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Does abnormal glycogen structure contribute to increased susceptibility to seizures in epilepsy?

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Abstract

Epilepsy is a family of brain disorders with a largely unknown etiology and high percentage of pharmacoresistance. The clinical manifestations of epilepsy are seizures, which originate from aberrant neuronal synchronization and hyperexcitability. Reactive astrocytosis, a hallmark of the epileptic tissue, develops into loss-of-function of glutamine synthetase, impairment of glutamate-glutamine cycle and increase in extracellular and astrocytic glutamate concentration. Here, we argue that chronically elevated intracellular glutamate level in astrocytes is instrumental to alterations in the metabolism of glycogen and leads to the synthesis of polyglucosans. Unaccessibility of glycogen-degrading enzymes to these insoluble molecules compromises the glycogenolysis-dependent reuptake of extracellular K^+ by astrocytes, thereby leading to increased extracellular K^+ and associated membrane depolarization. Based on current knowledge, we propose that the deterioration in structural homogeneity of glycogen particles is relevant to disruption of brain K^+ homeostasis and increased susceptibility to seizures in epilepsy.

Keywords

astrocytes; glycogen; potassium; glutamate; epilepsy

Introduction

The epileptic cerebral tissue is characterized by hypertrophy and proliferation of reactive astrocytes and release of growth factors by these cells that ultimately result in alterations of neuronal phenotype, neuronal hyperexcitability, glutamate excitoxicity and eventually neuronal cell death (de Lanerolle et al., 2010). Myriad of interdependent genetic, structural and metabolic mechanisms potentially contribute to the progressive generation of neuronal synchrony and recruitment of initially small hyperactive neuronal clusters during seizures (Jiruska et al., 2013). Although it is difficult to isolate a single identified mechanism without

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affecting the behavior of the whole system, we specifically examined the contribution of brain glycogen to the factors favoring neuronal hyperexcitability in epilepsy.

Glycogen is a polysaccharide of glucose and in the brain is confined to astrocytes (Ibrahim, 1975). Glycogen content in the mammalian brain ranges from 2.5 to 13 μmol/g, with higher values observed in hippocampus relative to grey and white matter (Cruz and Dienel, 2002; Dalsgaard et al., 2007; Oz et al., 2007). Since astrocytes occupy about 20% of brain volume, the amount of glycogen in astrocytic processes would range from 10 to 65 μmol/g, which is considerably higher than euglycemic glucose level (1–2 μmol/g). Glycogen can be mobilized several-fold faster than glucose in response to brain stimulation, and brief glycogenolytic bursts are capable of sustaining transiently elevated metabolic rates without substantial changes in glucose concentration (DiNuzzo et al., 2010). As an illustration, assuming an astrocytic glycogen content of 20 μmol/g (4 μmol/g in whole tissue) a seemingly negligible 5% degradation of glycogen would allow glycogenolysis to keep going for 30 seconds at 10 times the glucose consumption rate of resting human brain astrocytes (~0.2 μmol/g/min). These unique features of glycogenolysis contributed to revise the long-held concept of glycogen as a small emergency depot used exclusively in case of energy failure (for a review, see DiNuzzo et al., 2011). Rather, as it will be clear below glycogen occupies a fundamental position in the maintenance of ion homeostasis in the brain and hence it is a potential target in the pathophysiology of epilepsy. In the following, we describe some aspects of glycogen metabolism that might configure a role for this polysaccharide in increased susceptibility to seizures.

Requirement of glycogenolysis for active astrocytic K^+ uptake

Astrocytes provide the principal route to K^+ homeostasis in the brain (Hertz et al., 2013b). Active uptake of extracellular K^+ by astrocytes is critical for neuronal activity and disturbance in this fundamental astrocytic function is an important causal factor for epileptic seizures (D'Ambrosio, 2004). Net K^+ uptake by astrocytic Na^+/K^+-activated adenosyntrisphosphatase (NKA) occurs whenever extracellular K^+ increase (Amzica et al., 2002; Ballanyi et al., 1987; Chever et al., 2010; Dufour et al., 2011; Hertz, 1978; Xu et al., 2013). Similarly, many studies have consistently reported that glycogen is rapidly degraded when extracellular K^+ rise (reviewed by DiNuzzo et al., 2012). K^+-induced glycogen degradation has recently been reported in rat hippocampal slice or primary astrocyte preparations (Choi et al., 2012; Xu et al., 2013). These findings indicate that clearance of extracellular K^+ is fueled preferentially by glycogenolysis.

