Physiologic coupling of glial glycogen metabolism to neuronal activity in brain[1]

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Brain glycogen is localized almost exclusively to glia, where it undergoes continuous utilization and resynthesis. We have shown that glycogen utilization increases during tactile stimulation of the rat face and vibrissae. Conversely, decreased neuronal activity during hibernation and anesthesia is accompanied by a marked increase in brain glycogen content. These observations support a link between neuronal activity and glial glycogen metabolism. The energetics of glycogen metabolism suggest that glial glycogen is mobilized to meet increased metabolic demands of glia rather than to serve as a substrate for neuronal activity. An advantage to the use of glycogen may be the potentially faster generation of ATP from glycogen than from glucose. Alternatively, glycogen could be utilized if glucose supply is transiently insufficient during the onset of increased metabolic activity. Brain glycogen may have a dynamic role as a buffer between the abrupt increases in focal metabolic demands that occur during normal brain activity and the compensatory changes in focal cerebral blood flow or oxidative metabolism.

Key words: brain, glia, glycogen, glycolysis, hibernation.

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Le glycogène cérébral est localisé presque exclusivement dans les cellules gliales, où il est utilisé et resynthétisé continuellement. Nous avons constaté que l'utilisation du glycogène augmente durant la stimulation tactile du faciès et des moustaches du rat. À l'opposé, l'activité neuronale réduite durant l'hibernation et l'anesthésie est accompagnée d'une augmentation marquée de la teneur en glycogène cérébral. Ces observations appuient l'hypothèse d'un lien entre l'activité neuronale et le métabolisme du glycogène glial. L'énergétique du métabolisme du glycogène suggère que le glycogène glial est mobilisé davantage pour répondre aux besoins métaboliques accrus des cellules gliales que pour servir de substrat pour l'activité neuronale. Un avantage lié à l'utilisation du glycogène serait la production d'ATP potentiellement plus rapide par le glycogène que par le glucose. Le glycogène pourrait aussi être utilisé au début d'une activité métabolique accrue lorsque l'apport de glucose est temporairement insuffisant. Le glycogène cérébral pourrait jouer un rôle tampon dynamique entre les augmentations subites des besoins métaboliques focaux, observées durant une activité cérébrale normale, et les variations compensatrices du métabolisme oxydatif ou du débit sanguin cérébral focal.

Mots clés : cerveau, cellule gliale, glycogène, glycolyse, hibernation.

[Traduit par la rédaction]

Introduction

Glycogen is the largest energy store in brain (Lowry et al. 1964). It has long been recognized that brain glycogen reserves are rapidly consumed during failure of substrate supply, and these energy stores may serve a protective function during ischemia or hypoglycemia (Swanson et al. 1990; Swanson and Choi 1993). However, an additional and perhaps primary function of brain glycogen is suggested by recent findings pointing toward an active, stimulus-responsive metabolism of glycogen under normal conditions in brain. As glycogen is localized almost exclusively to glia, these findings imply a significant metabolic interaction between neurons and glia.

The localization of glycogen to glia in adult mammalian brain has been established in several ways. Firstly, studies using electron microscopy show that glycogen granules are localized only to glial processes, with the exception of certain large motor neurons in brainstem nuclei (Phelps 1972; Borke and Nau 1984; Cataldo and Broadwell 1986). The glial localization is maintained under conditions known to cause a generalized increase in brain glycogen content (Guth and Watson 1968; Phelps 1972). Secondly, primary cultures of astrocytes contain appreciable glycogen stores, whereas cultured neurons contain little or no glycogen (Rosenberg and Dichter 1985; Swanson and Choi 1993). Thirdly, immunohistochemical studies of glycogen phosphorylase *in vitro* (Reinhart et al. 1990) and *in vivo* (Kato et al. 1989; Ignacio et al. 1990) show it is restricted to glia. Whether these glycogen stores serve to fuel glial metabolism or are in some form transferred to neurons has not yet been established (see below).

