



Diabetes, Nutrition & Metabolism

Clinical and Experimental

Vol. 15, N. 5

October 2002



SYMPOSIUM ON

“Hypoglycaemia 2001: From Research to Practice”

Assisi, Italy
26-29 May, 2001

Part I



Published by
Editrice Kurtis
Via Luigi Zoja, 30
20153 Milano, Italy

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journals online at
www.kurtis.it

Brain glycogen: An insulin-sensitive carbohydrate store

E.R. Seaquist and R. Gruetter

INTRODUCTION

Glucose is the primary fuel used by the brain under normal metabolic conditions and a constant source of this hexose is essential for normal brain function. Traditional thinking has been that the brain depends on the continuous delivery of glucose across the blood brain barrier for the generation of energy (1), although it has been recognized for sometime that cerebral tissue contains glycogen in micromolar concentrations. Indeed, recent measures in the rat have demonstrated that the concentration of glycogen in the brain exceeds that of glucose under euglycaemic conditions (2).

The transport of blood glucose across the blood brain barrier has been shown to occur via carrier-mediated facilitated diffusion that is stereospecific for D-glucose and not dependent on energy (1, 3-7). The glucose transporter GLUT-1 has been detected in high concentrations in brain endothelial cells (8), and most, if not all, glucose transport across the blood brain barrier has been ascribed to GLUT-1. Interestingly, the insulin sensitive glucose transporter GLUT-4 has also been found at the blood brain barrier (9-13), raising the possibility that insulin may participate in the regulation of glucose transport across the blood brain barrier. Insulin receptors have also been identified at the blood brain barrier and in cerebral tissue (14-16). In these locations, the receptors provide a rapid mechanism through which insulin can participate in the regulation of cerebral energy. Insulin is important in maintaining glycogen content in tissues such as liver and muscle. It is likely that insulin may perform such a similar function in brain as well. In this mini-review, the regulation of *in vivo* cerebral energy metabolism will be discussed. We will first concentrate on a discussion of the regulation of glucose transport across the blood brain barrier and then present information about glycogen metabolism in the brain. Our observations suggest that glycogen may provide an important source of glucosyl units that are sensitive to changes in insulin concentration. We hypothesize that these glucosyl units may sustain brain energy metabolism during periods of profound insulin-induced hypoglycaemia.

GLUCOSE TRANSPORT ACROSS THE BLOOD BRAIN BARRIER

To enter the brain, glucose must first cross the blood brain barrier. Since glucose enters the cerebral compartment by facilitated diffusion, the net glucose flux across the blood brain barrier – which at steady-state equals the glucose consumption rate (CMR_{glc}) – is dictated by the concentration difference between the blood and brain compartments and the permeability-surface area product of the blood brain barrier. Calculation of the rates of cerebral glucose transport and metabolism from the concentrations of glucose in the blood and brain compartments can be accomplished by using the symmetric Michaelis-Menten model (17).

We recently reported a series of experiments in which *in vivo* glucose transport rates were measured in healthy human volunteers (17, 18). In these experiments, steady-state brain glucose concentrations were measured by high field ¹H-magnetic resonance spectroscopy from a gray matter rich volume while plasma concentrations of glucose and insulin were controlled using the glucose clamp technique with somatostatin (17). The relationship between the concentrations of glucose in plasma and brain was found to be linear for the entire range of plasma glucose concentrations studied (4-30 mM), with brain concentrations always substantially below plasma (Fig. 1). The linear relationship between plasma glucose and brain glucose was shown to be a consequence of extending the mechanism to reversible Michaelis-Menten kinetics, which takes into account that at

Diab. Nutr. Metab. 15: 285-290, 2002.

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Key words: Brain glucose, brain glycogen, hypoglycaemia.

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steady-state, the transported glucose is a substrate for backtransport (the reverse reaction). The K_m for glucose transport was 0.6 ± 2 mM and the maximum transport rate was estimated to be 0.69 ± 0.09 $\mu\text{mmol/g/min}$. This K_m for glucose transport is substantially lower than that measured using classical Michaelis-Menten kinetics. However, such a low K_m is consistent with that measured for GLUT-1 mediated glucose transport in the erythrocyte (19), supporting that glucose transport into the brain is largely mediated by the GLUT-1 transporter.

