

NOT ALL SWEETNESS AND LIGHT: THE ROLE OF GLYCOGEN IN HYPOGLYCEMIC SEIZURES

Factors Which Abolish Hypoglycemic Seizures Do Not Increase Cerebral Glycogen Content *In Vitro*. Abdelmalik PA, Liang P, Weisspapir M, Samoilova M, Burnham WM, Carlen PL. *Neurobiol Dis* 2008;29(2):201–209. Epub 2007 Aug 29. The brain is heavily dependant on glucose for its function and survival. Hypoglycemia can have severe, irreversible consequences, including seizures, coma and death. However, the *in vivo* content of brain glycogen, the storage form of glucose, is meager and is a function of both neuronal activity and glucose concentration. In the intact *in vitro* hippocampus isolated from mice aged postnatal days 8–13, we have recently characterized a novel model of hypoglycemic seizures, wherein seizures were abolished by various neuroprotective strategies. We had hypothesized that these strategies might act, in part, by increasing cerebral glycogen content. In the present experiments, it was found that neither decreasing temperature nor increasing glucose concentrations (above 2 mM) significantly increased hippocampal glycogen content. Preparations of isolated frontal neocortex *in vitro* do not produce hypoglycemic seizures yet it was found they contained significantly lower glycogen content as compared to the isolated intact hippocampus. Further, the application of either TTX, or a cocktail containing APV, CNQX and gabazine, to block synaptic activity, did not increase, but paradoxically decreased, hippocampal glycogen content in the isolated intact hippocampus. Significant decreases in glycogen were noted when neuronal activity was increased via incubation with l-aspartate (500 μ M) or low Mg^{2+} . Lastly, we examined the incidence of hypoglycemic seizures in hippocampi isolated from mice aged 15–19 and 22–24 days, and compared it to the incidence of hypoglycemic seizures of hippocampi isolated from mice aged 8–13 days described previously (Abdelmalik et al., 2007 *Neurobiol Dis* 26(3):646–660). It was noted that hypoglycemic seizures were generated less frequently, and had less impact on synaptic transmission in hippocampi from PD 22–24 as compared to hippocampi from mice PD 15–19 or PD 8–13. However, hippocampi from 8- to 13-day-old mice had significantly more glycogen than the other two age groups. The present data suggest that none of the interventions which abolish hypoglycemic seizures increases glycogen content, and that low glycogen content, *per se*, may not predispose to the generation of hypoglycemic seizures.

COMMENTARY

Matthew Arnold's 1869 essay, "Culture and Anarchy," posits that every culture strives for the ideals of sweetness (beauty) and light (truth, clarity). When applied to the

role of glucose and glycogen in cerebral energy regulation, the sweetness is obvious but the light still eludes our grasp.

Seizures that are due to hypoglycemia can lead to permanent neuronal damage and even death. In the clinical setting, hypoglycemic seizures occur most commonly in individuals with poorly regulated type 1 diabetes, especially at times of altered insulin availability or function. Hypoglycemic seizures also occur frequently in infants of diabetic mothers and in newborns

with asphyxia, sepsis, congenital heart disease, and a variety of hereditary metabolic disorders and endocrinopathies (1,2). A hypoglycemic seizure, as with any seizure, increases energy demand for the very substrate that is lacking—glucose. This ironic situation places the brain at risk for multiple neurologic impairments (3). The outcome of hypoglycemic seizures depends upon the etiology, duration, severity, and recurrence of the hypoglycemia. Because of the potentially severe consequences of hypoglycemic seizures, it is important to understand their pathophysiology as well as to design ways to treat and prevent them (4). Despite the obvious proximate cause (i.e., decreased serum glucose concentration), the exact mechanism by which low glucose induces seizures is not fully understood.

Abdelmalik et al. previously showed that hippocampal explants from young mice (P8–13) exposed to low extracellular glucose (4 mM) developed prolonged spontaneous seizure-like activity in the CA1 region (5). Following this seizure-like activity, there was an irreversible reduction of synaptic activity accompanied by spreading depression-like events. Preventing hypoglycemic seizures with midazolam or nonglycolytic, oxidative substrates (e.g., pyruvate) lessened the extent of the synaptic activity deficit. These observations suggest that preservation of a cerebral energy source might ameliorate hypoglycemic seizure-induced synaptic transmission abnormalities. In this article, the authors asked whether glycogen could serve such a protective function.

Glycogen is the storage form of glucose throughout the body. Most glycogen is stored in liver and muscle, but the brain also contains a small amount of glycogen that can be released at times of glucose need (6). As the brain depends largely on the oxidation of glucose for its energy needs, a readily available reserve of glucose could attenuate the effects of cerebral hypoglycemia. Cerebral glycogen and glucose are in a dynamic equilibrium whereby glycogen stores are regulated by both neuronal activity and by glucose concentration.

