

Astrocyte Glycogen and Brain Energy Metabolism

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KEY WORDS

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ABSTRACT

The brain contains glycogen but at low concentration compared with liver and muscle. In the adult brain, glycogen is found predominately in astrocytes. Astrocyte glycogen content is modulated by a number of factors including some neurotransmitters and ambient glucose concentration. Compelling evidence indicates that astrocyte glycogen breaks down during hypoglycemia to lactate that is transferred to adjacent neurons or axons where it is used aerobically as fuel. In the case of CNS white matter, this source of energy can extend axon function for 20 min or longer. Likewise, during periods of intense neural activity when energy demand exceeds glucose supply, astrocyte glycogen is degraded to lactate, a portion of which is transferred to axons for fuel. Astrocyte glycogen, therefore, offers some protection against hypoglycemic neural injury and ensures that neurons and axons can maintain their function during very intense periods of activation. These emerging principles about the roles of astrocyte glycogen contradict the long held belief that this metabolic pool has little or no functional significance. © 2007 Wiley-Liss, Inc.

INTRODUCTION

Glycogen is the repository for stored glucose in the mammalian body and is found in most tissues in the body, including brain. However, its main depots reside in liver and skeletal muscle, and it is from these two tissues that the majority of our knowledge of glycogen and its function is derived (Garrett and Grisham, 1999). The principal function of glycogen is to act as a cellular storage depot for glucose, either for the cells own use or for export to other cells. In times of plenty, then, glucose in excess of what is immediately required may be stored as glycogen. When glucose intake is reduced below immediate requirements, as during a period of starvation, glycogen is metabolized to release energy substrate in the form of glucose (or glucose-related substrates). From an evolutionary standpoint the advantages of this system are obvious. Ingested food beyond what is immediately needed can be stored so that precious excess calories do not go to waste; the carbohydrates are ultimately stored in the form of glycogen, and excess fat is stored in adipose tissue as subcutaneous fat. It should be noted however, that a fast of 24 h is sufficient to completely deplete liver glycogen stores (Champe and Harvey,

1994), and it is the fat stores that ensure survival during extended periods of food deprivation.

The precise function of glycogen is dependent upon its location. Liver glycogen maintains blood glucose levels within the normal (normoglycemic) range (4–7.2 mM; 72–130 mg/dL), when ingested glucose is insufficient to maintain these levels (Champe and Harvey, 1994). Under such conditions, liver glycogen is metabolized to glucose, which is then released into the systemic circulation to sustain normoglycemic blood levels (Champe and Harvey, 1994). In this way the liver glycogen acts in an egalitarian manner to support glucose-dependent function in the whole body. Skeletal muscle glycogen has a different function. It is metabolized during increased energy demand, e.g. exercise, to provide energy to the muscle via glycolysis, and the waste product lactate is excreted into the blood (Newsholme and Leech, 1983). Thus the dogmatic view is that liver glycogen is used for the benefit of the entire body, whereas skeletal muscle glycogen is used solely by that tissue. However, recent data have indicated that glycogen metabolism in skeletal muscle is more interesting than previously thought. During intense exercise systemic blood glucose levels can drop to hypoglycemic levels (<4 mM), but blood lactate levels can increase tenfold from 1 to 10 mM. Measurements of arterial–venous lactate differences have indicated that under these conditions net lactate transfer switches from an efflux from the brain to a net uptake of lactate into the brain (i.e. the arterial–venous difference goes from negative to positive) (Secher and Quistorff, 2005). Taking into account that the lactate generated from muscle glycogen may be used as an energy substrate by other organs (e.g., brain), muscle and liver glycogen can be seen to have some functional overlap. That lactate can be taken up by brain as an energy substrate is also worth noting. It indicates that the time-honored notion that the adult brain uses glucose exclusively has exceptions. As we shall see later, this idea is important for the story of brain glycogen.

The presence of glycogen in the brain implies that it has a definite role within the brain parenchyma. Brain glycogen levels of 6–12 μmol (Cruz and Dienel, 2002) are lower than those in liver (100–500 μmol) (McKenna

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et al., 2006) and skeletal muscle (300–350 μmol) (Chrysanthopoulos et al., 2004). This implies that brain glycogen is not of any practical use as a glucose reservoir to support blood glucose levels, as occurs in liver. Consistent with this expectation, the levels of brain glycogen change very little during starvation while those in liver fall precipitously (Nilsson and Hultman, 1973). The much lower amount of glycogen in brain, compared with liver, is also consistent with the simple reality that the rigid skull limits fluctuations in intracranial contents; massive up and down regulation of bulk glycogen would simply not be possible without disruptive changes in intracranial pressure (Ames, 2000). The brain takes up only about 10% of glucose in the blood (McKenna et al., 2006) and glucose extracted that is surplus to requirements is transported back into the venous system and transported out of the brain (Bachelard, 1980). From a theoretical standpoint, it seems most probable that brain glycogen is intended for local use only (Brown, 2004). In other words, brain glycogen would function more like muscle glycogen than liver glycogen. That said, the details prove to be unexpectedly interesting. Brain, unlike muscle and liver, has great cellular heterogeneity which is essential for its complex functions (Nicholls et al., 1992). This cellular heterogeneity implies that the role of brain glycogen is more specialized and subtle than that of muscle.