The presence of a linear relationship between brain and plasma glucose in humans was recently confirmed by additional experiments done in our laboratory and in the laboratory of deGraaf in which ^1H NMR spectroscopy was used to examine gray and white matter rich volumes (18, 20). We have also recently shown that relationship between brain and plasma glucose is linear in the rat brain using ^{13}C NMR spectroscopy (21). In these last experiments we also demonstrated that the reversible model was able to accurately predict the point at which brain glucose concentrations approached zero during insulin-induced hypoglycaemia. Taken together, these studies suggest that the apparent K_m for glucose transport in the *in vivo* brain is on the order of 1-3 mM, which suggests that under normo- and hyperglycaemic conditions, glucose influx into the brain is close to the maximal transport rate, T_{max} . In addition, the low K_m of glucose transport implies that the steady supply of glucose for brain cells becomes only progressively impaired with decreasing plasma glucose concentrations, with a gradually increasing deficit in glucose supply. Supporting these conclusions is our observation that glucose transport becomes rate limiting for metabolism in the lightly anesthetized rat at 2.1 mM plasma concentration (22), which was found to be consistent with the plasma glucose concentration at which cerebral blood flow was acutely increased (21).

THE EFFECT OF INSULIN ON RATES OF GLUCOSE TRANSPORT/METABOLISM IN HUMANS

The bulk of glucose transport is believed to be insulin-independent, however, the abundance of receptors for insulin at the blood-brain barrier and in the brain suggests that insulin may act on cerebral carbohydrate metabolism. To determine whether in-

ulin alters the kinetics of glucose transport in humans, we examined the relationship between blood and brain glucose concentrations in a series of glucose clamp studies with somatostatin in which insulin was infused at a rate of 1.0 mU/kg/min (18). In this experiment, the plasma glucose concentration at which steady state brain glucose concentrations were measured varied from 4-25 mM. The concentration of glucose in the occipital cortex was determined from proton spectra acquired using a 4 Tesla magnet. In this study we also compared glucose content in gray matter to that in white matter, which was found to be identical within the experimental scatter. This observation is consistent with the frequently made assumption that brain glucose distributes fairly evenly in the brain between intracellular and extracellular compartments (1, 23). The relationship between blood and brain glucose concentrations in these studies done in the presence of a high physiological level of insulin (108 ± 6 pM) was linear and not different from that defined by the previous studies performed in the absence of insulin (insulin < 30 pM) (Fig. 1). The kinetic constants calculated from these data also did not differ from those calculated from data obtained in the absence of insulin (Table 1). This observation was further sub-

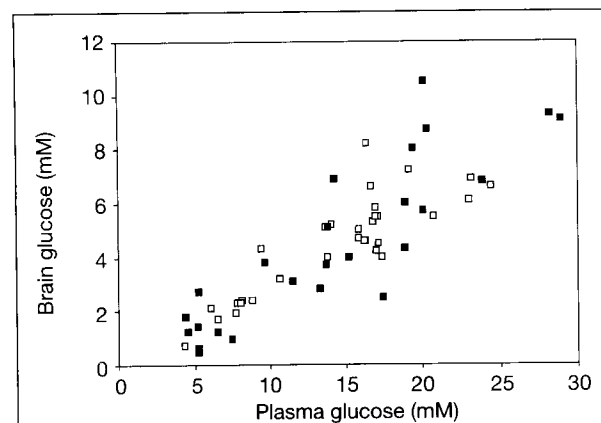


Fig. 1 - Relationship between glucose concentrations in plasma and brain in the presence and absence of insulin. Healthy subjects participated in glucose clamp experiments in which somatostatin was infused at a rate of 0.16 $\mu\text{g/kg/min}$ alone to suppress endogenous insulin secretion (■) or with an insulin infusion at a rate of 1.0 mU/kg/min (□). Each box represents a single experiment. A linear relationship was found between plasma and brain glucose concentrations and the relationship was not altered by insulin. Data are reproduced with permission from (17) and (18).

stantiated by glucose clamp studies in which insulin secretion was suppressed by the infusion of somatostatin and plasma glucose concentrations were maintained at a constant level. In this experiment, the concentration of glucose in the occipital cortex was first measured by ^1H NMR during somatostatin infusion alone when serum insulin concentrations were <20 pM and then again after a 60 min infusion of insulin at a rate of 2.0 mU/kg/min, which raised serum insulin to above 600 pM. The brain glucose concentrations measured under these 2 conditions were not different, supporting the hypothesis that glucose transport across the blood brain barrier is primarily an insulin independent process.