Unlike peripheral tissues, brain glycogen is relatively insensitive to changes in plasma glucose or hormone levels (Nelson et al. 1968). The concentration of glycogen in rodent brain, expressed as glucosyl equivalents, is approximately 3 mM (Lowry et al. 1964). This may be compared with values of approximately 30 mM in skeletal muscle and 300 mM in fed liver (Nelson et al. 1968). Within brain, glycogen concentrations vary between gray matter structures (Swanson et al. 1989*a*) and between individual cell layers within structures (McCandless et al. 1987). However, these measures of glycogen content provide no information about the relative rates of glycogen metabolism.

Brain glycogen is an active pool, with a half-life in the awake, resting rat of approximately 4.4 h (Watanabe and Passonneau 1973). Turnover of glucose residues at the outer tiers of glycogen granules proceeds at a faster rate than at the limit dextrin, and turnover is slowed during phenobarbital anesthesia. Under normal conditions, the flux of glucose into and out of the glycogen pool represents about 6% of the total brain glycolytic flux (Watanabe and Passonneau 1973). This rapid, continuous turnover suggests that glycogen may play a dynamic role in

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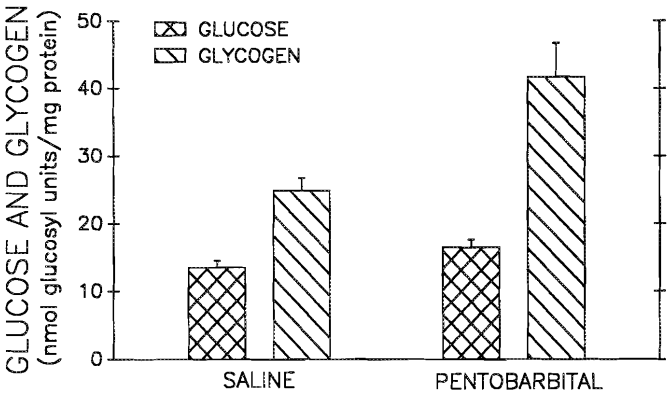

FIG. 1. Glycogen content is increased by 85% in rat cortex during pentobarbital anesthesia ($p < 0.01$, Student's t-test). The small (12%) increase in glucose content did not achieve statistical significance. Bars are means ± SEM. Fasted 200-g Sprague–Dawley rats were given saline or saline plus pentobarbital (65 mg/kg) i.p.; $n = 5$ in each group. Euthermia was maintained with a heat lamp. After 6 h the rats were killed by focused microwave irradiation using a Gherling-Moore model 4104 microwave fixation system (Cober Electronics, Stamford, Conn). (Swanson et al. 1989*a*). Glycogen and glucose were determined by the amyloglucosidase method of Passonneau and Lauderdale (1974) and normalized to protein (Lowry et al. 1951).