LOCATION OF GLYCOGEN IN THE BRAIN

Given that the function of the brain is achieved by the complex interactions of a variety of cells, it is not surprising that the cellular location of brain glycogen is specialized, whereas glycogen is expressed in virtually all liver and skeletal muscle cells. It is routinely stated that glycogen is present only in astrocytes in the brain (Cataldo and Broadwell, 1986). While it is widely accepted that brain glycogen is almost exclusively expressed in astrocytes in the adult, in younger animals this is not the case and the expression of glycogen in specific populations of brain cells is a function of age. Brain glycogen is expressed in embryonic neural and glial tissue (Bloom and Fawcett, 1968), but with maturity the degree of neuronal expression fades, and by adulthood only ependymal and choroid plexus cells, beyond astrocytes, express brain glycogen (Magistretti et al., 1993). The function of brain glycogen in neural cells at birth is unknown, but this may imply that these cells have higher metabolic demand or a less secure glucose supply at that stage of development. This would correlate with the neonatal brain receiving about half of its energy requirements from nonglucose substrates, primarily in the form of lactate (Nehlig and Pereira de Vasconcelos, 1993), which crosses the blood brain barrier via monocarboxylate transporters (MCTs). The MCTs are expressed in the neonatal blood brain barrier, but their expression decreases post-weaning (Pellerin et al., 1998).

In adults, brain glycogen is expressed in astrocytes, but is it expressed in all astrocytes and at the same concentration? To answer these questions we must delve down to the cellular level. Astrocytes are expressed in all brain regions and in fact outnumber neurons (although they have a smaller somatic volume). Because they are expressed in all brain regions, astrocyte location, *per se*, does not dictate regional glycogen expression. Astrocyte subtypes have been defined based on morphology or functional properties. Anatomically distinctive astrocytes, called fibrous and protoplasmic, are found in white and grey matter, respectively. Both of these cell types contain glycogen although it is not known if all cells of each class express glycogen (Kong et al., 2002; Wender et al., 2000). At least two types of astrocytes are recognized based on functional properties. One type expresses high concentrations of glutamate transporters and gap junctions (these are “classical” astrocytes), the other type lacks both of these properties and was more recently identified (Huang and Bergles, 2004; Matthias et al., 2003). Certainly some, perhaps all, functionally classic astrocytes contain glycogen, while the issue simply has not been studied in the other group. In tissue culture experiments, some astrocytes appear not to express glycogen (Rosenberg and Dichter, 1987; Verge et al., 1996) but it remains to be determined *in vivo* if there is a glycogen negative astrocyte subtype. The cellular enzymes that metabolize brain glycogen are also localized to astrocytes (Pellegrini et al., 1996; Pfeiffer-Guglielmi et al., 2003). The brain isozyme of glycogen phosphorylase, which breaks down glycogen, is located predominately in astrocytes, but interestingly is also located in the few nonastrocytic cells that express brain glycogen, namely choroid plexus and ependymal cells (Magistretti et al., 1993). Glycogen synthase, which synthesizes glycogen, is likewise expressed in astrocytes but surprisingly is also expressed in neurons where it is likely to have a role distinct from glycogen metabolism (Pellegrini et al., 1996).

Brain glycogen is not uniformly distributed throughout the brain. Electron microscopic study of brain glycogen granules suggests that glycogen is highest in brain areas with the greatest synaptic density (Phelps, 1972), perhaps implying a role in the energy dependent process of synaptic transmission. However, high concentrations of brain glycogen are also reported in white matter (Cruz and Dienel, 2002) an area devoid of synapses. Most studies find that brain glycogen concentration is about twice as high in grey than white matter (a single paper finds the opposite; (Kong et al., 2002)). The grey matter structures associated with high brain glycogen levels are pons/medulla, cerebellum, hippocampus, hypothalamus, thalamus, medulla, cerebral cortex, and striatum (Sagar et al., 1987). It is interesting to note that hypoglycemic injury progresses from rostral to caudal areas (although the cerebellum is relatively unaffected) and does show at least some correlation with brain glycogen (Auer, 2004), supporting the hypothesis that brain areas with least glycogen are most susceptible to hypoglycemic damage (see later).