INSULIN AND BRAIN GLYCOGEN

Glycogen serves as an important carbohydrate store and its level varies greatly between tissues. Compared to other tissues, the brain has relatively low glycogen content and the ratio of glycogen content in liver, muscle, and brain is estimated to be 100:10:1 (24). Brain glycogen is localized nearly entirely to the astrocytes in adult brain, but its precise function is unknown. Because of a low phosphatase activity, in brain, most of the glycogenolysis results in additional fuel for glycolysis, that is available for neurons as lactate (25). Lactate is readily transported across cell membranes and is increasingly recognized as a fuel for neuronal metabolism (26). Glial glycogen may also provide fuel to allow continued glutamate uptake into astrocytes and thus reduce hypoglycemic brain injury by reducing glutamate excitotoxicity. Indeed, Swanson and Choi have shown in cell cultures that, *in vitro*, neuronal survival during glucose deprivation is influenced by the glycogen content of glial tissue (27). The role of brain glycogen in maintaining neuronal survival *in vivo* is unknown, but to address this question, particularly in humans, will require the development of new approaches to directly and non-invasively measure

in vivo brain glycogen that can overcome the limitations posed by the *post-mortem* analysis and tissue extraction methods used in the past.

Recently, we demonstrated that these limitations can be overcome by non-invasively measuring glycogen concentrations in rat brain, using newly developed 3-D localization methods (28) and ^{13}C -magnetic resonance spectroscopy (2). In these experiments, lightly anesthetized rats were infused [^{13}C] glucose and the incorporation of ^{13}C into the glycogen C1 resonance was followed for more than 16 hr using a 9.4 Tesla magnet. Incorporation of ^{13}C -glucose into glycogen occurred at a significantly higher rate in the first 60 min compared to 3 hours later. Subsequent infusion with unlabelled glucose (pulse-chase experiment) resulted in a substantially slower rate of label washout (Fig. 2). These experiments demonstrated that basal glycogen turnover was approximately $0.5 \mu\text{mol/g/h}$, and we estimated that 7-10 hours were required to achieve complete isotopic turnover of brain glycogen (2). The discrepancy between label incorporation and washout implied that net glycogen synthesis must have occurred, which was estimated at $\sim 3 \mu\text{mol/g}$ over the first 4 hr of the experiment. The net glycogen synthesis was likely a result of hyperinsulinaemia/hyperglycaemia, since the experimental conditions resulted in an exogenously induced hyperglycaemia of ~ 15 mM. Because pancreatic β -cell function was not controlled, the animals likely responded to the hyperglycaemia with increased insulin secretion, thereby resulting in significant hyperinsulinaemia. This study demonstrates that *in vivo* alterations in plasma glucose/insulin concentrations can affect cerebral carbohydrate metabolism in a substantial fashion. These observations are significant in the context of diabetes, because derangements of plasma glucose and insulin homeostasis are one of the hallmarks of this disease. Therefore, it appears that glycogen metabolism in the brain, like that in other organs, is sensitive to changes in the plasma concentrations of glucose and/or insulin.

Table 1 - Kinetic constants calculated using reversible Michaelis-Menten model.

	K_t (mM)	$T_{\text{max}}/\text{CMR}_{\text{glc}}$
White matter rich region in the presence of insulin*	1.96 ± 2.45	2.15 ± 0.25
Gray matter rich cortex in the presence of insulin*	-0.98 ± 2.13	2.24 ± 0.23
Gray matter rich cortex in the absence of insulin**	0.6 ± 2.0	2.3 ± 0.2

*from 18; **from 17.