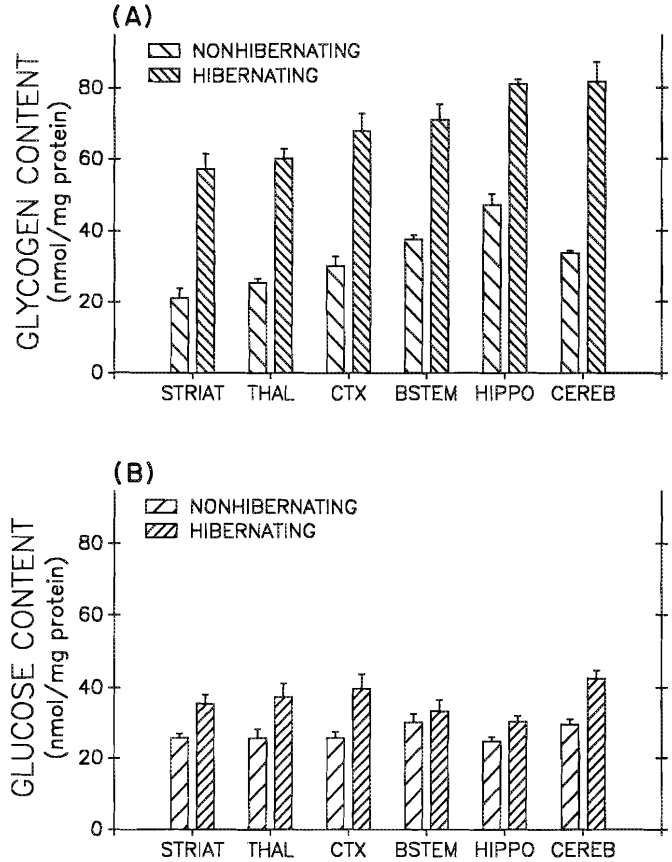

FIG. 2. Glycogen content (A) in ground squirrel brain is markedly increased during hibernation ($p < 0.01$ for each region, Student's t-test), with only modest elevations in regional brain glucose (B). Bars are means ± SEM. STRIAT, striatum; THAL, thalamus; CTX, cortex; BSTEM, brainstem; HIPPO, hippocampus; CEREB, cerebellum. The ground squirrels (*Citellus lateralis*) were generously supplied by Dr. T. S. Kilduff, Stanford University. They were captured in summer 1988 and housed in a 12 h light : 12 h dark photoperiod with food and water ad libitum. Hibernating animals ($n = 7$) were maintained at 4°C and were sacrificed in December 1988. The hibernating state was confirmed by rectal temperatures below 10°C. Euthermic (nonhibernating) animals ($n = 6$) had undergone one cycle of hibernation in captivity and were maintained at 25°C ambient temperature at the time of sacrifice in May 1989. Animals were killed and biochemical measurements made as described in Fig. 1. Regional brain dissections were performed as described by Glowinski and Iversen (1966).

normal brain metabolism rather than acting solely as an emergency energy reserve.

Several authors (Pentreath et al. 1986; Magistretti et al. 1981, 1986) have proposed an active role for glycogen in cerebral energy metabolism on the basis of neuromodulator effects on glycogen turnover. Adenosine, noradrenaline (NE), serotonin, histamine, and vasoactive intestinal peptide (VIP) have been shown to induce glycogenolysis in brain slice preparations (Quach et al. 1978; Magistretti et al. 1986). These agents have also been shown to induce glycogenolysis in primary astrocyte cultures in a dose-dependent manner (Magistretti et al. 1983; Cambray-Deakin et al. 1988*b*). *In vivo*, NE depletion by unilateral locus caeruleus lesions causes slowed glycogenolysis during seizures and ischemia (Harik et al. 1982). The glycogenolytic response to NE and VIP is of particular interest in light of the demonstration by Magistretti and colleagues (1981) of a radially arranged VIP neuronal network in cortex. These authors point out that the radially arranged VIP system could interact with the tangentially oriented noradrenergic fibers to allow focal induction of glycogenolysis in discrete brain regions to accompany or precede increased metabolic demand.

In brain, as in other tissues, glycogen phosphorylase may exist in two forms: phosphorylase b or phosphorylase a. Phosphorylase b is inhibited at normal concentrations of ATP and stimulated by elevated AMP and P_i, such that it is active only when high-energy phosphates are depleted (Lowry et al. 1967; Siesjö 1978). Normally more than 85% of brain phosphorylase is in the b form (Breckenridge and Norman 1965). However, under the influence of adrenergic and other neuromodulators, phosphorylase b is converted to the a form. Phosphorylase a is active under normal intracellular conditions, allowing glycogen to be metabolized prior to a fall in high-energy phosphates (Lowry et al. 1967; Siesjö 1978). Thus, brain appears to share with muscle and other tissues the capacity to mobilize glycogen in preparation for energy demand as well as during energy failure. As described by Magistretti and colleagues, the VIP and noradrenergic systems provide a neuroanatomic substrate that would allow this "anticipatory" mobilization of glycogen in local, functionally discrete regions of brain.