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Regulation of Brain Glycogen

The two key enzymes that regulate synthesis and degradation of brain glycogen are glycogen synthase and glycogen phosphorylase, respectively. Glycogen synthase is found in both astrocytes and neurons, but its neuronal function is suspected to be unrelated to glycogen metabolism. The cDNA for brain glycogen synthase shares 96% homology with the muscle isozyme, but less with the liver isozyme (Pellegri et al., 1996). The cDNA and presumably the functional enzyme are widely distributed throughout the brain, but are most robustly expressed in hippocampus, cerebellum, and olfactory bulb (Pellegri et al., 1996). The enzyme exists in two forms, an inactive phosphorylated form (GSb), and an active dephosphorylated form (GSa), which allows more control over glycogen metabolism. A family of phosphatases controls interconversion of the phosphorylated and dephosphorylated forms. Protein phosphatase 1 (PP1) is abundantly expressed in astrocytes and binds glycogen, resulting in dephosphorylation of glycogen synthase and Protein to Glycogen (PTG) protein, which facilitates binding of protein phosphatase 1 to glycogen (Fig. 1) (Allaman et al., 2000).

Glycogen phosphorylase has three isoforms, with each isoform deriving its name from the tissue of its predominant expression, muscle, liver, and brain (Ignacio et al., 1990). Interestingly the muscle isoform, as well as the brain isoform, are expressed in brain tissue, specifically in astrocytes, correlating with the predominantly astrocytic expression of glycogen (Pfeiffer-Guglielmi et al., 2003). The cDNA for brain glycogen phosphorylase shares 83% homology with the muscle and 80% with the liver isozymes. Like glycogen synthase, glycogen phosphorylase exists in phosphorylated (GP_a) and dephosphorylated states (GP_b), but unlike glycogen synthase it is the phosphorylated state that is active and the dephosphorylated state that is inactive. Interconversion of these states is regulated by phosphorylase kinase.

There are numerous compounds that have been reported to either up or down regulate glycogen content in the brain. These include vasoactive intestinal peptide, nor-adrenaline and adenosine, which upregulate glycogen synthase (Sorg and Magistretti, 1992). These compounds exert their effects by synthesizing new PTG protein. IGF I and IGF II, as well as insulin, increase brain glycogen content presumably by acting on insulin receptors in a similar way to how this happens elsewhere in the body. Extracellular glucose concentration also determines glycogen content, which is not surprising considering that glucose molecules are the building blocks of glycogen (e.g., Brown et al., 2003).

ROLE OF ASTROCYTIC GLYCOGEN

When compared with the concentrations of glycogen in the liver and skeletal muscle the glycogen stores of the brain appear negligible and thus a possible role for brain glycogen has been dismissed (Stryer, 1995). Liver

glycogen can maintain systemic euglycemia for up to 24 h in the absence of exogenously ingested glucose (or precursors), but the much lower concentration of glycogen in the brain implies a very limited period of support. Brain tissue consumes about 100 μ moles of oxygen per g of tissue per hour implying complete oxidative metabolism would consume 600 μ moles glucose/g/h. The concentration of brain glycogen is between 5 and 15 pmoles/ μ g protein ($\sim$ 0.5–1.5 μ moles/g tissue). Thus, in the absence of glucose, these figures suggest that brain glycogen would be consumed within a few minutes. Although brain glycogen acts in the same manner as liver and skeletal muscle glycogen in providing energy substrate, key differences also apply (see below). To date the two main roles that have been identified for glycogen in the brain are to provide energy substrate (1) under hypoglycemic conditions when delivery of blood-borne glucose is insufficient to meet immediate energy requirements (Brown et al., 2003; Choi et al., 2003), and (2) during periods of increased tissue energy demand when ambient glucose is insufficient to meet the transient elevation in energy demand (Brown et al. 2003).

Hypoglycemia

Because of excellent homeostatic control of blood glucose level, spontaneous systemic hypoglycemia is a rare occurrence predominantly seen with tumors of insulin producing cells. However, since the advent of insulin therapy as a clinical treatment for Type 1 diabetes, insulin-induced hypoglycemia has become commonplace among Type 1 diabetics (Frier and Fisher, 1999). It occurs when insulin is administered in excess of that required to prevent hyperglycemia (Frier and Fisher, 1999). Initial clues to a potential role for brain glycogen in hypoglycemia resulted from studies on cell culture techniques, where it was observed that neurons co-cultured with glial cells survived longer than neurons cultured in the absence of glia (Whatley et al., 1981). The reason for this extended neuronal survival was elucidated in another cell culture study, in which the key factor that determined neuronal survival during hypoglycemia was astrocytic glycogen content (Swanson and Choi, 1993). The mere presence of astrocytes was not enough to prolong neuronal survival as the astrocytes had to contain significant glycogen. This was the first time that a direct link had been made between astrocytic glycogen and neuronal survival during energy substrate deprivation. The mechanism of this benefit, however, was not clear, and included the possibility that glycogen in astrocytes simply allowed these cells to maintain ATP levels longer so that they could continue to protect neurons by taking up glutamate (Swanson and Choi, 1993). While it was also a possibility that astrocyte glycogen might be metabolized to an energy substrate that could be shared with deprived neurons, this was not directly tested.

