen synthase EC 2.4.1.11) and degrade (glycogen phosphorylase EC 2.4.1.1) glycogen correlates with the presence of glycogen. The brain isozyme of glycogen phosphorylase is located predominantly in astrocytes (Ignacio *et al.* 1990; Pfeiffer-Guglielmi *et al.* 2003) and weakly in choroid plexus and ependymal cells (Magistretti *et al.* 1993). Surprisingly glycogen synthase, while present in astrocytes (Inoue *et al.* 1988; Pellegri *et al.* 1996), is also expressed in neurones (Inoue *et al.* 1988), suggesting an alternative role unrelated to glycogen metabolism.

Regional

The concentration of glycogen in the rat brain is reported as between 2 and 5 μmol/g of wet tissue (Lipton 1988; Choi *et al.* 1999), although higher values of up to 8–12 μmol/g of wet tissue have recently been reported (Cruz and Dienel 2002). Glycogen is not distributed uniformly throughout the brain but displays regional variability. Grey matter structures tend to have a higher glycogen content than white matter structures (Duffy *et al.* 1972; Koizumi 1974; Swanson *et al.* 1989a), although a single study has demonstrated higher glycogen content in white matter than grey matter structures (Kong *et al.* 2002). The grey matter structures with the highest glycogen content are, in descending order; pons/medulla, cerebellum, hippocampi, then hypothalamus, thalamus followed by mid brain, cerebral cortex and striatum, which all have similar concentrations (Duffy *et al.* 1972; Sagar *et al.* 1987). Early Russian studies however, found higher glycogen content in cortex than cerebellum (Palladin 1965). Large accumulations of glycogen have also been shown in ependymal fibres, and in the meninges of the base of the brain (Koizumi 1974). Glycogen content does vary with age, which must be taken into account when making regional comparisons (Chesler and Himwich 1943). Even within individual brain structures there is variation in glycogen content with dentate gyrus possessing double the glycogen content of the rest of the hippocampus (Lipton 1988). Likewise, in mouse cerebral cortex glycogen content is higher in the superficial layers than in the deeper layers

(Folbergrova *et al*. 1970), and in four areas of medulla oblongata glycogen levels vary significantly (Duffy *et al*. 1972). The reason for this variability in glycogen content is unclear, but may be related to astrocytic density or local metabolic requirements of the tissue.

Glycogen metabolism

The general pathways of glycogenesis and glycogenolysis are identical in all tissues, but the enzymes that are involved and their control are specifically tailored to the individual requirements of the specific tissue. Glycogen synthesis proceeds with conversion of glucose-6-phosphate to glucose-1-phosphate by phosphoglucomutase (EC 5.4.2.2). Uridine triphosphate (UTP) then combines with glucose-1-phosphate, a reaction catalyzed by UDP glucose pyrophosphorylase (EC 2.7.7.9), to form UDP-glucose with the release of two inorganic phosphate molecules. UDP glucose is the source of all glucosyl residues added to the glycogen molecule. The glucose molecules are attached via the hydroxyl group on a specific tyrosine side chain of the glycogenin molecule. Transfer of glucose to glycogenin is catalyzed by glycogen initiator synthase (EC 2.4.1.112), which enables the glucosyl residue to act as an acceptor for subsequent glucose molecules. Elongation of the chain is accomplished by formation of an α-1,4-glycosidic bond between carbon 1 of the free glucose molecule and carbon 4 of the accepting glucosyl residue, a process that is catalyzed by glycogen synthase, with UDP released and converted to UTP by nucleoside diphosphate kinase (EC 2.7.4.6), ready for recombination with a glucose molecule. If every glucose molecule was attached to the growing glycogen molecule in this way a linear molecule would be formed, and indeed such a compound, amylose, is found in plants, as well as similar glycan structures in Syrian hamster over-expressing the prion protein (Rudd *et al*. 1999). However, on average at about every eighth glucosyl unit, a branch occurs due to an α-1,6-glycosidic bond forming between the carbon 1 of the glucose molecule and carbon 6 of the accepting glucosyl residue. This branching has two advantages, increasing glycogen solubility, and vastly increases the number of non-reducing ends to which new glucose molecules can be added (note: this structural modification also permits simultaneous liberation of multiple glucosyl residues). The branches are formed by the action of the branching enzyme amylo-(α-1,4–α-1,6)-transglycosylase (EC 3.2.1.3), which transfers between 4 and 6 glucosyl units from the non-reducing end of the chain to the main glycogen molecule forming an α-1,6 glycosidic bond. Glycogen degradation (glycogenolysis) is not simply a reversal of glycogen synthesis but requires independent enzymes, thereby providing a greater degree of control of glycogen content. The product of glycogen breakdown is mainly glucose-1-phosphate, subsequently converted to glucose-6-phosphate by phosphoglucomutase, but glucose is also liberated, with the ratio of glucose-1-phosphate:glucose dependent upon the number of glucosyl units between branch points (see above). The fate of these compounds is tissue specific and dependent upon the presence of glucose-6-phosphatase. Glycogen phosphorylase cleaves the α-1,4 glycosidic bond between the glucosyl residues at the non-reducing ends of the glycogen chain to form glucose-1-phosphate. The action of glycogen phosphorylase proceeds until four glucosyl units remain before a branch point, where the reaction ceases leaving limit dextran, which can be degraded no further. The branches can be removed by the action of two enzymes, and although their presence in the brain has not been shown it is implied due to the complete depletion of brain glycogen during anoxia/ischaemia. The first, oligo-(α-1,4–α-1,4)-glucantransferase (glucosyl 4 : 4 transferase, EC 3.2.1.33) removes the outer three glucosyl residues at a branch point and attaches them to the non-reducing end of the molecule leaving a sole glucosyl residue at the branch point. The glucosyl residues are acted on by glycogen phosphorylase as mentioned above. The remaining glucosyl residue (attached to the main glycogen molecule by an α-1,6 bond) is removed by amylo-α-(1,6)-glucosidase (EC 3.2.1.33) liberating free glucose. The glucosyl chain can now be phosphorylated by glycogen phosphorylase liberating glucose-1-phosphate until four glucosyl units remain before a branch point (see above) and the process is repeated. The glycogen degradation product is ultimately glucose-6-phosphate (and glucose), whose metabolism depends on the energy status of the cell. Glycogen synthesis and degradation occur simultaneously, therefore the net glycogen content varies depending upon immediate energy needs. For a more detailed description of astrocyte glycogen metabolism see (Wiesinger *et al*. 1997), and standard biochemistry textbooks for general descriptions of glycogen (Clarke 1991; Champe and Harvey 1994; Stryer 1995). As glycogen regulation has been predominantly studied in liver and muscle, a brief description of these tissues, highlighting intertissue differences follows, concluding with what has been defined in the brain.