Several lines of evidence suggest that glial glycogenolysis accompanies neuronal activity *in vivo*. Firstly, glial glycogen is known to accumulate during conditions of tonically depressed neuronal activity. These conditions include neuronal loss (Shimizu and Hanuro 1958; Guth and Watson 1968; Pudenz et al. 1975), slow-wave sleep (Karnovsky 1983), and anesthesia (Brunner et al. 1971; Phelps 1972; Watanabe and Passonneau 1973). Our laboratory has studied the effects of barbiturates on rat brain glycogen content *in vivo* and in primary astrocyte cultures devoid of neurons. As seen in Fig. 1, 6 h of pentobarbital anesthesia led to an 85% increase in glycogen in rat cerebral cortex. In contrast, phenobarbital has no effect on cortical astrocytes cultured without neurons (Swanson

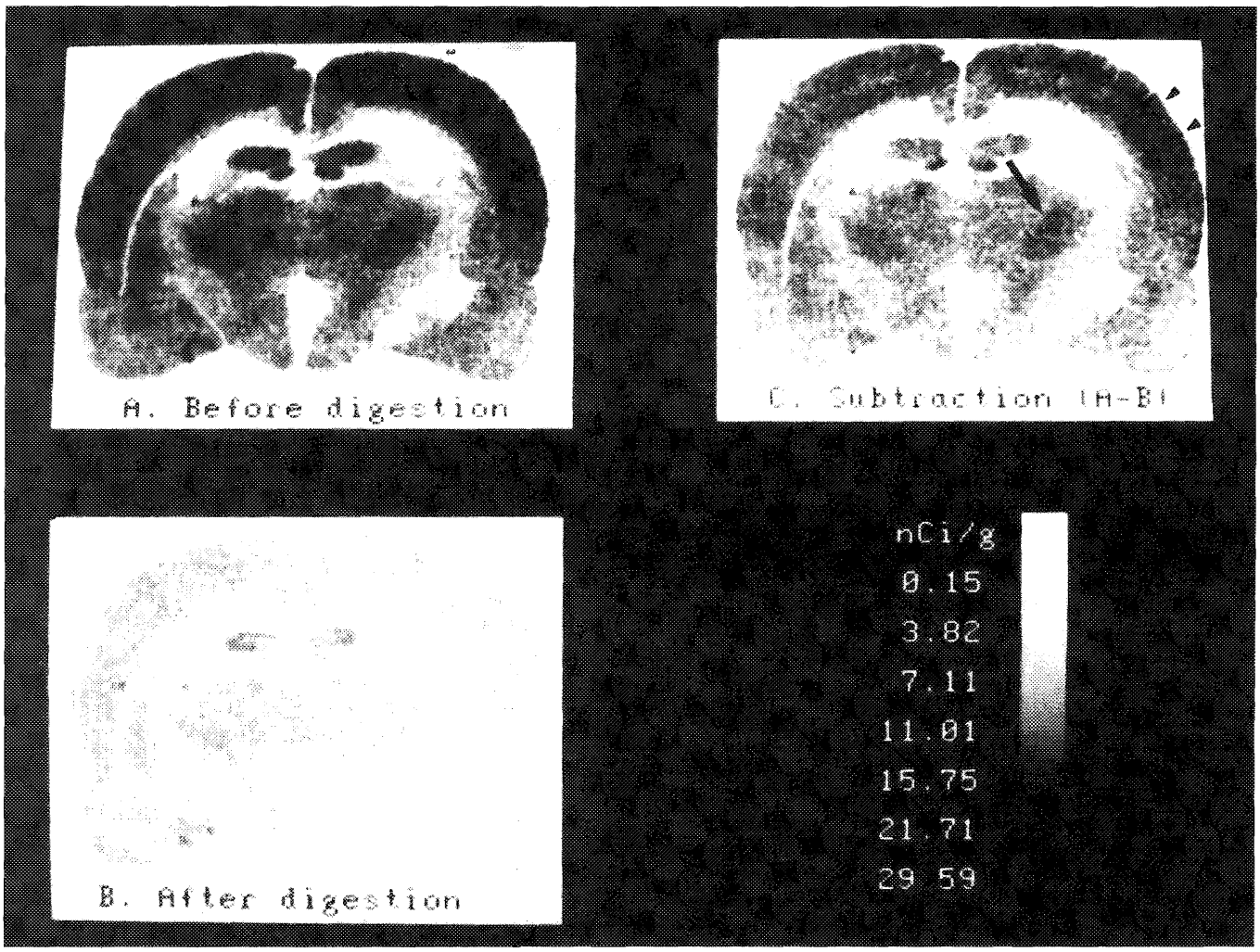

FIG. 3. Utilization of ^{14}C-labeled glycogen is shown by autoradiography. Autoradiographs were prepared before (A) and after (B) removal of glycogen from the section by digestion in amyloglucosidase. (C) Digital subtraction of autoradiograph (B) from (A), showing the distribution of ^{14}C-labeled glycogen in the section. Tactile stimulation of the right face and vibrassae caused a loss of ^{14}C-labeled glycogen from contralateral forebrain structures known to receive sensory input from these areas. Arrowheads (barrel field cortex) and arrow (ventromedial thalamus) denote structures ipsilateral to the tactile stimulation.

et al. 1989*b*). Insulin, methionine sulfoximine, and glucose, which have direct effects on glial metabolism, do increase glycogen stores in cultured astrocytes (Passonneau and Crites 1976; Swanson et al. 1989*b*; Hevor and Delorme 1991). These findings suggest that the effect of barbiturate anesthesia on glial glycogen content *in vivo* may be secondary to depression of neuronal activity rather than a direct effect on glial metabolism.

We have also studied glycogen metabolism in hibernation, another condition in which neuronal activity is reduced. Figure 2 shows brain glycogen and glucose levels in several brain regions of hibernating and nonhibernating ground squirrels. Hibernating animals had markedly elevated glycogen levels in all brain regions examined, with only modest increases in brain glucose levels.

