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Cerebral glucose metabolism in diabetes mellitus

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Abstract

The brain uses glucose as its primary fuel. Cerebral metabolism of glucose requires transport through the blood–brain barrier, glycolytic conversion to pyruvate, metabolism via the tricarboxylic acid cycle and ultimately oxidation to carbon dioxide and water for full provision of adenosine triphosphate (ATP) and its high-energy equivalents. When deprived of glucose, the brain becomes dysfunctional or can be even permanently damaged. Glucose is stored as glycogen within astrocytes with potential importance for tolerance of hypoglycemia. Glycogen may also be important for the metabolic response to somatosensory stimulation and coupling of blood flow and cellular metabolism. Uncontrolled diabetes has a variety of adverse effects upon brain metabolism and function. Many aspects of function that affect the brain may be indirectly linked to cerebral glucose metabolism. Neurotransmitter metabolism, cerebral blood flow, blood–brain barrier and microvascular function may all be affected to varying degrees by either hypoglycemia or uncontrolled diabetes mellitus.

Keywords: Cerebral glucose metabolism; Diabetes mellitus; Hypoglycemia

1. Introduction

The brain uses glucose, largely through oxidative metabolism, as its primary fuel for energy generation. When blood glucose levels are reduced by insulin administration to levels below ~3 mmol/l, subtle brain dysfunction occurs in humans within minutes. Prolonged or profound hypoglycemia leads to coma, seizures and potentially permanent brain damage. There is little short-term flexibility of the brain for facultative use of alternative fuels. Metabolism of glucose non-oxidatively to lactate also occurs in brain and may be important for rapid responses to synaptic activity. The role and quantity of glycogen in the brain are being re-evaluated because of new measurement methods. There are also new conceptual insights into how glycogen helps the brain deal with rapidly changing metabolic circumstances and defend against hypoglycemia.

Normally, the brain depends upon a continuous supply of glucose from the blood stream. Glucose must be transported into the brain through the endothelial cells of the brain microvasculature using facilitated diffusion by transport carrier proteins. Glucose transport and its regulation, which uses many isoforms of glucose transporters, are being recognized as increasingly complex. Energy use within neurons is tightly coupled with local blood flow to brain regions. At least in part this is mediated by signals organized by astroglial cells, which also serve as the primary repository of brain glycogen.

Diabetes mellitus is a disorder caused by a relative (Type 2 diabetes mellitus) or absolute deficiency (Type 1 diabetes mellitus) of the hormone insulin. Diabetes mellitus has long-term complications including accelerated atherosclerosis, retinal microvascular damage causing vision loss, glomerular injury causing renal failure, and peripheral neuropathy leading to amputations. Diabetes mellitus and its most common treatment side effect, hypoglycemia, have multiple effects on the central nervous system. This review summarizes selected aspects of recent understanding of brain metabolism in diabetes and hypoglycemia.

2. Overview of brain metabolism

2.1. Brain metabolic activity and energy use

The brain is very metabolically active; it consumes metabolic energy disproportionate to its size. Representing

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about 2% of body weight in adults, in the resting state, the brain consumes 20% of the body's oxygen use and 15% of the cardiac output (Clarke and Sokoloff, 1999). Developmentally, even more disproportionate energy consumption by the brain occurs (Kinnala et al., 1996). In children by 5–6 years of age the brain's consumption of oxygen may approach 50% of total body consumption at rest. These high rates of energy consumption during development may place children at an increased risk of fuel deprivation. Certainly, much of the evidence that long-term damage occurs as a result of hypoglycemia is noted in children, especially when associated with severe hypoglycemia induced seizures (Kaufman et al., 1999; Rovet and Ehrlich, 1999).

Consumption of energy by the brain is not accounted for by mechanical work as with muscle or by osmotic work as with the kidney. Instead, energy consumption by the brain is largely needed to maintain and restore ionic gradients associated with synaptic transmission. Electrical potentials and active reuptake of neurotransmitters, perhaps especially excitatory neurotransmitters such as glutamate, by energy-dependent processes also consume significant energy. Some synthetic processes also account for significant energy use.

Chemical energy within the brain exists primarily in the form of high-energy phosphate bonds contained in creatine phosphate and adenosine triphosphate (ATP). The levels of creatine phosphate and ATP are extremely labile postmortem and with acute deprivation of glucose and/or oxygen for any extended period. Creatine phosphate levels in brain are higher than those of ATP. The enzyme creatine kinase is very active in brain. Labile high-energy phosphate bonds from creatine phosphate help buffer ATP by providing a ready source of ~P to phosphorylate ADP.