The first demonstration that astrocyte glycogen could, in fact, supply neighboring neural elements with fuel came in studies on the rodent optic nerve (Fig. 2)

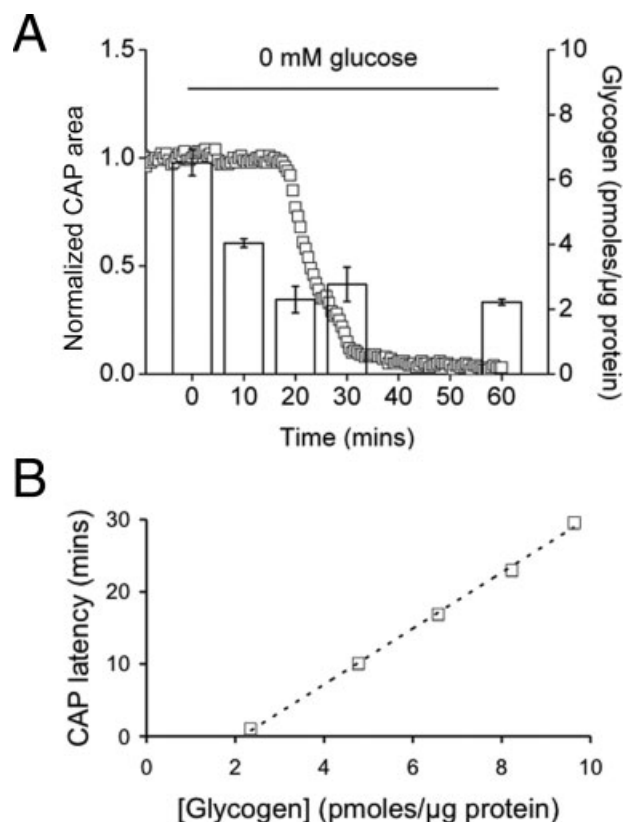


Fig. 2. In MON the glycogen content of the tissue determines latency to CAP failure during glucose withdrawal. (A) In the MON aglycemia resulted in delayed failure of the stimulus evoked compound action potential (CAP) after about 20 min. On exposure to aglycemia glycogen content decreased from control levels of about 7 pmoles/μg protein to its nadir of 2 pmoles/μg protein after 20 min, coinciding with CAP failure. The left axis indicates CAP area (open squares) and the right axis indicates the glycogen content (open columns). Error bars indicate the S.E.M. (B) Relationship between glycogen content immediately prior to aglycaemia and latency to onset of CAP failure after aglycemia onset. The dotted line is a best fit of data points (from Brown et al., 2003).

(Wender et al., 2000). This central white matter tract is devoid of the complications of synapses or neuronal cell bodies, and as such is an ideal preparation in which to investigate astrocyte–axon interactions (Ransom and Fern, 1997). Manipulations that either increased or decreased glycogen content influenced the duration that the stimulus-evoked CAP (reflecting axon function) could be maintained when glucose was withdrawn in rat optic nerve (Wender et al., 2000). Increasing glycogen content by pre-incubation in elevated glucose aCSF delayed the duration to CAP failure, whereas down-regulating glycogen content accelerated CAP failure (Fig. 2). Follow on studies in mouse optic nerve revealed that blocking glycogen metabolism using the glycogen phosphorylase inhibitor isofagomine prevented the neuroprotective effects of elevated brain glycogen content (Fig. 3) (Brown et al., 2005). In addition to supporting function during aglycemia, brain glycogen also supported function during hypoglycemia. MONs were fully supported in 2 mM glucose aCSF for several hours, but prior depletion of brain glycogen by a period of aglycemia rendered