Liver

Glycogen is stored in the liver as a glucose reservoir to be released into the blood stream in response to falling blood glucose levels. In this way liver glycogen maintains euglycaemia to the benefit of the whole organism. On the first day of starvation liver glycogen is responsible for as much as 40–80% of blood glucose, but this decreases as liver stores become exhausted (Bollen *et al*. 1998). Glucose is transported into hepatocytes by the GLUT2 glucose transporter, which has a high K_m ensuring that the extra- and intracellular concentrations of glucose are in equilibrium, conferring on hepatocytes the capacity to sense blood glucose levels. Glucose is converted to glucose-6-phosphate by glucokinase, which has a K_m above the euglycaemic blood glucose levels,

and is not inhibited by the reaction product (glucose-6-phosphate, Ferrer *et al*. 2003). Hepatocytes also possess hexokinase, which has a much lower K_m than glucokinase. The interaction of glucokinase and hexokinase is such that at blood glucose levels lower than 5 mM, there is no significant glucokinase activity and the only source of glucose-6-phosphate is via hexokinase. However, the glucose-6-phosphate thus generated cannot be diverted towards glycogen formation as this glucose-6-phosphate cannot activate glycogen synthase, and only at elevated blood glucose levels (such as after a carbohydrate rich meal) is glucokinase activated and glucose-6-phosphate produced (Ferrer *et al*. 2003). Thus, in liver glycogen formation is not controlled by glucose transport but by glucokinase ensuring that glycogen is formed only when glucose is in excess of requirements (Bollen *et al*. 1998; Ferrer *et al*. 2003).

Regulation of liver glycogen

Glycogen synthase exists in three isoforms, muscle, liver and brain, with the liver isoenzyme specific for the liver, the muscle isoenzyme expressed in most other tissues, and the brain isoenzyme specific for the brain (Pellegri *et al*. 1996). Glycogen synthase can be phosphorylated to the inactive b form by a variety of protein kinases, including protein kinase A and phosphorylase kinase. Phosphorylation leads to a decrease in the V_{max} of the glycogen synthase and reduces glycogen formation (Ferrer *et al*. 2003). Glycogen synthase is activated by dephosphorylation by protein phosphatase 1, resulting in conversion to the active a form. Glucose-6-phosphate binds directly to inactivated glycogen synthase and converts it to an active form, and also allows phosphatases to dephosphorylate glycogen synthase and produce an active enzyme that no longer requires the presence of glucose-6-phosphate to maintain an active state.

Glycogen phosphorylase exists in three forms, muscle, liver and brain (Pfeiffer-Guglielmi *et al*. 2003). All forms are converted from the inactive form b to the phosphorylated active form a by phosphorylase kinase. Liver glycogen phosphorylase is more tightly controlled by phosphorylation than muscle (see later), allowing the liver to respond to extracellular changes in glucose, as glucose binds directly to the active site of phosphorylase a preventing glycogen breakdown (Bollen *et al*. 1998; Ferrer *et al*. 2003; references therein). This is a potential target for type 2 diabetes therapies, and small glucose related compounds including isofagomine, have been developed that inhibit glycogen breakdown and thus prevent release of hepatic glucose (Waagepetersen *et al*. 2000; Treadway *et al*. 2001).

Muscle

Glycogen is stored in skeletal muscle as a local energy reserve for exclusive use by skeletal muscle cells. Glucose is transported into the cell via the insulin-sensitive GLUT4 glucose transporter (Ferrer *et al*. 2003). On stimulation by insulin binding to surface insulin receptors the GLUT4 transporter is translocated from intracellular vesicles to the cell membrane, where it facilitates transmembrane glucose influx, thus glucose transport into muscle cells is dependent upon insulin (a key feature of type-1 diabetes is muscle wasting). Glucose is phosphorylated to glucose-6-phosphate by hexokinase, which has a lower K_m than liver glucokinase (Yeaman *et al*. 2001; Ferrer *et al*. 2003).

Regulation of muscle glycogen

Muscle differs from liver in that glycogen synthesis is controlled by both glucose transport and glycogen synthase, as shown by the elevated glycogen levels in transgenic animals overexpressing glycogen synthase, at the expense of UDP glucose levels (Ferrer *et al*. 2003). Muscle glycogen synthase is controlled by dephosphorylation, similar to liver. In both liver and muscle insulin stimulates glycogen synthase to promote glycogen synthesis, independent of its capacity to increase transmembrane glucose influx. The binding of insulin to target receptors leads to inactivation of glycogen synthase kinase 3 by phosphorylation by protein kinase b, shifting the balance towards dephosphorylation, and hence activation of glycogen synthase (Yeaman *et al*. 2001).

Glycogen phosphorylase in muscle is significantly different than in liver, as it is regulated by both allosteric binding and phosphorylation, whereas liver is controlled by phosphorylation alone. In muscle glycogen phosphorylase is activated by allosteric binding of AMP and inhibited by glucose-6-phosphate, enabling the skeletal muscle cells to respond to cellular energy deficits (Bollen *et al*. 1998).

Brain

The majority of glycogen in the brain is present in astrocytes, but unlike liver or muscle it is difficult to define a particular function for brain glycogen, although this situation is changing (see below). Astrocytes possess the GLUT1 transporter (Vannucci *et al*. 1997) but there is good evidence that they also possess insulin receptors and the insulin-sensitive GLUT4 transporter (Brown *et al*. 2002), leading to the intriguing concept that astrocytes take up glucose in both insulin-dependent and insulin-independent ways. This has been demonstrated in tissue culture where both hyperglycaemia (Magistretti *et al*. 1993) and insulin (Dringen and Hamprecht 1992) elevated glycogen content. The reasons for this are unclear, but it does suggest that brain glycogen may be adversely affected during type-1 diabetes.

Astrocytes express both muscle and brain isoenzymes of glycogen phosphorylase, but not the liver isoenzyme (Pfeiffer-Guglielmi *et al*. 2003). This suggests that in astrocytes glycogen phosphorylase is under allosteric control and is responsive to the energy state of the cell and can rapidly liberate glycogen derived metabolites during times of need, which appears to be borne out in functional experiments (Brown *et al*. 2003). It appears that the brain isoform requires

AMP for full activation, and that the affinity for glycogen is lower than the muscle isoform, suggesting that the brain isoform is sensitive to cellular AMP levels, an index of cellular energy levels (Pfeiffer-Guglielmi *et al*. 2003).

Regulation of brain glycogen

The cDNA for brain glycogen synthase has been isolated and shares 96% amino acid homology with the muscle isoenzyme, but a lower identity with the liver isozyme (Pellegri *et al*. 1996). It is widely distributed throughout the brain but has highest expression in the cerebellum, hippocampi and olfactory bulb (Pellegri *et al*. 1996). Interestingly it is located in both astrocytes and neurones, although the neuronal function is a mystery. The expression of glycogen synthase is up-regulated by vasoactive intestinal peptide (VIP) and noradrenaline, compounds which induce not only glycogenolysis but also delayed glycogen resynthesis (Sorg and Magistretti 1992; Pellegri *et al*. 1996). Glycogen synthase exists in two forms, the inactive phosphorylated and the active phosphorylated forms. Studies by Magistretti's group have demonstrated agonist-induced increases in glycogen are at least in part mediated by protein targeting to glycogen mRNA expression. Noradrenaline and VIP cause a cAMP dependent activation of transcription and synthesis of new proteins, specifically the protein to glycogen (PTG) protein (Allaman *et al*. 2000). Dephosphorylation of glycogen synthase is controlled by a family of phosphatases (Roach 2002), of which the PPP1R6, a glycogen targeting subunit of protein phosphatase 1, is found in many tissues including brain (Armstrong *et al*. 1997). PTG is abundantly expressed in astrocytes and acts to facilitate binding of protein phosphatase 1 to glycogen resulting in dephosphorylation and activation of glycogen synthase (Allaman *et al*. 2000). Adenosine also acts to elevate astrocyte glycogen via action on the A_{2B} adenosine receptor, via the same mechanism (Allaman *et al*. 2003).