Glycogenolysis differs from glycolysis only with respect to the first reaction, namely the substrate-level phosphorylation of glucose to glucose-6-phosphate catalyzed by hexokinase, the remaining nine steps being identical. Thus, in principle K^+ uptake should be equally well fueled by glucose. However, this does not seem to be the case, as glucose has been found to be unable to support astrocytic K^+ uptake during inhibition of glycogenolysis (Xu et al., 2013). This finding is important and advances the complex but fascinating idea that inhibition of glycogenolysis blocks the process that stimulates its mobilization via a yet unknown signaling mechanism (Hertz et al., this volume). Importantly, Na^+ loading in astrocytes resulting from increased extracellular Na^+ level and/or astrocytic Na^+

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permeability has been found to rescue the competence of these cells for K^+ uptake even during inhibition of glycogenolysis (Xu et al., 2013). However, this event is unlikely to happen during seizures, as extracellular Na^+ decreases (Hablitz and Heinemann, 1989) and astrocytes experience Na^+ loss as extracellular K^+ rises (Chow et al., 1991). Whether and how reduced Na^+ levels can stop glucose phosphorylation and force glycogenolysis is not known. Another mechanism that we proposed for requirement of glycogenolysis by K^+ uptake is based on the absence of mitochondrial creatine kinase in astrocytes, which prevents (or postpones) ADP signaling to mitochondria, where active hexokinase resides, after increased ATP hydrolysis by NKA (DiNuzzo et al., 2012). So, the observation that astrocytic Na^+ loading succeeds in activating phosphorylation of glucose by hexokinase would be explained by acceleration (or anticipation) of cytosol-to-mitochondria ADP signaling. Certainly the mechanisms coupling glycogenolysis and K^+ uptake are not fully understood and their elucidation requires further research (DiNuzzo et al., 2013). Nonetheless, it is conceivable that unavailability of the fast glycogenolytic response impairs K^+ uptake in astrocytes and contributes to the increased concentration of the ion in the extracellular space reported in epileptic brain.

Although the amount of charge moved during signal generation and propagation in individual neurons is very small, in the long run repetitive neuronal activity might result in protracted alterations of ionic concentrations. During seizures, extracellular K^+ concentration reaches 12–15 mM from a baseline value of 3 mM (Heinemann and Lux, 1977; Pedley et al., 1976). The changes in extracellular K^+ can modify in parallel the cell membrane potential of many neurons to persistently depolarized values, a situation that might evolve in “autogenic” paroxysmal discharges (Lebovitz, 1996). Conversion of regular firing of pyramidal neurons into burst firing upon elevation of extracellular K^+ has been observed in hippocampal slices (Jensen et al., 1994). The role of extracellular K^+ is especially important in maintaining hypersynchronous activity of neuronal populations, which is evidenced by the fact that propagation of epileptiform events is untouched by inhibition of chemical neurotransmission (Heinemann et al., 1986; Konnerth et al., 1986; Nelken and Yaari, 1987; Yaari et al., 1986). Overall, it seems well established that increases in extracellular K^+ perpetuate and perhaps cause epileptic seizures within a feedback loop with multiple synaptic factors (Fisher et al., 1976; McNamara, 1994) and that, at least in certain brain regions, chemical synaptic transmission is not necessary for synchronization of neuronal activity (reviewed by Dudek et al., 1998).