More direct evidence for an association between neuronal activity and glial glycogen metabolism comes from studies performed in retinal preparations. The anatomic separation of neurons and the specialized glia of retina (Müller cells) facilitates discrimination between biochemical events occurring in the two cell types. As shown by Tsacopoulos et al. (1988), glycogen in the honeybee retina is stored in the Müller cells and is absent from retinal neurons. These authors found that repetitive light stimulation accelerates glycogenolysis in the Müller cells, implying a link between retinal neuronal activity and Müller cell metabolism. In further studies using mammalian (guinea pig) retina, glycolysis was found to occur preferentially in the Müller cells (Poitry-Yamate and Tsacopoulos 1991).

Our laboratory has recently developed an autoradiographic method for observing glycogen turnover in the rat brain *in situ*. (Swanson et al. 1987, 1992). In brief, awake rats are injected with ^{14}C labeled glucose, of which a small fraction is incorporated into cerebral glycogen. One hour later one side of the face and vibrissae are gently stimulated with a cotton-tipped applicator for 10 min. This repetitive stimulation has previously been shown to increase glucose utilization in the corresponding sensory regions of brain (Sharp et al. 1988). Immediately following the stimulation the rats are sacrificed by focused microwave irradiation (to abruptly halt brain metabolism) and the brain is anhydrously sectioned onto cover slips. Glucose and small molecular weight metabolites are washed out of the sections in a series of 85% isopropanol washes, and the sections

are placed on X-ray film for 2 weeks to produce autoradiographs (Fig. 3A). The sections are then placed in a solution of amyloglucosidase to remove glycogen, dried, and again placed on X-ray film (Fig. 3B). The autoradiographs of amyloglucosidase-treated sections are digitally subtracted from autoradiographs prepared before glycogen removal to obtain an image corresponding to ^{14}C-labeled glycogen distribution (Fig. 3C). To confirm that the subtraction images reflect ^{14}C labeled glycogen, this procedure was also performed using brains depleted of glycogen by 30 min. ischemia (decapitation) prior to microwave fixation. Sections prepared from the glycogen-depleted brains showed essentially no ^{14}C in the subtraction autoradiograph.