Glycogen phosphorylase catalyses the breakdown of glycogen and exists in three isoforms; muscle, liver and brain (Ignacio *et al*. 1990). Both the muscle and brain isoforms are expressed throughout brain and spinal cord, and are expressed predominantly in astrocytes (Pfeiffer-Guglielmi *et al*. 2003), in agreement with the astrocytic location of glycogen (Cataldo and Broadwell 1986). The cDNA for brain glycogen phosphorylase shares 83% amino acid homology with the muscle isoenzyme, and 80% homology with the liver isoenzyme (Newgard *et al*. 1988). Glycogen phosphorylase exists in the phosphorylated active state and in the dephosphorylated inactive state. Phosphorylation is controlled by phosphorylase kinase, but brain glycogen phosphorylase is also regulated by allosteric binding of AMP and glucose similar to muscle, resulting in brain glycogen phosphorylase being sensitive to cellular energy needs.

Compounds that increase astrocytic glycogen levels include glucose, IGF I, IGF II, insulin, glutamate, and methionine sulfoximine. The excitatory neurotransmitter glutamate produces elevated glycogen levels (Hamai *et al*. 1999), but this action appears to be mediated by increased transmembrane glucose transport (Magistretti *et al*. 1999). Methionine sulfoximine (MSO) has also been shown to increase glycogen levels although its mechanism is more complicated. MSO inhibits glutamine synthetase (EC 6.3.1.2), which converts glutamate to glutamine; a reaction that consumes one molecule of ATP, thus the increase in glycogen may be a secondary effect caused by increased intracellular ATP (Swanson *et al*. 1989a).

Perhaps most relevant to the role of peripheral glycogen as an energy source is a putative role for insulin in regulating brain glycogen content. Insulin is present in the brain parenchyma, although at concentrations less than in plasma (Baskin *et al*. 1987). The source of CNS insulin is presumably peripheral as there are facilitated insulin transporters on the BBB (Banks *et al*. 1997), although there is evidence that it may also have a central origin (Unger *et al*. 1991). An insulin-dependent 'gain' of brain glucose was demonstrated almost 30 years ago (Daniel *et al*. 1975), suggesting that insulin does promote glycogenesis in the brain, although that study used supra-physiological concentrations of insulin, and also demonstrated an insulin-independent hyperglycaemia-induced glucose 'gain'. A later study by the same group demonstrated that insulin's effects on glycogen content were only achieved in hyperglycaemic conditions (Daniel *et al*. 1977). Studies carried out in culture have shown that insulin, at fairly high concentrations, does cause elevations in astrocytic glycogen levels (Dringen and Hamprecht 1992; Magistretti *et al*. 1993; Swanson and Choi 1993). In a separate study however, it was proposed that insulin's actions were mediated via activation of glycogen synthase, an effect that could be inhibited by wortmannin, a phosphatidyl inositol (PI) 3-kinase inhibitor (Hamai *et al*. 1999). Several studies have investigated the presence of insulin receptors in the brain and have demonstrated a primarily neuronal expression, but with uneven regional distribution (Wickelgren 1998; Zhao *et al*. 1999; Schulingkamp *et al*. 2000). The apparent absence of the insulin sensitive GLUT4 glucose transporter from astrocytes (El Messari *et al*. 1998; Apelt *et al*. 1999) suggests that the actions of insulin on astrocytes may be independent of increased transmembrane glucose transport (Hamai *et al*. 1999). However, recent studies using the advantageous preparation of transgenic mice that express green fluorescent protein (GFP) solely in glial fibrillary acidic protein (GFAP) expressing astrocytes (Zhuo *et al*. 1997), have demonstrated the presence of both insulin receptors and the GLUT4 transporter on hippocampal and corpus callosum astrocytes, but surprisingly not in optic nerve astrocytes (Brown *et al*. 2002). Thus insulin receptors display selective regional expression, although the significance of this is unclear. The insulin receptors were functional as application of exogenous insulin significantly increased glycogen in both hippocampus and corpus callosum, but not

in optic nerve. However whether insulin's actions were mediated by translocation of the GLUT4 transporter to the astrocyte membrane is unknown. Similarly, whether insulin activates glycogen synthase in astrocytes as occurs in hepatocytes awaits investigation.

Thus, while the regulation and function of astrocytic glycogen shares similarities with both muscle and liver, astrocytes more closely resemble muscle cells. Glucose transport into astrocytes is via insulin-insensitive GLUT1 glucose transporter, but evidence now suggests there is also an insulin-sensitive GLUT4 pathway (Brown *et al*. 2002). Intracellular glucose is phosphorylated to glucose-6-phosphate by hexokinase, as astrocytes do not possess glucokinase, present in the liver, and brain phosphorylase regulation by allosteric interactions resemble muscle cells. Astrocytes (which do not possess glucose-6-phosphatase) participate in an intercellular lactate shuttle within the same tissue, as seen in muscle (Brooks 2002), releasing glycogen-derived lactate for use by neighbouring cells.

Identification and measurement of glycogen

The presence of glycogen in tissue can be ascertained using both cytochemical techniques that visually identify the presence of glycogen, and quantitative techniques that estimate/measure absolute glycogen content. The most common staining method relies on the interaction of glycogen and osmium tetroxide, which acts as a contrast stain for glycogen. Glycogen in brain sections treated with osmium tetroxide and periodic acid–thiocarbohydrazide–silver protein appears as electron dense particles. These particles are between 10 and 40 nm in diameter, and serve to indicate the presence of glycogen (Koizumi 1974; Cataldo and Broadwell 1986; Peters *et al*. 1991; Wender *et al*. 2000), but do not yield quantitative information. An alternative staining method uses periodic acid–Schiff's base (PAS) after treatment with dimedone, an agent that blocks aldehyde groups on non-glycogen substances. A recent study employing this technique demonstrated that sleep deprivation depletes glycogen content (Kong *et al*. 2002).

Measurements of glycogen content have traditionally been carried out using biochemical assay on post mortem tissue. One extremely important aspect of glycogen measurement is that glycogen is degraded extremely rapidly, thus optimization of tissue extraction is vital (Palladin 1965). Prior to measurement, degradation must be halted by disabling glycogen metabolism. Exposing live brain tissue to a fatal high strength dose of focused microwave energy denatures enzymes responsible for glycogen metabolism preserving glycogen content at time of irradiation (Sagar *et al*. 1987). Conversely freezing the entire animal immediately by rapid immersion into liquid nitrogen (Passonneau and Lauderdale 1974) or Freon-12 (King *et al*. 1967; Nelson *et al*. 1968; Folbergrova *et al*. 1969), or rapidly removing brain tissue at 0°C followed by immediate freezing (Henry and Lowry 1985; Henriksson *et al*. 1986; Wender *et al*. 2000) halts glycogen metabolism, although it may permit some agonal glycogen metabolism. A similar technique, known as funnel freezing has been developed to very rapidly freeze brain tissue, and minimize glycogen breakdown (Cruz and Dienel 2002). The most accurate (Passonneau and Lauderdale 1974), and thus most popular assay technique for measuring