Excess glutamate affects the metabolism of glycogen in astrocytes

One of the main features of reactive astrocytosis is the loss-of-function of glutamine synthetase (GS), whose consequences are depletion of glutamine and accumulation of glutamate in the tissue (Cavus et al., 2005; Eid et al., 2004; Petroff et al., 2003; Petroff et al., 2002; van der Hel et al., 2005). Since glutamatergic neurotransmission largely depends on availability of glutamine (Tani et al., 2010), protracted suppression of GS activity eventually mitigates the neuronal release of glutamate in the extracellular space (Devinsky et al., 2013). In addition, the Na^+-dependent, high-capacity and high-affinity glutamate uptake by astrocytes maintains inward transmitter flux in astrocytes even at the high K^+ level attainable during seizures (Claudio et al., 2002 and references therein). Accordingly, the

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concentration of glutamate in GS-deficient hippocampal formation of laboratory rats has been found to increase specifically in astrocytes (Perez et al., 2012).

Metabolism of glutamate in brain astrocytes is significantly correlated with synthesis and degradation of glycogen. In particular, glycogenolysis has been found to be essential for glutamate formation (see Hertz et al., 2013a and references therein). Glutamate production from glycogen precursor requires the anaplerotic reaction catalyzed by pyruvate carboxylase (PC), which is selectively expressed by astrocytes (reviewed by Mangia et al., 2012). PC converts a molecule of pyruvate into oxalacetate, thereby replenishing the tricarboxylic acid (TCA) cycle after a molecule of α-ketoglutarate has exited the cycle to form glutamate (note that glutamate is in equilibrium with α-ketoglutarate). Significant reduction in activity of PC relative to pyruvate dehydrogenase has been reported during inhibition of astrocytic glycogenolysis (Sickmann et al., 2012). PC is also the first enzyme of the gluconeogenic pathway (i.e. the pathway converting pyruvate to glucose), which in the brain is followed by activity of phosphoenolpyruvate carboxykinase (PEPCK), fructose-1,6-bisphosphatase (FBPase) to yield phosphoenolpyruvate and fructose-6-phosphate, respectively (see DiNuzzo et al., 2011 and references therein). Glutamate-induced activation of PEPCK and glycogen synthesis has been first observed in skeletal muscle (Odedra and Palmer, 1981). Noticeably, pathological (see below) but not physiological (Qu et al., 2001) accumulation of glutamate in astrocytes has been found to stimulate gluconeogenesis and glycogen synthesis (sometimes these pathways are collectively named glyconeogenesis, a term which is not used here to avoid confusion). Support to this view comes from *in vitro* studies on cultured rodent cerebral cortical astrocytes. Incorporation of label from glutamate to lactate has been observed in astrocyte exposed to high (0.5 mM) but not low (0.1 mM) glutamate concentration (McKenna et al., 1996; Sonnewald et al., 1993). Incorporation of label from lactate to glycogen has been reported as well, which was abolished by the PEPCK inhibitor 3-mercaptopicolinate (Dringen et al., 1993; Schmoll et al., 1995). Thus, the biochemical relationship between glutamate and glycogen appears to be reciprocal and likely mediated by gluconeogenic enzymes.

The above-mentioned facts apply to intracellular astrocytic glutamate. Increases in extracellular glutamate *per se* do not stimulate glycogenolysis in astrocytes (Magistretti, 1988) or perhaps are even glycogenic (Swanson et al., 1990). Certainly, astrocytic glutamate uptake is glucose-sparing for these cells, which is often interpreted as a result of its own use as alternative energy substrate to glucose (Dienel, 2013; McKenna, 2013). However, when glutamate concentration is pathologically elevated, part of the glutamate carbons are not oxidized but rather incorporated into glycogen. Importantly, increases in extracellular K^+ stimulate astrocytic PC (Kaufman and Driscoll, 1992) and FBPase (Verge and Hevor, 1995), thereby supporting the hypothesis of a stimulation of the gluconeogenic pathway in epilepsy. For PC to be effective for anaplerosis, it needs to have a supply of pyruvate, and K^+ is also involved in stimulating the glycolytic enzymes pyruvate kinase (Outlaw and Lowry, 1979). A direct effect of K^+ in stimulating gluconeogenesis and glycogen synthesis from glutamate but not from lactate has been demonstrated in amphibian retinal Muller glial cells (Goldman, 1988), where initiation of gluconeogenesis and blockade of glycolysis has been observed in response to vasoactive intestinal peptide (VIP) (Goldman, 1990). Interestingly, increased

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levels of VIP type-2 receptor is a features of reactive astrocytes (Nishimoto et al., 2011). VIP is known to induce glycogenolysis in cerebral cortical astrocytes (Magistretti, 1990). Whether the effect of VIP on reactive astrocytes is glycogenic remains to be established.