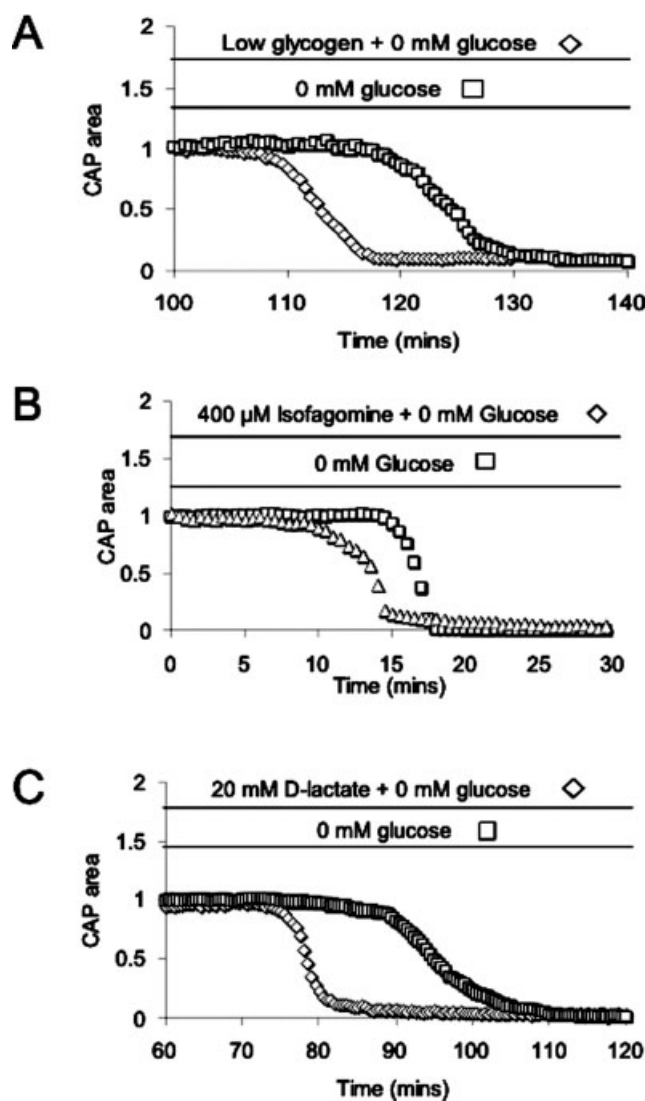


Fig. 3. The ability of glycogen to sustain CAP function during aglycemia. (A) Under control conditions in which MONs were bathed in 10 mM glucose for 1 h prior to removal of glucose, the CAP was sustained for 20 min after onset of aglycemia before failing (□). Prior reduction in glycogen levels by bathing MONs in 0 glucose for 20 min accelerated CAP failure during a subsequent period of aglycemia (◇). Onset of aglycemia at 100 mins. (B) Inhibiting glycogen metabolism by pre-incubation with isofagomine, an inhibitor of glycogen phosphorylase, results in accelerated CAP failure during aglycemia (◇) compared with control conditions with MONs bathed in 10 mM glucose prior to aglycemia (□), implying glycogen metabolism supports CAP function during aglycemia. Onset of aglycemia at 0 mins. (C) Introduction of D-lactate which competes at the MCT transporter with endogenous L-lactate from glycogen metabolism accelerated CAP failure during aglycemia (◇) compared with control conditions (□), implying it is L-lactate uptake, presumably from astrocytic glycogen, that is supporting axonal function. Onset of aglycemia at 60 mins (from Brown et al., 2003; Brown et al., 2005; Tekkok et al., 2005).

2 mM glucose incapable of supporting function, implying that brain glycogen was providing an energy substrate to supplement the hypoglycemic concentrations of glucose (Brown et al., 2003). More recent data have demonstrated in the mouse optic nerve that in the presence of an energy substrate that cannot support function, in this case fructose, glycogen will act to provide substrate

to maintain function (Meakin et al., 2007). However once glycogen stores are exhausted function fails in the presence of fructose.

Glycogen content can be measured using NMR technology (Choi and Gruetter, 2003). This involves infusing labelled [$1\text{-}^{13}\text{C}$] glucose, which is incorporated into the glycogen molecule, providing a measurable signal for the glycogen. Such experiments have for the first time allowed measurements of glycogen content in human brain, and have shown a slow incorporation of the label into glycogen over a period of 6 h (Oz et al., 2003). Similar techniques applied to rats demonstrate that glycogen is a labile store with a relatively low overall turnover (although this may be accelerated during increased tissue energy demand—see later). In rats, an episode of hypoglycemia resulted in a drop in glycogen concentration, but only after glucose concentration in the brain had fallen to zero (Choi et al., 2003). These data imply that glycogen is rapidly metabolized when glucose falls towards the K_m of hexokinase, and the point at which glucose transport becomes rate limiting for metabolism. Thus glycogen provides significant energy substrate during hypoglycemia. On restoration of normoglycemia glycogen content rose until it exceeded by several fold the pre-hypoglycemia baseline level (Choi et al., 2003), and this super compensation has been implied as a possible mechanism for hypoglycemia unawareness (Criego et al., 2005).