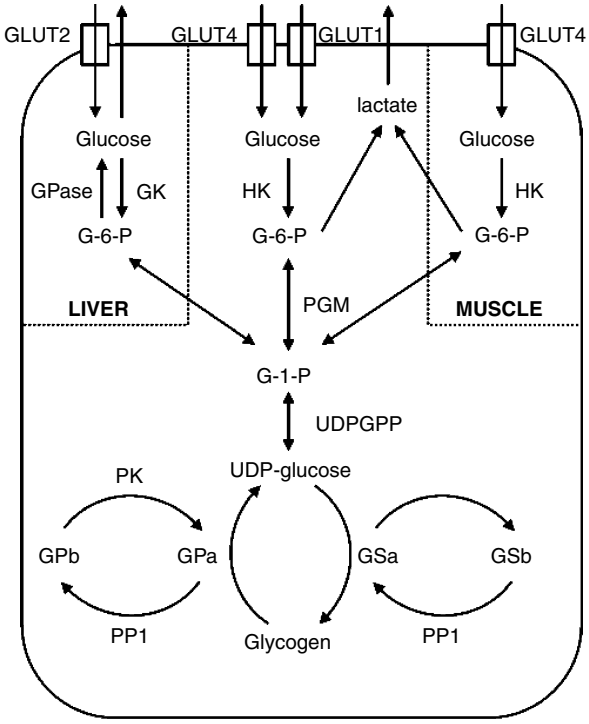

Fig. 2 Schematic illustration of glucose entry and glycogen formation in the brain, with additional aspects particular to liver and muscle enclosed within dotted lines. In liver glucose is transported intracellularly via the GLUT2 glucose transporter (GLUT2) and phosphorylated by glucokinase (GK) to glucose-6-phosphate (G-6-P), whereas in muscle glucose is transported via the insulin-sensitive GLUT4 (GLUT4) transporter, and phosphorylated by hexokinase (HK). A similar scheme applies to brain with the exception that glucose is also transported via the insulin-insensitive GLUT1 transporter (GLUT1). In all three tissues G-6-P is converted to glucose-1-phosphate (G-1-P) by phosphoglucomutase (PGM) and then to UDP glucose by UDP glucose pyrophosphate (UDPGPP). UDP glucose is converted to glycogen by glycogen synthase (GS), which exists in the active dephosphorylated form (GSa) or the inactive phosphorylated form (GSb). Protein phosphatase 1, targeted to glycogen by regulatory subunits such as protein targeting to glycogen (PTG), converts the inactive GSb to the active GSa promoting glycogen accumulation. Glycogen is broken down by glycogen phosphorylase (GP), which exists in the active phosphorylated form (GPa), or the inactive dephosphorylated form (GPb). Phosphorylase kinase dephosphorylates GPb to the active form. In muscle and brain the glycogen-derived product released is lactate, as these cells lack glucose-6-phosphatase (GPase). Liver cells however, do contain this enzyme and release glucose into the blood stream.

glycogen is to use debranching enzymes that metabolize glycogen to glucose followed by standard biochemical assay of glucose (Passonneau *et al*. 1967). Glycogen is usually expressed as glucosyl units, as the debranching enzymes yields one glucose molecule per glucosyl unit of the glycogen molecule. Recent improvements in this technique have been reported, the critical step apparently to minimize animal handling (∼ sensory stimulation) prior to assay. Studies using this technique have reported measurements of brain glycogen twice as high as those previously reported (Cruz and Dienel 2002).

NMR detection of ^{13}C glucose incorporation into the brain glycogen is a non-invasive technique that can estimate glycogen content *in vivo* in both laboratory animals and human volunteers. ^{13}C-labelled glucose is injected systemically and blood glucose levels monitored. The labelled glucose is slowly incorporated into the brain glycogen pool producing a detectable glycogen signal that can be continuously monitored (Choi *et al*. 1999). Mathematical manipulations using a glycogen standard estimate the glycogen concentration in the brain, values that are consistent with glycogen content measured by assay (Choi *et al*. 1999). This technology has been applied to demonstrate the presence of ^{13}C-glucose-labelled glycogen in human brain (Oz *et al*. 2003), as well as the sensitivity of the rat brain glycogen pool to systemic hypoglycaemia (Seaquist and Gruetter 2002; Choi *et al*. 2003). This non-invasive technology could be used in humans to record glycogen measurements under both pathological and physiological conditions, although such studies will obviously be limited to the few establishments with human head magnets.

The manner in which glycogen content is expressed can lead to confusion. Given the prevalence of the amyloglucosidase method glycogen is expressed as glucosyl units either relative to a normalized protein concentration (pmol glycogen/μg protein), or relative to the wet or dry weight of the tissue (μmol glycogen/g wet tissue). It is commonly assumed that wet brain tissue contains about 12% protein content (Sagar *et al*. 1987), and that dry brain tissue contains about half the protein of wet brain tissue, thus a simple calculation allows interconversion. Other literature refers to tissue glycogen content in mg glycogen/g tissue, which requires a conversion factor of 181 g/mol of free glucose, but 162 g/mol glucosyl units for glycogen due to the glycosidic bonds in glycogen.

Function of CNS glycogen

Pathological conditions

While the physiological function of brain glycogen has not been fully elucidated, the effects of various pathological events on glycogen have been investigated. These studies have been essentially reactive in nature and investigate the effects that various experimentally imposed paradigms have on glycogen content. As such they do not specifically define a role for glycogen, rather roles for glycogen are implied from the response to the insult.

Hypoglycaemia

While the primary use of insulin therapy is to treat type 1 diabetes, systemic insulin-induced hypoglycaemia was introduced in the 1930s as a therapy to alleviate the symptoms of schizophrenia (Rinkel and Himwich 1959), a practice now discontinued. Such clinical therapy prompted inquiries as to the effect of limiting glucose delivery on brain function and biochemistry. In the brain of adult dogs insulin-induced systemic hypoglycaemia resulted in a fall in glycogen content first in the areas of the brain with the highest metabolic rate, the depletion of the glycogen depending on the metabolic rate of the tissue rather that its initial concentration (Chesler and Himwich 1944). Thus, for the first time a link was established between glycogen metabolism and brain function, albeit under pathological conditions. Similar studies carried out in rabbit showed a fall of 35% of control levels after insulin-induced hypoglycaemic seizure (Goncharova 1957). More recent data using NMR spectroscopy to measure ^{13}C-glucose incorporation into brain glycogen have demonstrated similar results; insulin-induced hypoglycaemia depleted brain glycogen levels in anaesthetized rats, but only after the brain glucose concentration had fallen to zero (Choi *et al*. 2003). During insulin-induced hypoglycaemia, when the brain's demand for energy exceeds blood-borne glucose supply glycogen is degraded to support brain function. However, once glycogen content is depleted during constant hypoglycaemia, brain function fails and may suffer irreversible injury (Auer 1986; Frier and Fisher 1999).

The above studies have shown an indirect link between glucose availability, brain glycogen content and brain function, but demonstration of a clear link between brain glycogen and function was lacking. An initial clue emerged from tissue culture studies in which hypothalamic neurones were cultured in the presence or absence of glial cells. Neurones grown in glia confluent conditions survived for longer than neurones grown in the absence of glial cells (Whatley *et al*. 1981). The conclusion drawn was that neuronal survival in glia-confluent conditions was due to a metabolic coupling of unknown mechanism between the cell types. A subsequent study in cultured cortical neurones determined it was the glycogen content of astrocytes that was the decisive factor in neuronal survival. In a confluent mixed neuronal glial co-culture depleting astrocytic glycogen content reduced neuronal survival illustrating that it was not the number of astrocytes in culture, rather the glycogen content of the astrocytes, that promoted neuronal survival (Swanson and Choi 1993). This was effectively the first

study in which glycogen content was manipulated to determine the effect on neuronal function.