Synthesis of unmetabolizable glycogen is correlated with epileptic seizures

L-methionine-SR-sulfoximine (MSO) is a convulsant agent that acts primarily by inhibiting GS in astrocytes, although other proepileptic effects of MSO have been reported (e.g., Sellinger et al., 1984). In addition to its ability to elicit seizures, MSO is a powerful glycogenic agent (Folbergrova, 1973; Folbergrova et al., 1969; Phelps, 1975; Seidel and Shuttleworth, 2011; Swanson et al., 1989). Increase in gluconeogenesis and de novo synthesis of glycogen are features of the MSO epileptogenic rodent brain. Indeed, MSO-induced glycogen synthesis has been found to be a consequence of increased activity of the astrocytic gluconeogenic enzyme FBPase (Delorme and Hevor, 1985; Hevor et al., 1986). The consistency of these metabolic effects in cultured astrocytes (Verge and Hevor, 1995), i.e. in the absence of neuronal hyperactivity, supports the notion that glycogen accumulation is not an effect of seizures but simply of high intracellular glutamate concentration. This conclusion is supported by the fact that in MSO-dependent seizures glycogen increase is observed during the pre-convulsive period before epileptic crisis (reviewed by Cloix and Hevor, 2009). On this basis, a possible causal link between glycogen metabolism and epileptogenesis has been proposed (Cloix and Hevor, 2011). However, studies aimed at establishing such a role for glycogen failed to demonstrate consistent effects of seizures on glycogen levels (see Walling et al., 2007). The lack of success in correlating epilepsy and glycogen content has been evidenced by the finding that some animals subjected to MSO developed violent seizures without any variation in tissue glycogen (Bernard-Helary et al., 2000). Furthermore, animals capable of accumulating glycogen after MSO administration but before seizures exhibited different resistance to convulsions depending on whether they were able to utilize glycogen (Bernard-Helary et al., 2000). Among MSO-treated animals, those that are seizure-prone accumulate aberrant glycogen particles, while those that are seizure-resistant exhibit normal-appearing glycogen particles whatever the change in glycogen levels (Delorme and Hevor, 1985; Folbergrova et al., 1996; Phelps, 1975). Finally, there are many models of induced seizures characterized by unchanged or even decreased tissue glycogen content (see Cloix and Hevor, 2009). We believe that experiments directed to prove such hypothesis should examine not only glycogen content, as is commonly done, but also glycogen structure.

Brain glycogen appears to be subjected to a “quality-check” control mechanism by the laforin-malin protein complex. The laforin-malin complex mediates the continual proteasome-dependent degradation of abnormal glycogen particles and the associated enzymes necessary for glycogen synthesis (Liu et al., 2013; Vilchez et al., 2007). Mutations in either laforin- or malin-encoding genes, as it occurs in Lafora Disease (LD), results in synthesis of malformed glycogen-like polymer named polyglucosans (Spuch et al., 2012). Polyglucosans contain large quantity of phosphate esters, where biosynthetic errors of glycogen synthase are normally removed by laforin (Roach, 2011). Phosphate is found as glucose C2 and C3 monoesters, possibly after formation of glucose-1,2- or glucose-1,3-cyclic phosphate in the active site of glycogen synthase (Tagliabracci et al., 2011). However,