Recent findings indicate that the benefits of glycogen noted *in vitro* extend to whole animals. Manipulation of glycogen with the unique glycogen phosphorylase inhibitor CP316819 resulted in an increase in brain glycogen that staved off the pathological effects of insulin-induced systemic hypoglycemia by delaying the onset of isoelectric EEG, a recognizable sign of trans-membrane ion gradient breakdown. This phosphorylase inhibitor has the special feature of allowing glycogen breakdown to occur in the face of profound hypoglycemia. The benefit of glycogen was surprising strong and preserved brain function for up to 90 min (Suh et al., 2007). This demonstration of greater than an hour of neural protection from astrocyte glycogen *in vivo* speaks to the practical importance of this energy reserve and repudiates the time-worn notion that brain glycogen content is too low to influence brain function.

Increased Tissue Energy Demand

The roles of glycogen in liver and muscle are intuitively easier to grasp than the role of glycogen in brain, given the smaller amounts of brain glycogen and the fact that it is solely confined to astrocytes in adults. The brain has a high resting metabolic rate, second only to heart muscle and kidney cortex (McKenna et al., 2006), that increases during localized brain activation. These increases in metabolic demand are met by increased blood flow to the activated area, thereby delivering additional glucose and O_2 to meet the demand (McKenna et al., 2006). At this point it may be useful to take a

step back and assess glucose usage by the brain, to determine if there are any situations where delivery of energy substrate would be better met by glycogen metabolism than increased delivery of glucose. Under activated conditions the brain has the same metabolic demand as exercising muscle, although this rate is only about double the metabolic rate of resting brain (Shulman et al., 2004). The brain's increased energy demands tend to be almost instantaneous (Leybaert, 2005). The latency of the signals to increase blood flow results in a sudden shortfall in glucose delivery relative to demand. This situation jeopardizes or limits brain function as ATP levels fall below what is required to maintain proper transmembrane ion gradients necessary for the integrity of neural signalling. Because astrocytes have intimate anatomic relationships with neurons and axons throughout the brain, separated by only 200 Å of extracellular space, they are ideally positioned to share substrate with their neural neighbors. More specifically, to share an energy substrate derived from glycogen with neurons or axons that have momentarily exhausted their immediate supply of glucose. The disadvantages of depending entirely on glucose are not restricted to the delay between the increase in demand and delivery of glucose, but also include the fact that glucose requires initial phosphorylation before its metabolism can proceed. Hexokinase is one of the slower glycolytic enzymes (Champe and Harvey, 1994), suggesting that glucose phosphorylation may be rate limiting as glucose concentrations fall towards the K_m of hexokinase (50–100 μM). Brain glycogen, in contrast, is predominantly metabolized via glucose-6-phosphate, i.e. after the hexokinase step, and does not require ATP. Additionally, glycogen phosphorylase is a fast enzyme (Shulman et al., 2001) and under increased energy demand the product/substrate ratio of the glycolytic intermediaries would decree a rapid metabolism of brain glycogen (Garrett and Grisham, 1999). In the MON preparation, increased tissue energy demand induced by imposing a high frequency stimulus on the tissue, did indeed result in a rapid decrease in glycogen content, even in the presence of normoglycemic concentrations of bath glucose (Brown et al., 2003). We conclude that even under normoglycemic glucose conditions, increases in tissue energy demand are in part met by glycogen degradation. This view was supported by experiments where blockade of lactate transport into axons prevented glycogen from supporting function (Brown et al., 2005).

Astrocyte Glycogen can be Metabolized to Lactate for Transfer to Axons

How might astrocyte glycogen benefit its neural neighbors in terms of energy metabolism? Several observations contribute to the current working hypothesis that astrocyte glycogen breaks down to lactate that is exported to the extracellular space and then taken up by neurons or axons as an aerobic fuel (Brown et al., 2004). These include the fact that neurons and axons op-