Using the sensitive amyloglucosidase method to measure tissue glycogen concentration for this investigation, Abdelmalik et al. showed that glycogen content did not correlate with extracellular glucose concentration. Based on the presumed role of neuronal activity in glycogen regulation, the authors hypothesized that hypoglycemic seizures would decrease glycogen content and that suppressing hypoglycemic seizures would increase glycogen content. However, their results did not confirm that hypothesis. Glycogen content was increased not decreased by blocking neuronal activity (with tetrodotoxin), blocking synaptic transmission (with glutamate or GABA receptor antagonists), or inducing seizures (with iodoacetate, aspartate, or low magnesium).

To test the converse of the hypothesis, the authors measured glycogen content in hippocampal explants under conditions of normoglycemia, hypoglycemia, and hypoglycemia with

neuroprotective substrates added (i.e., pyruvate, midazolam, or the NMDA antagonist aminophosphonovaleric acid [APV]). Compared with normoglycemic controls, there was no difference in glycogen content in any of the conditions, suggesting that hypoglycemia does not alter glycogen stores. Furthermore, in hippocampal explants from slightly older mice (P22–24) that are much less susceptible to hypoglycemic seizures, there was significantly less glycogen than in hypoglycemic, seizure-prone P8–13 mice. Therefore, glycogen content does not seem to correlate with hypoglycemic seizures and the question persists—why does the brain have glycogen (7)?

This article demonstrates that, contrary to expectation, neither increasing neuronal activity via hypoglycemic seizures nor decreasing neuronal activity leads to a compensatory increase in glycogen storage in the hippocampus *in vitro*. The authors conclude that glycogenesis does not necessarily protect against the consequences of hypoglycemia. These results must be interpreted with caution. The experiments were conducted on intact hippocampi *in vitro*, with preservation of hippocampal structure and neural networks; however, the experimental design leaves open room for concern about the possibility of variable oxygen penetration within the thick tissue. This point is important because so much epilepsy research has been carried out in brain slices. Correlation must always be made with the *in vivo* condition (8). As the authors show, when glycogen levels are measured in hippocampal explants incubated for various lengths of time, there is an initial decrease in glycogen content at 1 hour (a commonly used starting time point for *in vitro* electrophysiology) and a return to baseline glycogen level by 2 hours of incubation. This observation has implications for *in vitro* experiments, either slices or explants, especially those involving hypoxia or hypoglycemia (or both). Animal age is also a critical variable: very young mice are more susceptible to hypoglycemic seizures and their irreversible effects on synaptic transmission. Finally, results obtained from the hippocampus may not reflect the effects of hypoglycemic seizures or glycogen content elsewhere in the brain. Indeed, the authors show that blocks of frontal neocortex tissue (1,000 μm thick) are resistant to hypoglycemic seizures and that the neocortex stores even less glycogen than does the hippocampus. Before concluding that a certain neural structure is a critical site of seizure generation, sampling of numerous brain regions during *in vivo* seizure initiation would be necessary (8).

The role of glycogen in cerebral energy metabolism and excitability regulation is certainly complex and not yet fully explained. While the long-term regulation of cerebral energy substrates undoubtedly involves glycogen storage and release, during acute seizures resulting from hypoglycemia, the brain's energy needs might be unmet, leading to the adverse consequences for seizure susceptibility and cognition. Prompt treatment of hypoglycemic seizures may help avert those

complications, possibly in conjunction with neuroprotective agents that work independently of glycogen (e.g., NMDA receptors antagonists, GABA receptor agonists).

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References

1. Yager JY. Hypoglycemic injury to the immature brain. *Clin Perinatol* 2002;29:651–674.
2. Jones TW, Davis EA. Hypoglycemia in children with type 1 diabetes: current issues and controversies. *Pediatr Diabetes* 2003;4:143–150.
3. McNay EC, Williamson A, McCrimmon RJ, Sherwin RS. Cognitive and neural hippocampal effects of long-term moderate recurrent hypoglycemia. *Diabetes* 2006;55:1088–1095.
4. Yamada KA, Rensing N, Thio LL. Ketogenic diet reduces hypoglycemia-induced neuronal death in young rats. *Neurosci Lett* 2005;385:210–214.
5. Abdelmalik PA, Shannon P, Yiu A, Liang P, Adamchik Y, Weiss-papir M, Samoilova M, Burnham WM, Carlen PC. Hypoglycemic seizures during transient hypoglycemia exacerbate hippocampal dysfunction. *Neurobiol Dis* 2007;26:646–660.
6. Gruetter R. Glycogen: the forgotten cerebral energy store. *J Neurosci Res* 2003;74:179–183.
7. Brown AM. Brain glycogen re-awakened. *J Neurochem* 2004;89:537–552.
8. Kirchner A, Velísková J, Velísek L. Differential effects of low glucose concentrations on seizures and epileptiform activity in vivo and in vitro. *Eur J Neurosci* 2006;23:1512–1522.