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this notion has been recently challenged by the finding that glycogen phosphorylation is not mediated by glycogen synthase (Nitschke et al., 2013). The latter study also reported C6 esterification (in addition to C2 and C3) and substantial increase in C6 monophosphate esters, a substrate of laforin, during LD. Since 1,6 cyclization is impossible, these findings leave the mechanism for glycogen phosphorylation unexplained. Although previously postulated, no evidence for the presence of phosphodiesters in glycogen has been reported, which is consistent with the fact that laforin does not apparently display phosphodiesterase activity (Nitschke et al., 2013; Tagliabracci et al., 2011). Elevated phosphate levels in glycogen are associated with reduced branching frequency and increased chain length. This results in formation of irregularly branched polymers that are partly insoluble and thus inaccessible to glycogen-degrading enzymes (see below). Interestingly, the presence of phosphate has been found to increase the priming ability of glycogen for *de novo* synthesis (Lomako et al., 1994), which is in agreement with the predominant location of C6 monoesters in the centre of the molecule (Nitschke et al., 2013). These outcomes suggest that phosphate might be a signal for glycogen growth, eventually interrupted at cessation of biosynthesis. Thus elevated levels of phosphate monoesters would impair degradation of glycogen not directly, but after promoting excessive glucosyl chain elongation. Accordingly, standard biochemical assays (e.g., amyloglucosidases or glycogen phosphorylase plus debranching enzyme) are likely insensitive to glycogen structure, regardless of the specificity for glucosyl residues with or without phosphate. However, complete degradation of abnormal glycogen to glucose normally takes place within lysosomes (e.g., Raben et al., 2002). Therefore, a biochemical assay based on acid α-glucosidase (acid maltase) possibly combined with other lysosomal digestive enzymes at low pH could provide an estimate of total (soluble and insoluble) glycogen. Comparison between this outcome and that obtained with phosphorylase plus debranching enzyme should thus give a quantitative estimate of the abnormal vs total glycogen. Interestingly enough, recent evidence supports that autophagy, a primary mechanism for the transport of abnormal glycogen molecules to lysosomes, is impaired in some epilepsies (McMahon et al., 2012) and in LD (Duran et al., 2014; Knecht et al., 2010), probably due to the lack of malin-laforin complexes (Criado et al., 2012).

Abnormal polyglucosan bodies (PGBs) accumulation has been implicated as the major cause of LD, a disorder characterized by a severe, progressive myoclonus epilepsy (Tagliabracci et al., 2008). Noticeably, PGBs have been found to appear first in astrocytes and much later in neurons in the malin-knockout mouse model of LD (Valles-Ortega et al., 2011). This finding is important, because formation of polyglucosans is commonly thought to take place specifically in neurons, a notion that links the adverse effects of their accumulation to neuronal apoptosis (Duran et al., 2012; Vilchez et al., 2007). Indeed, the glycogenic process is not confined to astrocytes, because also neurons express glycogen synthase and glycogen synthase kinase-3 (GSK3), a key element in activation of glycogen synthase, while they do not express glycogen phosphorylase (Inoue et al., 1988; Pfeiffer et al., 1990). Due to the role of GSK3 in regulating many cellular signaling pathways, dysregulation of this kinase is commonly an adverse event which eventually leads to apoptosis and cell death (reviewed by Takahashi-Yanaga, 2013). The early appearance of unmetabolizable glycogen in astrocytes advances the possibility that the impairment of the physiological processes dependent on astrocytic glycogenolysis, i.e. K^+ uptake, is an initial event in the pathology. Presence of

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polyglucosans in neurons *per se* is possibly not the main factor leading to epilepsy, as neuronal PGBs are also found in patients with glycogen branching enzyme deficiency (glycogen storage disease type IV), which suffer from motor, sensory and autonomic deficits but not epilepsy (Robitaille et al., 1980). Inclusions of polyglucosans, named *corpora amylacea* in some old and new studies (for details on the nomenclature, see Cavanagh, 1999), have been frequently observed in cerebral astrocytes of patients suffering of several epileptic disorders and often correlated with seizure duration (Abel et al., 2010; Agari et al., 2012; Cherian et al., 2003; Chung and Horoupian, 1996; Das et al., 2011; Erdamar et al., 2000; Kakita et al., 2005; Kawamura et al., 2002; Nishio et al., 2001; Palmucci et al., 1982; Radhakrishnan et al., 2007; Radhakrishnan et al., 1999; Ribeiro Mde et al., 2003; Van Paesschen et al., 1997; Vanderhaeghen, 1971), although their occurrence has also been reported in various brain regions and even in other tissues either in the presence or in the absence of specific pathology (see Leel-Ossy, 1998). The aggregation of glycogen-like polymers is a complex and relatively unexplored phenomenon, which includes abnormal polyglucosans but also membrane-bound aggregates of normal β-glycogen (glycogenosomes) observed under various conditions such as lysosomal disorders and aging (Cavanagh and Jones, 2000). Whether accumulation of polyglucosans directly predisposes to seizures remains to be demostrated.