In more intact preparations further indirect evidence was accumulating to link glycogen content with brain function. Stimulus evoked EPSPs from the CA1 region of hippocampal slices were sustained in artificial cerebrospinal fluid (aCSF) containing 10 mM glucose. Introduction of glucose free aCSF resulted in a delayed decrease in the slope of stimulus evoked EPSPs. The limited duration of sustainable function in the absence of exogenously applied glucose (it is important to note that under *in vivo* conditions glucose can still be transported across the BBB, whereas under *in vitro* aglycaemia glucose phosphorylation ceases) may be explained by degradation of the limited glycogen stores to support function. Increased credence is given to this assumption by experimental manoeuvres that would deplete glycogen resulting in accelerated EPSP decrease during subsequent periods of glucose withdrawal (Izumi *et al*. 1997). In organotypic hippocampal slice cultures removal of exogenous glucose for one hour did not significantly affect stimulus evoked population spikes (Cater *et al*. 2001), whereas in acutely isolated hippocampal slices reduction of glucose to 2 mM greatly attenuated the population spike amplitude (Cox and Bachelard 1982). However, the organotypic slices were incubated in 30 mM glucose for 14 days prior to experimental procedure, which would be expected to greatly increase glycogen content, and explain the prolonged maintenance of function in the absence of exogenously applied glucose (Cater *et al*. 2001).

In the rodent optic nerve the relationship between glycogen content and stimulus-evoked function has been studied in detail, finally demonstrating a link between brain glycogen content and function. On removal of exogenously applied glucose function is sustained for 20 min in mouse optic nerve (Brown *et al*. 2003), and for 30 min in the rat optic nerve (RON, Wender *et al*. 2000). This is in contradiction to the widely held belief that brain function can only survive for a few minutes on glycogen reserves in the absence of glucose (Siesjö 1978; Clarke and Sokoloff 1999). However, the experimental conditions were designed to place minimal metabolic demand on the tissue, with function defined as the area of the compound action potential (CAP) evoked every 30 s. Increasing the firing rate resulted in accelerated CAP failure (Brown *et al*. 2003) reinforcing earlier findings that the metabolic rate of the tissue determines the rate of glycogen breakdown (Chesler and Himwich 1944). Up-regulation of glycogen content resulted in delayed latency to CAP failure, whereas down-regulation of glycogen content accelerated CAP failure (Wender *et al*. 2000; Brown *et al*. 2003). Measurement of glycogen content prior to aglycaemia, and latency to functional failure during subsequent aglycaemia, have demonstrated a linear correlation, indicating that it is the glycogen content present in the tissue at the onset of aglycaemia that determines the duration of function (Brown *et al*. 2003), a relationship that may have profound implications for the type-1 diabetic condition (see later), i.e. excess glycogen is neuroprotective during hypoglycaemia. The ability of glycogen to support brain function during periods of aglycaemia has been established, but spontaneous hypoglycaemia is a rare occurrence and iatrogenic hypoglycaemia is a result of insulin therapy: it is unlikely that brain glycogen's principal role is to act as an energy reserve during such pathological conditions.

Ischaemia

Experimentally induced ischaemia (combined aglycaemia and anoxia, designed to mimic the effects of stroke, where blood supply to the brain is interrupted by a thrombus) also results in decreased glycogen levels. In addition to the reduction in ambient glucose there is also a decrease in ambient oxygen. During aglycaemia where glucose is absent, other available energy reserves (e.g. lactate, pyruvate) can be metabolized aerobically, whereas during ischaemia aerobic metabolism cannot occur. It is widely accepted that the mammalian CNS cannot tolerate anoxic conditions even for short periods of time (Hansen 1985), although a recent study has surprisingly shown central white matter to be resistant to anoxia (Baltan Tekkök *et al*. 2003), thus the energy yield from anaerobically metabolized glycogen is insufficient to support function. The rapid decrease in glycogen seen in hippocampus argues that the decrease is mediated by activation of glycogenolysis rather than inhibition of glycogen synthesis (Lipton 1988). Qualitatively similar results were shown in single cells from spinal cord exposed to ischaemia (Passonneau and Lowry 1971). Thus, under conditions of combined hypoglycaemia and anoxia there is compelling evidence to support the hypothesis that glycogen is broken down in order to yield glycolytic energy in the face of the decreased exogenous energy supply.

Other miscellaneous pathologies

Glycogen storage diseases comprise several inherited diseases that are the result of malfunction of the enzymes that regulate glycogen metabolism (Wolfsdorf and Weinstein 2003). The majority of information on these diseases has been on the effects on liver and muscle glycogen, with the effects on brain glycogen unknown. Recent reports have shown that brain glycogen is increased, but whether these effects are related to the morbidity/mortality associated with the diseases in unknown (Salvan *et al*. 1997; Martini *et al*. 2001; Shotelersuk *et al*. 2002). All glycogen storage diseases with the exception of type 0 result in augmentation of glycogen content, suggesting that the absence of peripheral glycogen is fatal (Roach 2002). Whether this applies to the brain is an unresolved but fascinating issue.

Glycogen levels are also affected by other pathological events including seizures. In two strains of genetically susceptible mice administration of methionine sulfoximine

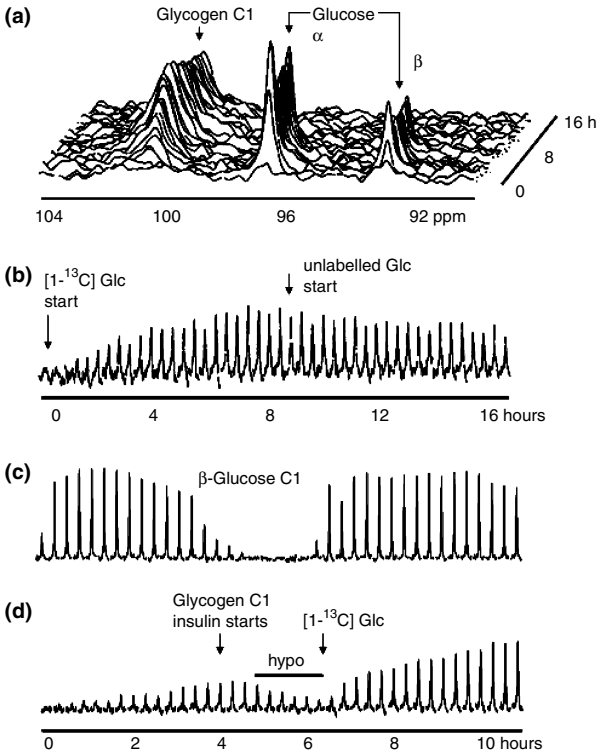

Fig. 3 Incorporation of ^{13}C-labelled glucose into brain glycogen in anaesthetized rats. (a) A stack plot of ^{13}C spectra recorded over a 16-h period. [1-^{13}C]Glucose infusion was started at 0 h and switched to unlabelled glucose at 8.5 h. The net accumulation of glycogen label, along with the detection of labelled glucose is illustrated. (b) A horizontal stack plot of glycogen C1 resonance at 100.5 p.p.m. measured over time, taken from the same experiments as in A (from Choi *et al.* 1999). Insulin infusion results in decreased brain glucose signal (hypoglycaemia) in anaesthetized rats, which was restored upon re-infusion of [1-^{13}C] glucose (c). (d) Insulin infusion also resulted in a decreased glycogen signal. However the glycogen was depleted only when the glucose signal was zero, and glycogen content rebounded above prehypoglycaemia levels upon re-infusion of [1-^{13}C]glucose (from Choi *et al.* 2003).