Figure 3 is representative of glycogen autoradiographs prepared from forebrain sections. Somatosensory barrel field cortex and ventromedial thalamus contralateral to the stimulated rat vibrissae show a loss of ^{14}C-labeled glycogen relative to the ipsilateral structures. No asymmetry is seen in hippocampus or basal forebrain. These findings demonstrate a mobilization of glial glycogen stores in brain regions known to exhibit increased neuronal activity in response to the tactile stimulation (Durham and Woolsey 1978; Killackey 1980; Sharp et al. 1988).

The functional significance of glial glycogenolysis under normal conditions remains speculative. One proposed but as yet undemonstrated function of glycogen moblilization in glia is to produce metabolic intermediates for transfer to neurons. However, it is not clear what conditions would make such a transfer useful. Glial glycogen metabolism is maintained at some cost: in addition to maintenance and control of the glycogen metabolizing enzymes, each cycle of glucose into and out of glycogen costs one high-energy phosphate bond (Siesjö 1978). In the presence of extracellular glucose there is no apparent advantage in cycling glucose through a glial glycogen pool prior to its utilization by neurons. Under conditions of glucose insufficiency, glycogen stores could potentially be metabolized in glia and exported to neurons as metabolic intermediates. However, except during profound hypoglycemia, the delivery of glucose to brain greatly exceeds the delivery of extractable oxygen[2]. Consequently, under most conditions, in which glucose supply is inadequate, oxygen supply will also be inadequate and only anaerobic metabolism will be possible. As pyruvate, lactate, and tricarboxylic acid cycle intermediates can generate ATP only through oxidative metabolism, transfer of these intermediates from glia to neurons would not facilitate neuronal ATP production under anaerobic conditions. It is possible that the transfer of glucose or glycolytic intermediates could support glycolytic neuronal ATP production in the absence of oxygen. However, glycogen is cleaved by phosphorylase to form glucose-1-phosphate (G-1-P), not glucose. G-1-P and the other phosphorylated glycolytic intermediates are not known to pass across cell membranes, so rapid egress of these glycolytic intermediates from glia would require a brisk phosphatase activity. Hexose phosphatase activity is very low in brain (Sokoloff et al. 1977; Hawkins and Miller 1978). ATP itself is not known to enter cells (Burnstock 1990).

Rather than supply neuronal metabolism, mobilized glial glycogen stores could serve to fuel the metabolic demands of glia themselves. Neuronal activity increases glial metabolic demands in several ways. Active glial uptake of K^+ from extracellular space can be triggered by elevations in K^+ resulting from increased neuronal activity (Hertz 1978; Syková 1983; Walz 1989). Accordingly, elevated K^+ has been shown to induce glycogenolysis in cultured astrocytes (Cambray-Deakin et al. 1988*a*; Hof et al. 1988). In addition to active K^+ uptake, glial metabolic demands may result from uptake of neurotransmitters and from buffering of extracellular osmolality, volume, and pH (Walz 1989). Each of these processes is dependent on the transmembrane glial Na^+ gradient. Of interest in this regard are reports that in several tissues, including brain, membrane ion transport is fueled preferentially by glycolytically produced ATP (Paul et al. 1979; Lipton and Robacker 1983; Weiss and Lamp 1987; Raffin et al. 1988; Raffin et al. 1992). In addition, glia differ from neurons in that glia are able to survive and function normally for prolonged periods by glycolysis alone (Yu et al. 1989; Callahan et al. 1990), whereas neurons are exquisitely sensitive to hypoxia (Rothman 1984; Choi 1990).

As noted above, there is no energetic benefit to mobilization of glycogen when glucose is available as a substrate. One other potential advantage to the use of glycogen stores is that it may allow compartmentation of glucose metabolism. Evidence for compartmentalization exists in hepatocytes, in which glucuronidation has been reported to occur preferentially with glycogen-derived glucose (Christ and Jungermann 1987; Bánhegyi et al. 1988).