It would be interesting to examine if chronically elevated concentration of glutamate in astrocytes leads to aberrant glycogen synthesis in these cells, thereby compromising the physiological role of this polysaccharide in normal brain function. FBPase activation might underlie a change in astrocytic phenotype triggered by high glutamate and K^+. Increase of glutamate oxidation in astrocytes has been found to decrease glucose utilization, which is in agreement with the fact that interictal phase is characterized by glucose hypometabolism (Engel et al., 1982; Lai et al., 2010; Sitoh and Tien, 1998; Theodore et al., 1983). It is possible that during the interictal phase, excess glutamate is channeled into astrocytic TCA cycle and converted to glycogen via gluconeogenesis, as previously suggested (Phelps, 1975). FBPase is an ancient evolutionary enzyme known to regulate the synthesis of glycogen from non-carbohydrates by targeting GSK3 (Gizak et al., 2013). Note that GSK3 phosphorylation is in turn regulated by several signaling pathways that are not discussed here. FBPase in the brain is expressed by both neurons and astrocytes (Loffler et al., 2001). The reason for neuronal expression is unknown but it can be speculated having to do with the maintenance of high substrate flow through pentose phosphate pathway, which is especially important for maintaining antioxidant levels in neurons (Bolanos and Almeida, 2010). The mechanism of action of FBPase includes the near complete desensitization to inhibition by AMP and Ca^{2+} after binding to the ubiquitous glycolytic enzyme aldolase (Rakus et al., 2004). The FBPase-aldolase complex ensures the channeling of the aldolase product fructose-1,6-bisphosphate to FBPase, thus protecting the intermediate from cytosolic degradation. The correlation between increased activity of FBPase and glycogen synthesis after MSO administration (Helary-Bernard et al., 2000; Hevor et al., 1986) suggests that at least under some circumstances the FBPase-aldolase complex translocates to regions accommodating the machinery for glycogen synthesis as it happens in liver (Yanez et al., 2004). The stimulation of FBPase is likely accompanied by inhibition of glycolytic enzymes and/or compartmentation of the two pathways to prevent energy dissipation. The

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latter situation is plausible, as unchanged phosphofructokinase activity has been found under the gluconeogenic effect of MSO (Hevor and Gayet, 1978). However, high citrate (e.g. resulting from substantial entry of glutamate into TCA cycle) might also play a role as this compound inhibits glycolytic pyruvate kinase and activates FBPase (see DiNuzzo et al., 2011).