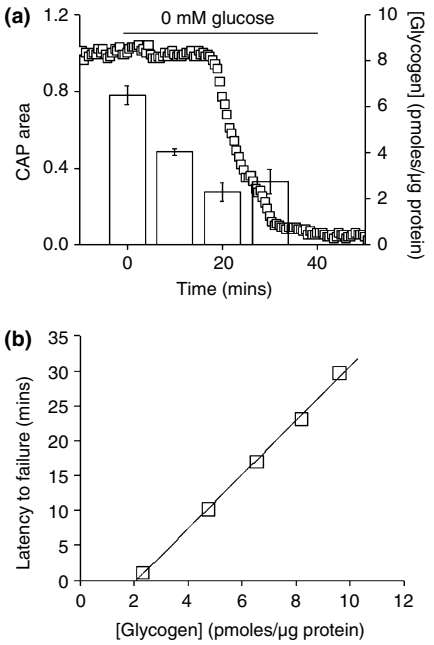

Fig. 4 Glycogen content determines latency to functional failure during aglycaemia. (a) In mouse optic nerve (MON) aglycaemia resulted in delayed functional failure, recorded as the stimulus evoked compound action potential (CAP), and with continued glucose deprivation the CAP fell rapidly to zero. On exposure to aglycaemia glycogen content fell until it reached its nadir at 20 min, coinciding with CAP failure. The left axis indicates CAP area (open squares) and the right axis indicates the glycogen content on the MONs (open columns). Error bars indicate the SEM. (b) The straight line is a best fit of data points (open squares), and indicates the relationship between glycogen content immediately prior to aglycaemia and latency to onset after aglycaemia onset (from Brown *et al.* 2003).

resulted in increased glycogen and delayed seizure onset in one strain, whereas in the other stain there was no effect on glycogen content and seizure onset was more rapid (Bernard-Helary *et al.* 2000). This suggests that increases in glycogen content may delay seizure onset, suggesting that brain glycogen may have anticonvulsant properties. However Lafora's disease is a progressive myoclonic epilepsy, resulting from defects in the EPM2A gene (Wang *et al.* 2002); a key characteristic of this disease is accumulation of polyglucosan (glycogen) bodies in the brain, where the Lafora bodies are localized to neurones (Loiseau *et al.* 1993).

Although type-1 diabetes results in low levels of hepatic glycogen, the effect of type-1 diabetes on brain glycogen is unknown. If human astrocytes display a similar pattern of insulin receptor expression as do mouse astrocytes (Brown *et al.* 2002), a role for brain glycogen in type-1 diabetes morbidity may be proposed, as the implied regulation of brain glycogen by both ambient glycaemic level and insulin suggests that in type-1 diabetes the prevalent hyperglycaemia coupled with the bolus injections of insulin may result in variable glycogen expression. This area awaits further investigation, but it is well known that type-1 diabetics who experience a single hypoglycaemic episode are more susceptible to succeeding periods of hypoglycaemia (Frier and Fisher 1999). The mechanism is unknown but in addition to the sympathetic nervous system and noradrenaline (Amiel 2001), glycogen may also be involved (Choi *et al.* 2003).

Physiological conditions

Sleep

The effects of sleep, sleep deprivation and wakefulness provides clues to glycogen's role during physiological activity. The first evidence that glycogen was affected by

sleep emerged from Russia in the 1940s and 1950s. The essential findings may be readily related as brain glycogen accumulation during sleep, depletion by sleep deprivation and mobilization on waking (Palladin 1965). It was found that sleep did not affect all brain areas uniformly, with hippocampi and caudal brain stem more affected that frontal or occipital cortex. Interestingly, the glycogen content accumulated very slowly during resumption of sleep after the period of sleep deprivation, until after 9 h it had returned to baseline levels, suggesting that glycogen may somehow be involved in the mechanism which starts, maintains or regulates sleep (Karadzic and Mrsulja 1969). The ability of glycogen to accumulate during sleep was also demonstrated by Karnovsky *et al*. (1983). In experiments carried out on rat brain, glycogen increased by 70% above waking levels within a few minutes of induction of slow wave sleep, again suggesting a role for brain glycogen during sleep. It has been hypothesized that 'sleep need involves depletion of brain glycogen' (Benington and Heller 1995). A number of neurotransmitters that stimulate glycogen phosphorylase including nor-adrenaline, serotonin and 5-HT are not only maximally released during waking, but are also released tonically during the waking state, presumably glycogenolysis is constantly stimulated resulting in a decrease in glycogen content. Thus, it is hypothesized that sleep has evolved as a state in which behaviour is suppressed and glycogen is replenished, and that glycogen depletion slowly occurs during waking and is slowly replenished during sleep. An increase in extracellular adenosine is seen as a key factor in glycogen-depletion mediated promotion of sleep drive. The decrease in intracellular ATP that accompanies depletion of astrocytic glycogen results in an increase in extracellular adenosine. This elevated adenosine acts via adenosine receptors to tonically hyperpolarize neurones and can account for many of the phenomena of sleepiness (Benington and Heller 1995; references therein). Complementary data in rats showed that brain glycogen decreased about 40% in rats deprived of sleep for 12–24 h and that 15 h of recovery following 12 h of sleep deprivation fully replenished glycogen stores (Kong *et al*. 2002). However, a more rapid accumulation of glycogen upon sleep onset has also been reported (Karnovsky *et al*. 1983). The expression of glycogen synthase and glycogen phosphorylase vary with the sleep wake cycle, with the maximal activity occurring in the middle of the light period and the minimal activity occurring in the middle of the dark period. Following sleep deprivation, a twofold increase in PTG mRNA, and a decrease of mRNAs encoding glycogen synthase and glycogen phosphorylase were observed, the functional consequences of which were a two point five fold increase in glycogen synthase activity (Petit *et al*. 2002).

In contradiction to prior work is a recent study showing that sleep deprivation does not affect glycogen content of the cortex, but depletes glycogen in the cerebellum, a brain area more associated with movement than sleep (Gip *et al*. 2002). More recent data suggest that the effect of sleep deprivation on glycogen content varies according to the strain of mouse, with glycogen content varying not only between different stains, but also between different brain areas, suggesting that glycogen content *per se* is an unlikely end point of sleep's functional role (Franken *et al*. 2003).

General anaesthesia

The effects of general anaesthetics on brain glycogen were first investigated in the 1950s, where it was found that administration of general anaesthetics to animals resulted in increased levels of glycogen in the brain (Nelson *et al*. 1968). In rats and mice, anaesthesia for 6 h resulted in a doubling or tripling of brain glycogen content (Hutchins and Rogers 1970). The degree of brain glycogen increase was related to both depth and duration of anaesthesia, and type of anaesthesia used, with pentobarbital producing greater increases in glycogen content than ether. In rabbits ethyl ether or halothane anaesthesia significantly increased glycogen in both grey and white matter (Rosengarten 1970). Anaesthetics are known to increase the ratio of brain to plasma glucose, and indeed increased plasma glucose in the absence of anaesthesia did increase glycogen levels, but the increase was only one-third to one-half as great as with pentobarbital, suggesting that anaesthesia, rather then elevated brain glucose causes the increased glycogen (Nelson *et al*. 1968). In the mouse, anaesthesia resulted in increases in the glycogen content of cerebral cortex and subjacent white matter, although the degree of increase was region specific (Folbergrova *et al*. 1970). In an electron microscopic study sodium pentobarbital resulted in increases in the number of glycogen granules in the hippocampi and cortex, with smaller increases in the molecular layer of the cerebellum. Most interestingly the response appeared to be greatest in areas of high synaptic density (Phelps 1972), suggesting a connection between the ability of anaesthetics to reduce neuronal activity reflected in decreased EEG responses, and the increase in glycogen. However, it should be borne in mind that glycogen assays may have been biased by rapid postmortem glycogenolysis, and that anaesthesia results in reduced brain glucose utilization, which in turn may spare glycogen content. Further evidence that the effect of anaesthetics on brain glycogen was related to neuronal activity came from tissue culture studies where anaesthetics, when applied to astrocyte cultures, did not affect glycogen content (Swanson *et al*. 1989b). The ability of anaesthetics to only increase glycogen when applied *in vivo* suggests that in the conscious, awake state neuronal activity leads to glycogen mobilization, supposedly to satisfy enhanced neuronal energy needs via transfer of substrates such as lactate, but when rendered quiescent reduce their utilization allowing glycogen to accumulate.