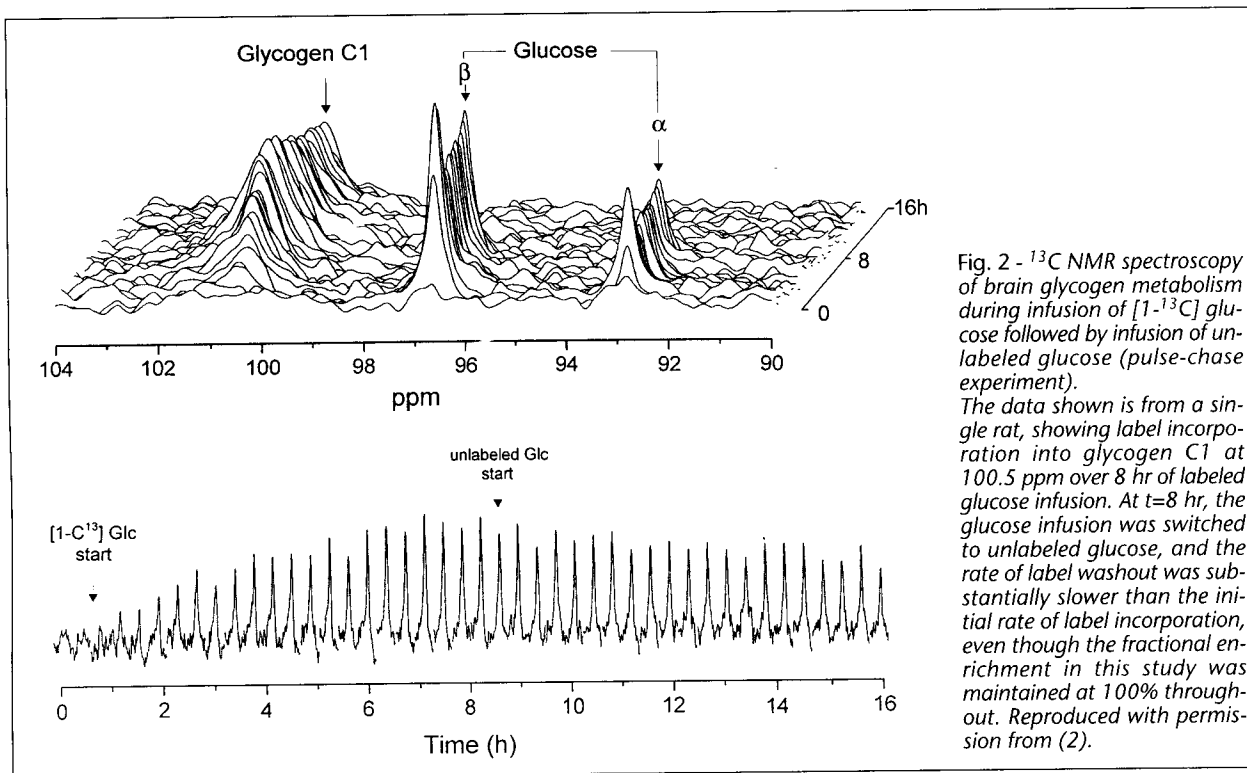


Fig. 2 - ¹³C NMR spectroscopy of brain glycogen metabolism during infusion of [1-¹³C] glucose followed by infusion of unlabeled glucose (pulse-chase experiment). The data shown is from a single rat, showing label incorporation into glycogen C1 at 100.5 ppm over 8 hr of labeled glucose infusion. At t=8 hr, the glucose infusion was switched to unlabeled glucose, and the rate of label washout was substantially slower than the initial rate of label incorporation, even though the fractional enrichment in this study was maintained at 100% throughout. Reproduced with permission from (2).

In a preliminary study, we investigated the effect of insulin-induced hypoglycaemia on brain glycogen concentrations (22). Following an i.v. dose of 6-10 IU of insulin, glycogen degradation began when brain glucose approached zero. The degradation of brain glycogen was gradual and, despite extensive hypoglycaemia for up to 2 hr, never complete. The slow rate of glycogen depletion is consistent with a partial impairment of brain glucose supply being matched at least in part by glycogenolysis. After resuming the glucose infusion and clamping plasma glucose at the pre-hypoglycemic concentration, brain glycogen was rapidly restored and the amount of ¹³C label exceeded the accepted range for normal brain glycogen concentrations by severalfold, leading to a rebound in brain glycogen content. Such a rebound in tissue glycogen content (also termed super-compensation) has been observed in heart, muscle and liver, when repleting in the presence of ample substrate and insulin.