It has been proposed that glycogen accumulation in epileptic tissue might represent an energy defense against seizures (e.g., Boissonnet et al., 2013). However, this is inconsistent with the hypermetabolism of epileptic focus in the ictal phase (Engel et al., 1983; Lai et al., 2010; Sitoh and Tien, 1998), i.e. when glycogen would be expected to be mobilized. High glucose uptake and metabolism during seizures, in spite of normal or elevated glycogen content, suggests that the polysaccharide might be resistant to phosphorolysis. In animals subjected to several brain insults including MSO-administration, the extra astrocytic glycogen synthesized during the post-insult period was not accessible during subsequent metabolic challenges (Folbergrova et al., 1996). Accordingly, after convulsions induced by MSO the newly formed glycogen in astrocytes has been identified as aggregates similar to α-particles in liver-like rosette form, contrary to the β-particle structure normally found in the brain (Delorme and Hevor, 1985; Phelps, 1975). This result is relevant to the capability of metabolizing glycogen, because the structure of the glycogen molecules is of paramount importance to their synthesis and degradation. Indeed, branching of glycogen confer to the polysaccharide a quasi-spherical shape at steady-state (i.e. when the β-particle has exactly 12 tiers) (Melendez et al., 1997). This spherical surface is at the basis of the high water solubility of glycogen, which ultimately is due to controlled chain length and available volume for enzyme action (DiNuzzo, 2013). In contrast, unbranched linear chains of glucose, such as amylose, form hydrogen-bond-mediated single or double helices that exhibit strongly reduced water solubility and hence are resistant to enzymatic hydrolysis (Hejazi et al., 2008).It has been reported that the synthesis of PGBs in LD is paralleled by increased levels of glycogen phosphorylase, suggesting that indeed the enzyme cannot bind the polysaccharide (Valles-Ortega et al., 2011). The latter study also showed that the activity of glycogen synthase within PGB was compromised. We have previously demonstrated using biophysical modeling that loss of affinity of this enzyme for glycogen inner chains is sufficient to produce aberrant particles (DiNuzzo, 2013). Overall, a defective capacity of degrading glycogen is likely to be proepileptogenic, regardless of whether its content is increased or not (Bernard-Helary et al., 2000). This argument might provide a common cue for epilepsy and LD (Boissonnet et al., 2013; Roach et al., 2012).

Epilepsy and the glycogen hypothesis of sleep

Sleep is a fundamental function of mammalian physiology. That sleep is functionally important is evidenced by its conservation throughout evolution, although it represents a “risk” for the animal as it is a form of rest with impaired vigilance. Sleep occupies one third of the human life span, still we do not know why we need to sleep. The ideas that have been advanced to explain the role of sleep have also been shown to have limited generality. However, in the context of the present paper it is worth mentioning the metabolic hypothesis of sleep (Benington and Heller, 1995). This hypothesis states that (i) sleep need is due to reductions in cellular energy charge, and (ii) sleep is essential for the replenishment of brain

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energy stores. Specifically, during waking cerebral glycogen would be depleted and adenosine release would increase, the latter event leading to reduced neuronal activity (due to inhibitory action of adenosine) and increased sleep need. Restoration of glycogen would take place during the non-rapid eye movement (NREM) sleep. NREM sleep is neurophysiologically distinct from waking and is characterized by recurrent population-level synchronized bursts of neuronal activity (Vanderwolf, 1988). The above-mentioned relation between glycogen and sleep is strengthened by the finding that glycogen level rises during sleep (Karnovsky et al., 1983) and falls during sleep deprivation (Kong et al., 2002).

Sleep is relevant to epilepsy, as extenuating circumstances such as stress or extreme sleep deprivation might induce epileptic seizures (Malow, 2004). The role of sleep deprivation in eliciting epileptiform activity has been recognized more than 50 years ago (Rodin et al., 1962) and then repeatedly documented (reviewed by Kotagal, 2001; Matos et al., 2010). In certain types of epileptic syndromes seizures predominantly occur after sleep deprivation (Derry and Duncan, 2013). Likewise, many seizures have a tendency to develop during sleep and in particular during NREM sleep (Kothare and Kaleyias, 2010), which is in agreement with the reduced concentration of extracellular adenosine observed during sleep compared with wakefulness (e.g., Porkka-Heiskanen et al., 2000). Adenosine has also been found to be elevated in patients *after* seizure cessation (During and Spencer, 1992; Schrader et al., 1980). These findings have led to the conclusion that adenosine mediates seizure arrest (Boison and Stewart, 2009), which is consistent with its anticonvulsant effects mediated by A1 receptors (Boison, 2011). Adenosine accumulates as a result of enhanced energy demand and hypermetabolism during seizures. Indeed, the concentration of adenosine is correlated to the cell energy status, because the nucleotidases responsible for adenosine formation are activated by ATP and inhibited by inorganic phosphate, a product of ATP hydrolysis (Newby et al., 1985). It is noted that another route for extracellular adenosine accumulation is represented by increased ATP release by astrocytes and as such could be independent of brain energetics. In fact, the increase in adenosine level upon sleep deprivation (Porkka-Heiskanen et al., 2000) has been found to be accompanied by an enhancement of cortical excitability (Badawy et al., 2006). So, it is unlikely that adenosine alone accounts for the reciprocal interactions between sleep and epilepsy, although a possible proconvulsant switch of adenosine mediated by A2 receptors cannot be excluded (see Scharf et al., 2008).