Transport of glycogen-derived metabolites

As previously stated glycogen is located predominantly in astrocytes, but its function appears to be to provide energy substrate for neural elements (Swanson and Choi 1993; Wender *et al*. 2000; Brown *et al*. 2003). Glycogen itself is too big to be transferred intercellularly, thus glycogen must be metabolized before any transfer can occur, and it is widely agreed that the ultimate breakdown product of astrocytic glycogen is lactate (Brown *et al*. 2003; Dringen *et al*. 1993; Wender *et al*. 2000). Astrocytes do not release glucose as glucose-6-phosphatase activity is very low (Dringen *et al*. 1993; Magistretti *et al*. 1993; Gotoh *et al*. 2000). In grey matter the transport of lactate from astrocytes to neurones has been tied in with astrocytic uptake of glutamate (Magistretti *et al*. 1999), and has recently been modified to incorporate glycogen (Shulman *et al*. 2001), as glycogen-derived glucose-6-phosphate is indistinguishable from glucose-derived glucose-6-phosphate. It is perhaps expedient at this point to briefly describe the background to the development of the concept of intercellular astrocyte-neurone metabolic coupling to place the role of glycogen in context. As long ago as the 19th century it was realized that the anatomical arrangement of astrocytes, capillaries and neurones suggested that astrocytes were involved in the transfer of metabolic substrate from capillary to neurone (Andriezen 1893), as astrocytic endfeet are in intimate contact with capillaries along almost their entire surface, and astrocytes entirely ensheath synapses. The endothelial cells, as well as the astrocytic endfeet, possess the GLUT1 glucose transporter (Morgello *et al*. 1995; Vannucci *et al*. 1997), which facilitates transfer of glucose from capillaries into astrocytes. Assuming all glucose is metabolized glycolytically, the net product of glucose metabolism is two molecules of ATP and two molecules of pyruvate. However astrocytes possess the isoform of the lactate dehydrogenase enzyme (LDH5) that preferentially converts pyruvate to lactate. Lactate dehydrogenase (LDH) is composed of various combinations of two subunits, H (or LDH1) and M (or LDH5). Neurones stain exclusively with anti-H (LDH1) antibodies, the subunit combination that preferentially oxidizes lactate to pyruvate (Bittar *et al*. 1996). Tetramers composed of the M subunit predominantly reduce pyruvate to lactate and astrocytes, which have less active oxidative enzymes (Friede 1962), stain for both M and H antibodies. Thus astrocytes, expressing at least some LDH5, can readily convert pyruvate to lactate, and neurones, expressing LDH1, are specialized to oxidize lactate to pyruvate. Monocarboxylate transporters (MCTs) shuttle lactate across cell membranes using proton symport (Poole and Halestrap 1993). There are several isoforms of MCT: MCT1 is expressed in tissue that exports lactate, and MCT2 is expressed in tissue that consumes lactate (Jackson and Halestrap 1996; Bröer *et al*. 1997). MCT1 is expressed in astrocytes and is thus ideally situated to transport lactate out of astrocytes (Morgello *et al*. 1995; Vannucci *et al*. 1997; Koehler-Stec *et al*. 1998; Halestrap and Price 1999; Pierre *et al*. 2000), whereas neurones express MCT2 and are ideally suited for lactate uptake at low substrate concentrations (Bergersen *et al*. 2001; Halestrap and Price 1999; Pierre *et al*. 2000; Pierre *et al*. 2002; Debernardi *et al*. 2003).

There is no doubt that both grey and white matter CNS structures under *in vitro* conditions can survive for extended periods of time with exogenously applied lactate as the sole energy source (Izumi *et al*. 1997; Brown *et al*. 2001). However, as lactate does not cross the adult BBB efficiently (Pardridge and Oldendorf 1977), any lactate within the CNS must be generated within the brain parenchyma (reports of systemic injections of lactate improving cognitive performance during hypoglycaemia have not controlled for liver gluconeogenesis of lactate, Maran *et al*. 1994; Smith *et al*. 2003). It has been postulated that given the arrangement of astrocytic endfeet surrounding capillaries a proportion of capillary glucose may pass through astrocytes before being available to neurones (Magistretti *et al*. 1999). Since glucose is immediately phosphorylated once it enters astrocytes, and leaves the astrocyte as lactate, it is implicit to the model that neurones take up at least some of their energy substrate in the form of astrocyte-derived lactate (Magistretti *et al*. 1999). It has been proposed that at rest neurones directly take up glucose (Pellerin and Magistretti 2003), but that during periods of increased activity neurones increase their uptake of lactate (Pellerin *et al*. 1998). In white matter there is convincing evidence of that glycogen-derived lactate is transferred from astrocytes to axons to support function during periods of increased axonal firing under normoglycaemic conditions (Brown *et al*. 2003). This intercellular transfer of lactate has been proposed as a mechanism for energy transfer between astrocytes and neural elements in both grey (Pellerin *et al*. 1998) and white matter (Wender *et al*. 2000), but it remains a controversial area (Chih *et al*. 2001; Vannucci and Simpson 2001; Chih and Roberts 2003; Pellerin and Magistretti 2003). Adding to the confusion are contradictory NMR data, which shows that there either is (Pfeuffer *et al*. 1999), or is not (Choi *et al*. 1999; Chhina *et al*. 2001) an increase in ^{13}C enrichment of lactate from ^{13}C-glucose, suggesting that glucose is (not) liberated as lactate. Neurones do possess the GLUT3 glucose transporter (Vannucci *et al*. 1997) and are obviously capable of surviving *in vitro* with glucose and not lactate as the sole energy reserve. Of great interest would be determination of a correlation between preferred neural energy substrate(s) and tissue energy demand, as evidence points to increased glycolysis during periods of increased tissue energy demand (Shulman *et al*. 2001).

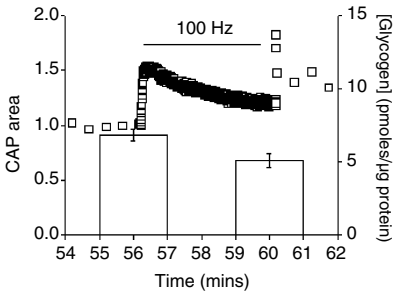

Fig. 5 Increased activity results in decreased glycogen content. In the MON 100 Hz stimulus does not result in a decrease in CAP area, indicative of functional failure, but glycogen content significantly decreased over the 4-min period of intense stimulus. The left axis indicates CAP are (open squares) and the right axis indicates the glycogen content on the MONs (open columns). Error bars indicate the SEM (from Brown *et al.* 2003).