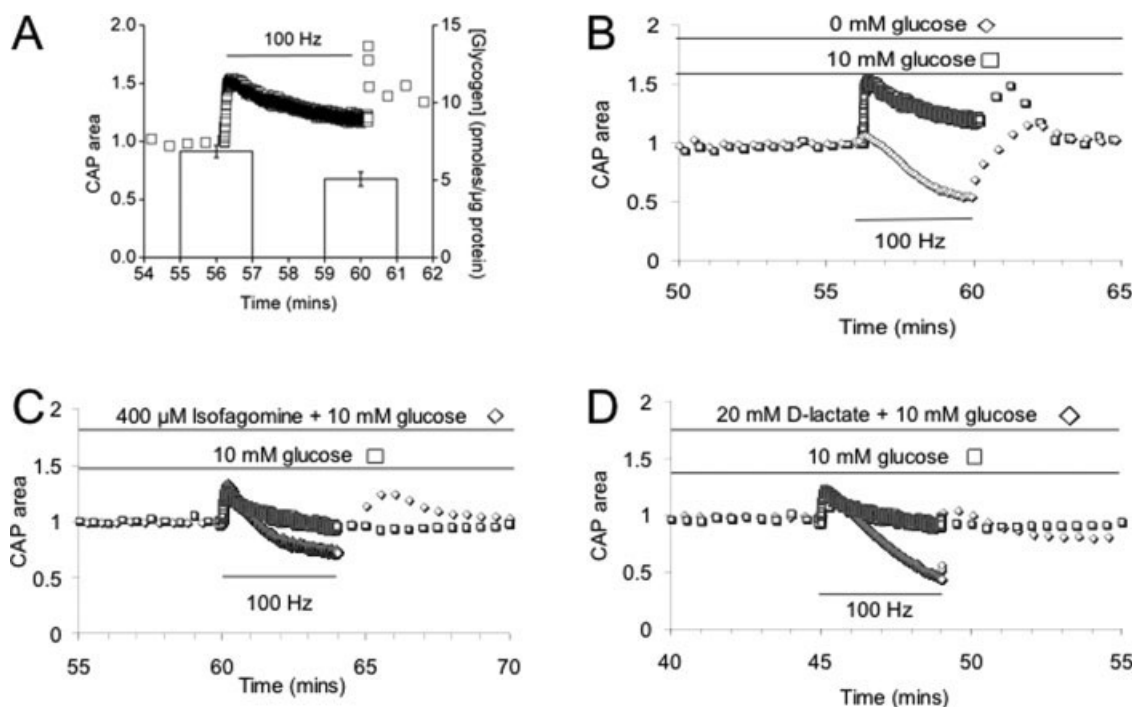


Fig. 4. CAP area during high frequency firing under conditions designed to disrupt astrocyte to axon lactate transfer. (A) In control conditions 100 Hz stimulus for 4 min resulted in an increase in the CAP area due to a spreading out of the CAP profile. After 4 min of 100 Hz firing the glycogen content of the MON was significantly reduced. (B) In MONs pre-treated with a 15 min period of 0 mM glucose to deplete glycogen content, the CAP fell below baseline during 100 Hz firing ($\diamond$), whereas in control MONs the CAP remained above baseline

($\square$). (C) In MONs bathed in 400 μ M isofagomine, an inhibitor of glycogen phosphorylase, the CAP fell below baseline during 100 Hz firing ($\diamond$), whereas in control MONs the CAP was maintained above baseline levels ($\square$). (D) In MONs bathed in 20 mM D-lactate, a competitive inhibitor of L-lactate at the MCT transporter, the CAP decreased below baseline during 100 Hz firing ($\diamond$), whereas in control MONs the CAP was maintained above baseline ($\square$, from Brown et al., 2003, 2005; Tekkök et al., 2005).

erate normally if lactate is substituted for glucose as fuel (Schurr et al., 1988; Wender et al., 2000) and that glycogen breaks down rapidly during hypoglycemia and appears mainly as extracellular lactate (Dringen et al., 1993). Pellerin and Magistretti proposed that lactate from astrocytes might be “shuttled” to neurons as a fuel to account for the coupling between glutamatergic neural transmission and glucose utilization in activated areas (Pellerin and Magistretti, 1994; also see Dringen et al., 1993). Glycogen is obviously too large a molecule to pass between cells, thus in order for glycogen located in astrocytes to benefit neural tissue an intermediary compound must be employed. The most likely candidate is lactate based on the following evidence. Unlike hepatocytes, astrocytes do not express the enzyme glucose-6-phosphatase and therefore cannot create free glucose from glycogen (Dringen and Hamprecht, 1993; Magistretti et al., 1993). However astrocytes do possess the enzyme lactate dehydrogenase, which converts pyruvate to lactate. Astrocytes in culture have been shown to release lactate into the culture medium (Dringen et al., 1993), which not only suggests lactate is the end product of glycogen metabolism, but also suggests that astrocytes possess the means to export lactate (Dringen et al., 1995). The MCT is necessary for transmembrane lactate movement, and the subtype associated with lactate efflux (MCT1) has been located on astrocytes (Koehler-Stec et al., 1998; Pierre et al., 2000). More indirect evi-

dence for lactate efflux from astrocytes is the extracellular space (ECS) lactate concentration of about 1 mM. Recordings with ion-sensitive electrodes show that during aglycemia there is an alkalisation of the ECS (Brown et al., 2001a), in contrast to anoxia and ischemia, which result in acidification of the ECS (Ransom and Philbin, 1992). This alkalization suggests that under normoglycemic conditions there is a constant efflux of lactate from astrocytes (lactate is co-transported with H^+), but during aglycemia the lactate source is limited and the ECS concentration of lactate and H^+ decreases. *In vitro* preparations of neural tissue including hippocampal slice (Cater et al., 2001; Izumi et al., 1997) and optic nerve (Brown et al., 2001b) can use lactate as an energy source in the absence of exogenously applied glucose, implying that neural tissue possesses both the ability to take up lactate as well as the ability to efficiently metabolize it. Indeed neurons and axons possess the MCT subtype (MCT2) expressed in tissue that take up lactate (Bergersen et al., 2005; Pierre and Pellerin, 2005; Pierre et al., 2000) as well as the lactate dehydrogenase subtype that preferentially converts lactate to pyruvate (Bittar et al., 1996), prior to oxidative phosphorylation.