Glycogen is a promising candidate underlying the relation between sleep and epilepsy. However, studies aimed at investigating this relation have only looked at glycogen content, without apparent success (Benington and Heller, 1995; Scharf et al., 2008). It could be interesting to test whether sleep is essential to restore glycogen homogeneity rather than to replenish the amount of the polysaccharyde. In particular, glucosyl chains that are partly metabolized during wakefulness might exhibit local inhomogeneities (e.g., due to the presence of phosphate monoesters) that need to be removed for proper storage and utilization of glycogen molecules. Thus, it is intriguing to speculate that during sleep an ordered alternation of synthesis and degradation of glycogen, for example induced by cyclic neuronal activity bursts during NREM sleep, ultimately restores the homogeneity of the glycogen pool in astrocytes. Support to the idea that aberrant glycogen metabolism is

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causative to epileptogenesis comes from the recent finding that stress, which shares many features with sleep deprivation, accelerates accumulation of polyglucosan bodies and LD-associated epilepsy (Wang et al., 2013).

Summary and perspectives

During epilepsy, loss-of-function of GS induced by reactive astrocytosis contributes to the rise of astrocytic glutamate concentration. Glutamate is tightly linked to glycogen metabolism and high glutamate levels are glycogenic. Furthermore, glutamate is a glucose-sparing agent and channeling of glutamate into astrocytic TCA cycle increases when the aminoacid is present at high concentrations. Excess glutamate possibly induces a switch of astrocytic metabolism from glycolysis to gluconeogenesis, which is likely controlled by regulation of several key enzymes, such as FBPase. Experimental evidence suggests that the glycogen formed as a result of the glutamate-induced activation of gluconeogenesis occurs through *de novo* synthesis, so probably after recruitment of primer proteins. We propose that under these unphysiological conditions the cellular machinery for glycogen metabolism fails to synthesize homogeneous glycogen β-particles and produces insoluble polyglucosans, which cannot be further metabolized. Besides the detrimental effects on neurons, this latter event would initially impair perhaps the main function of astrocytic glycogenolysis, namely K^+ uptake, thus increasing seizure susceptibility and eventually contributing to epileptogenesis.

Our hypothesis is consistent with an involvement of glycogen metabolism in epilepsy (Cloix and Hevor, 2009, 2011). It implies that glycogen particles gradually become unmetabolizable during epileptogenesis. Loss of solubility of glycogen would arise along with sustained cell depolarization due to accumulation of K^+ in the extracellular space. It would be interesting to examine the pattern of activity of glycogen synthase as well as the extent of phosphate incorporation within glycogen granules during and after seizures. A necessary (though not sufficient) prerequisite for the present hypothesis, is that massive and prolonged inhibition of brain glycogen phosphorylase should have proconvulsant effects. It will be straightforward to experimentally test whether astrocytes cultured from animal models of LD preserve the ability to take up K^+ when the extracellular concentration of the ion increases. In conclusion, the health of the pool of glycogen molecules in normal and diseased brain conceivably plays much more important roles than previously anticipated.

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Abbreviations used

FBPase	fructose-1,6-bisphosphatase
GS	glutamine synthetase
GSK3	glycogen synthase kinase 3

LD	Lafora disease
MSO	L-methionine-SR-sulfoximine
NKA	Na^+-K^+-activated adenosintrisphosphatase
NREM	non rapid eye movement
PC	pyruvate carboxylase
PEPCK	phosphoenolpyruvate carboxykinase
PGB	polyglucosan body
TCA	tricarboxylic acid
VIP	vasoactive intestinal peptide.

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