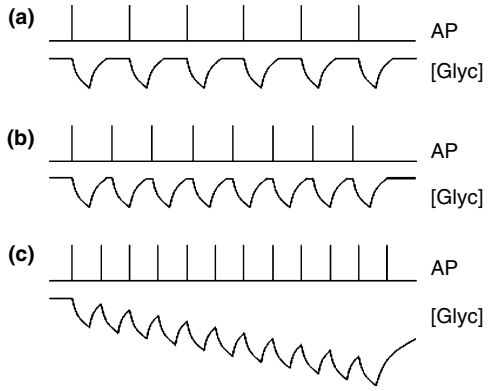

Fig. 6 Theoretical effect of increasing neuronal activity on glycogen levels. It is hypothesized that glycogen provides a rapid supplemental source of energy during increased tissue energy demand, such as elevated neuronal firing rates. The glycogen [Glyc] depleted by each action potential (AP) is replenished during the interspike interval. (a) and (b) During low frequency firing rates there is sufficient time between action potentials for full replenishment of glycogen, therefore there is no net decrease in glycogen content. (c) During rapid firing rates there is insufficient time to fully replenish glycogen content and it is hypothesized that during extended periods of firing the glycogen content would decrease (adapted from Shulman *et al.* 2001).

A physiological role for brain glycogen

A physiological role for brain glycogen has been suggested by various groups based on the following: (i) although glycogen can function as an energy metabolite to support function during periods of aglycaemia, the rarity of such events argues against glycogen's principal role as an emergency energy reserve; (ii) glycogen content accumulates *in vivo* during anaesthesia and sleep, consistent with the conscious, awake brain utilizing glycogen; (iii) glycogen content is under dynamic control by neurotransmitters, hormones and local energy status; (iv) the uptake rates of glucose into the brain are slightly higher than rates of consumption, and the difference between them is due to some efflux under normal conditions (Bachelard 1980), implying that the limited supply of brain glycogen does serve a specific function.

Sensory stimulation induces local glycogenolysis, linking physiological neuronal activity with glycogen utilization. Tactile stimulus of the rat face and vibrissae resulted in focal decreases in glycogen detected by autoradiographic techniques. Areas in which glycogen loss occurred included somatosensory cortex contralateral to stimulus site, and the trigeminal sensory nucleus and trigeminal motor nucleus ipsilateral to the stimulus site (Swanson *et al*. 1992). Sensory activation due to continual handling of rats reduced brain glycogen in dorsal cerebral cortex compared to control unhandled animals (Cruz and Dienel 2002). These two studies demonstrate that in the brain, sudden increases in local energy demand may be met by increased glycogenolysis. Recent supportive data have demonstrated that glycogenolysis does occur during increased tissue energy demand under normoglycaemic conditions, for the first time demonstrating that brain glycogen has a function during physiological activity (Brown *et al*. 2003). Using the mouse optic nerve as a model a role for glycogen in supporting function during high intensity stimulus was shown. Nerves subjected to 100 Hz stimulus in 10 mM glucose aCSF showed a rapid decrease in glycogen levels, suggesting glycogen mobilization to support function. Additional evidence was provided where: (i) inhibition of lactate transport out of astrocytes or into axons reduced function; (ii) downregulation of glycogen impaired high frequency firing during normoglycaemia. Increasing the ambient glucose to 30 mM allowed maintenance of full function indefinitely during such high intensity stimulus, indicating that the ability to maintain function during periods of high intensity stimulus is determined by the availability of adequate energy substrate. Thus a role for glycogen as an energy buffer (capable of providing short-term energy requirements), rather than an energy reserve (capable of providing long-term energy requirements), has been proposed (Brown *et al*. 2003). In this role glycogen provides supplemental energy substrate in the form of glycogen-derived lactate to support function when normoglycaemic concentrations of glucose are insufficient to adequately meet immediate tissue energy demands, and leads to the intriguing possibility that glycogen may abet the energy transactions associated with such phenomena as learning and memory where intense bursts of synaptic activity induce synaptic plasticity. Studies in neonatal chicks appear to support a role for glycogen in the formation of memory, which is modelled experimentally by brief high intensity stimuli to the hippocampus, where the energy requirements for the processing of memories result in decreased glycogen levels (O'Dowd *et al*. 1994). It has been

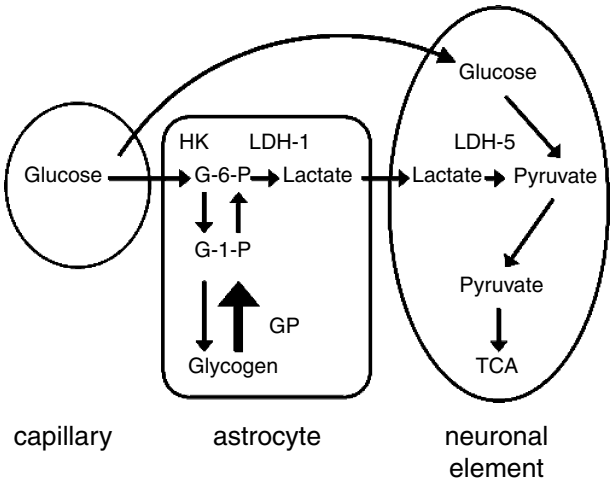

Fig. 7 The role of glycogen during increased tissue energy demand. Capillary glucose is transported across the endothelial cell and into astrocytes by the GLUT1 transporter although glucose may also be transported directly into neuronal elements via the GLUT3 glucose transporter. Glucose is phosphorylated by hexokinase (HK) to glucose-6-phosphate, which is the substrate for both glycogenesis and glycolysis. Glycogen can be rapidly degraded to glucose-1-phosphate by glycogen phosphorylase (GP) in response to increased demand, then to glucose-6-phosphate. Glucose-6-phosphate is then glycolytically metabolized ultimately to lactate via the action of lactate dehydrogenase-1 (LDH-1). The lactate is transported to neuronal element where it is oxidatively metabolized.

hypothesized that during periods of increased synaptic activity glycogen may fuel astrocytes in grey matter areas to clear synaptic glutamate, released during periods of increased synaptic activity (Shulman *et al*. 2001), however, no studies have directly investigated glycogen's role during such activity.

Conclusions and outlook

The last few years have seen a re-awakening of interest in brain glycogen, encouraged by techniques that can record *in vivo* brain glycogen for the first time, and data suggesting a physiological role for glycogen. Both of these developments raise interesting questions. Given that increased neuronal activity results in glycogenolysis, what are the signals that neurones send to the astrocyte to initiate glycogen breakdown? Prime suspects include elevated $[K^+]_o$ or $[glutamate]_o$, both indicators of increased neural activity, or release of glycogenolysis-promoting neurotransmitters. Is it possible that there is no signal, with the product/substrate ratio of glucose and its metabolites sufficient to determine the direction of key reactions that control glycogen content? How does insulin regulate brain glycogen? It appears likely that brain glycogen is regulated by both insulin-dependent and insulin-independent mechanisms, thus determining the relative contribution of each may have great relevance to type-1 diabetes. Can NMR spectroscopic techniques be used on human volunteers to correlate regional glycogen content with specific brain function, and can this technology be used in the diagnosis/treatment of such diverse disease states as epilepsy, hypoglycaemia and type-1 diabetes? The coming years promise increased enlightenment on brain glycogen and its role in brain energy metabolism, particularly with regard to its role in the burgeoning field of glia neuronal interaction.

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