If glycogen is supplying lactate to neural tissue certain predications can be made and experimentally validated. First, decreasing glycogen content in astrocytes should accelerate functional failure during aglycemia.

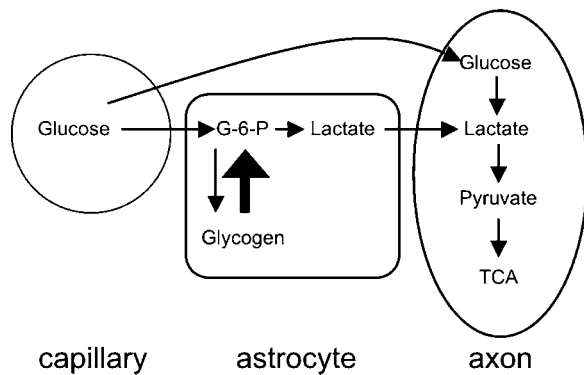


Fig. 5. Model illustrating the role of glycogen in supporting the CAP during increased tissue energy demand in MON. Glucose is transported across the endothelial cell from the capillary into astrocytes. Once inside the astrocyte glucose is phosphorylated to glucose-6-phosphate (G-6-P) by hexokinase. G-6-P is the substrate for both glycogenesis and glycolysis depending on several variables including intracellular G-6-P levels and metabolic status of the tissue. Glycogen can be rapidly degraded to G-6-P by glycogen phosphorylase, the target of isofagomine, which has a high K_m , and is thus a faster enzyme than hexokinase. A portion of G-6-P is metabolized to lactate, which is then transported out of the astrocyte via the MCT1 monocarboxylate transporter, and taken up into the axon by the MCT2 monocarboxylate transporter, the site of action of D-lactate. Intracellular axonal lactate is oxidized to pyruvate by lactate dehydrogenase and oxidatively metabolized. Glucose can also be transported directly into neural elements.

Second, the astrocytes should possess the MCT1 transporter and the axons should express the MCT2 transporter. Third, inhibiting glycogen metabolism should accelerate functional failure during aglycemia. Fourth, interrupting lactate uptake into neural elements should accelerate functional failure during aglycemia. Using the mouse optic nerve as a model we systematically tested these points. We found that glycogen can be up and down regulated dependent upon the concentration of glucose in the aCSF bathing the MON. A 2-hour incubation period in increasing concentrations of glucose resulted in increased glycogen content, the greatest glycogen content associated with the highest concentration of glucose. Subsequent periods of aglycemia resulted in failure of the stimulus evoked CAP, the fastest failure associated with the smallest glycogen content and the longest delay until failure associated with the greatest glycogen content (Brown et al., 2003). Immunohistochemical studies demonstrated the presence of the MCT1 transporter on MON astrocytes but not on axons, whereas the MCT2 transporter subtype was located predominantly on axons but not on astrocytes (Tekkok et al., 2005). The compound isofagomine was used to inhibit glycogen phosphorylase activity, which resulted in augmented glycogen content in MONs bathed in 10 mM glucose or 30 mM glucose. However the CAP failure was accelerated during aglycemia in MONs previously bathed in isofagomine compared with control conditions with nerves bathed in equivalent glucose concentrations in the absence of isofagomine (Fig. 3) (Brown et al., 2005). The stereoisomer of the biologically active compound L-lactate is D-lactate. We took advantage of the fact that MCTs transport D-lactate but it is metabolically inert to investigate L-lactate uptake. In MONS preincu-

bated with 20 mM D-lactate functional failure was accelerated during aglycemia in control MONs not incubated in D-lactate (Fig. 3). In addition, MONs subjected to high intensity stimulation in the presence of D-lactate showed a decline in CAP area whereas control MONs maintained CAP area at baseline levels (Fig. 4) (Tekkok et al., 2005). Taken together these experiments present a convincing argument that in the MON astrocytic glycogen is broken down to lactate and transported out of the astrocyte into the ECS, from which it can be taken up by axons and metabolized under conditions of aglycemia or increased tissue energy demand (Fig. 5). These functions of astrocyte glycogen represent important glial-neuronal interactions worthy of further experimental exploitation.

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