CONCLUSION

The brain relies on a continuous source of glucose from the blood to maintain function. Glucose trans-

port across the blood brain barrier appears to be largely an insulin independent process. However, the brain also contains substantial amounts of glycogen whose metabolism is sensitive to alterations in plasma concentrations of glucose and/or insulin. We propose that cerebral glycogen plays an important role in the brain during periods of severe insulin-induced hypoglycaemia.

ACKNOWLEDGEMENTS

Research reviewed herein was funded by grants from the National Institutes of Health [R01NS35192 (E.R.S.), M01RR00400, P41RR08079, R21DK58004 (R.G.)] and the Juvenile Diabetes Research Foundation [Grant 1-2001-722 (R.G.)].

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DISCUSSION

Question from the floor: Have you performed ³¹P-NMR to examine changes in cerebral ATP levels that occur as brain glucose concentrations approach zero and glycogen is mobilised?

Elizabeth Seaquist: No, we have not done any ³¹P stud-

ies. Doing so would require us to use a different MR procedure than the one done for the presented studies, but since methods are established we could attempt to do this in subsequent experiments.

Question from the floor: We are thoroughly convinced

that there is mobilisation of glycogen. Is the concentration sufficient to offset the energy loss that occurs during hypoglycaemia?

Elizabeth Seaquist: This is a very important question. I think the point is that plasma glucose need not go to zero before brain glycogen begins to break down. Our observations suggest that glycogen mobilisation begins when the plasma glucose concentration drops below the Km of glucose transport, which we have measured to be approximately 1-3 mM. Since we have estimated there to be about 2-4 μmol of glycogen per g of brain tissue, we believe this amount would be sufficient to sustain a 10% reduction in glucose uptake for more than one hour in humans.

Question from the floor: I was intrigued by the observation that 48 hours after hypoglycaemia you had an average 30% increase in brain glycogen concentration. Do you have any data that could correlate the amount of glycogen increase with the duration of hypoglycaemia? Do you have data that demonstrate how long that increase in glycogen lasts? Is it still elevated four, five or seven days after hypoglycaemia?

Elizabeth Seaquist: We have not looked at time points beyond 48 hours nor have we examined the correlation between the duration of hypoglycaemia and the amount ^{13}C incorporation into glycogen after hypoglycaemia.

Question from the floor: To what extent could the C1 in brain glycogen be influenced by changes in the enrichment of the plasma glucose?

Elizabeth Seaquist: In these studies we used 99% enriched $1-^{13}\text{C}$ -glucose and achieved fractional enrichments in the serum of more than 60% before the periods of hypoglycaemia. Based on our previous work, we know that a stable degree of label incorporation into glycogen is achieved using the protocol described. The loss of ^{13}C signal during hypoglycaemia tells us that glycogen is releasing glucosyl units as brain glucose concentrations drop during hypoglycaemia. The reappearance of the ^{13}C glycogen signal after hypoglycaemia may be influenced by the fractional enrichment initially, but the overall magnitude of the signal acquired after recovery exceeds that measured before hypoglycaemia when ^{13}C incorporation into glycogen was stable, telling us the net synthesis of glycogen had occurred.

Hypoglycemia and white matter: Pathophysiology of axon injury and role of glycogen

A.M. Brown, S.B. Tekkök and B.R. Ransom

The clinical introduction of exogenously applied insulin in 1922 has improved the quality of life for millions of sufferers of Type 1 diabetes mellitus. However it also introduced the specter of a potentially fatal side effect in the form of insulin-overdose hypoglycemia. The majority of attention in treating patients with Type 1 diabetes has been to prevent hyperglycemia, because hyperglycemia is associated with the long-term morbidity commonly seen in diabetics, including retinopathy, nephropathy and neuropathy. The use of insulin to rigidly maintain euglycemia prevents, or at least minimizes, these effects, but hypoglycemia can easily develop due to the inability to perfectly match insulin delivery with the minute-by-minute

fluctuations in blood glucose. If untreated, hypoglycemia can result in coma and even death. Fortunately, the body responds to impending hypoglycemia with a series of recognizable signs due

Diab. Nutr. Metab. 15: 290-294, 2002.
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Key-words: Glycogen, rat optic nerve, nifedipine, bepridil, lactate.

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