A second possible advantage is that metabolism of glycogen may be accomplished faster than metabolism of glucose. Utilization of glucose begins with carrier-mediated transport across cell membranes and phosphorylation at the expense of ATP. In contrast, glycogen phosphorylase produces G-1-P directly from inorganic phosphate and stored glycogen (Siesjö 1978). Lipton (1973), using a brain slice preparation, observed fluorescence changes suggesting glycogenolysis to occur specifically at the *onset* of neuronal activity, even in the presence of adequate glucose. Electrical stimulation of hippocampal slices in 10 mM glucose caused an immediate, brief reduction of NAD(P) to NAD(P)H, implying an initial mismatch between oxidative and glycolytic metabolism. This effect was abolished by dibutyryl cAMP or prolonged incubation of the slices, treatments that deplete glycogen.

Similarly, glycogen stores may be of critical importance in maintaining glial functions during activation of brain metabolism *in vivo*. A comparison of glucose and oxygen utilization during seizures shows an increase in glucose utilization out of proportion to oxygen consumption, particularly at the onset of seizures (Borgström et al. 1976). More recent studies using positron emission tomography (Fox et al. 1988) and autoradiography (Ackerman and Lear 1989) have shown that physiologic sensory stimuli also cause a disproportionate increase in glycolytic over oxidative metabolism. Sensory stimulation has also been reported to increase local glucose utilization more than local blood flow (Collins 1986; Ginsberg et al. 1987; Ueki et al. 1988).

Taken together, these findings suggest that increased local metabolic demands, particularly at onset, may be met primarily by increased glycolysis. Van den Berg (1983) has suggested that this may reflect a near maximal rate of oxidative brain metabolism at the basal state. Alternatively, the rate of *increase* in ATP production by glycolysis may exceed the rate at which

[2]Normal arterial glucose concentrations are 3–6 mM. Normal arterial O_2 is approximately 8.5 mM, of which a maximum of 35% (3 mM) can be extracted by brain (for review see Siesjö 1978, pp. 398–440. A 6:1 molar ratio of O_2 to glucose is required for oxidative metabolism, such that arterial blood supplies a several-fold excess of glucose over oxygen.

oxidative metabolism can increase in brain, analagous to differences in white and red muscle (Collins 1986). As noted above, the fact that G-1-P is formed directly from glycogen without consumption of ATP could favor the use of glycogen over glucose when a rapid increase in ATP production is needed.

Glycogen utilized under these conditions would be metabolized to lactic acid, analagous to generation of lactate in exercising muscle. Although cerebral blood flow would tend to prevent lactate accumulation, stimulation-induced local elevations in cerebral lactate concentrations have been noted by groups using tissue analysis (Ueki et al. 1988), microdialysis (Schasfoort et al. 1988), and spectroscopy (Prichard et al. 1991). Presumably the accumulated lactate is eventually metabolized oxidatively (Schurr et al. 1988), as net generation of lactate by brain is not known to occur under normal conditions. Borowsky and Collins (1989) have demonstrated a spatial heterogeneity in the ratios of oxidative to glycolytic enzymes in brain, and suggest that like muscle, brain may be metabolically segregated into red (oxidative) and white (glycolytic) compartments. Conceivably, lactate generated in one region may diffuse and be metabolized oxidatively in a neighboring region.

A further use for glycogen during focal brain activation could be to buffer any brief mismatch between metabolic demand and substrate supply. Increased local cerebral blood flow in response to stimulation is fast, but not instantaneous (Leniger-Follert and Hossmann 1979). Between the onsets of increased activity and increased perfusion, glial glycogenolysis could serve to spare extracellular glucose for neuronal use or could allow continued glial functioning during brief periods of glucose insufficiency.

In summary, brain glycogen stores are localized to glia and undergo continuous utilization and resynthesis. Glycogen accumulation is shown to occur during decreased neuronal activity, and repetitive sensory stimuli have been shown to increase glycogen utilization in both brain and retina. It is suggested that glycogen may serve to fuel brief increases in glial metabolic demands at the onset of increased neuronal activity. This role would account for the observations of continuous glycogen turnover under normal conditions in brain, and focal acceleration in turnover in response to repetitive sensory stimulation. Brain glycogen may thus act as a buffer between the abrupt increases in focal metabolic demands of normal brain activity and the compensatory changes in focal cerebral blood flow or oxidative metabolism.

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