2.2. Glucose as the primary fuel for brain metabolism

Glucose is the primary fuel for the brain (Clarke and Sokoloff, 1999). To a lesser degree, lactate derived from glucose metabolism may also play an important role (Pellerin et al., 2002). The metabolism of glucose in the brain is traditionally thought to be largely through its complete oxidation to CO_2 and water. In general, it is believed that long chain fatty acids are not used to support any significant contribution to brain energy metabolism. This view has been challenged recently by studies using ^{13}C-octanoate incorporation into metabolic pathways in rat brain. The results of these studies have led investigators to propose that up to 20% of brain metabolism, albeit in a compartmentalized fashion, may be provided by fatty acid metabolism (Ebert et al., 2003).

Ketone body (beta-hydroxybutyrate and acetoacetate) metabolism has been known for some time to be able to provide significant energy to the brain in several situations. During development (Cremer and Heath, 1974), with starvation (Owen et al., 1967), high fat feeding and ketogenic diets (Mitchell et al., 1995), and in severely uncontrolled diabetes (diabetic ketoacidosis), these short chain fatty acids may provide a quantitatively significant amount (~60%) of fuel for brain energy metabolism (Ruderman et al., 1974). Based on the distribution within the brain of radiolabeled products from different fuels (e.g., glucose, ketone bodies), it is likely, however, that metabolism of ketones to support energy generation may be restricted to certain areas within the brain (Hawkins and Biebuyck, 1979).

2.3. Brain oxidative metabolism and blood flow in altered glycemia

Brain oxidative energy metabolism in classic early studies was reported to be depressed as a result of extreme hyperglycemia associated with experimental diabetic ketoacidosis and coma (Kety et al., 1948a,b). Depressed fuel utilization could be caused partly by the comatose state and also could be influenced by hypothermia, which is often observed in ketoacidotic diabetes as well as in hypoglycemic coma (Kety et al., 1948a). Animal studies have reported reduced overall glucose metabolism and changes in glucose metabolism regionally with poorly controlled diabetes (Ruderman et al., 1974; Jakobsen et al., 1990). When ketonemia is present in uncontrolled diabetes, it may displace glucose utilization as a source for energy metabolism in brain in a glucose-fatty acid cycle manner. Such findings are not reproduced, in studies of brain metabolism in human diabetic subjects with less severe dysmetabolism however (Fanelli et al., 1998).

In animal models with insulin deficiency and marked hyperglycemia, there is a regionally specific decrease in cerebral blood flow. Duckrow found that hindbrain blood flow was more reduced than forebrain blood flow. Moreover, cerebral blood flow decreases regionally were dependent acutely and chronically upon the degree of hyperglycemia (Duckrow, 1987). In these studies, an osmotic effect was eliminated as a cause since control experiments with isosmotic mannitol showed no cerebral blood flow change. In contrast to hyperglycemia, characteristically in animal models, both acute and chronic hypoglycemia may increase cerebral blood flow, presumably as a compensatory mechanism to maintain adequate delivery of glucose fuel to the brain (Pelligrino, 1990). Chronic hypoglycemia may mitigate the cerebral blood flow increase of subsequent acute hypoglycemia through an adaptive response.

Human studies of cerebral blood flow in hyperglycemia and hypoglycemia are more inconsistent than animal studies. One reason may be that more extreme glucose ranges are often assessed in animal models. Findings of altered cerebral blood flow in human studies could also be confounded by the presence of vascular disease however, making the animal models a better index of functional changes related to glycemic alterations. Diabetes alters

cerebrovascular reactivity and resistance function properties (Lass et al., 1989; Tkac et al., 2001) of blood vessels in the brain. Decreases in cerebral blood flow (Wakisaka et al., 1990), no changes in basal cerebral blood flow but increases in cerebral blood flow with hypoglycemia (Neil et al., 1987) and increases in cerebral blood flow (Grill et al., 1990) of humans with diabetes are reported. Abnormalities in vasoreactivity are commonly reported in humans with diabetes. Abnormalities include impaired autoregulation in Type 1 diabetes mellitus (Kastrup et al., 1986), impaired microvascular reactivity (Kastrup et al., 1990), and abnormal autoregulation during cardiac bypass surgery (Croughwell et al., 1990).

2.4. Importance of glucose transport

To permit normal function and energy metabolism, the brain depends completely upon a continuous glucose supply. This supply must transfer glucose across the phospholipid cell membranes of microvascular endothelial cells that form the blood–brain barrier to reach neurons and glial cells (Clarke and Sokoloff, 1999). The primary method for transfer of glucose into the brain is by facilitated diffusion, an energy independent process. Carrier proteins mediate this transport of glucose.

Glucose transport proteins are thus the physiological basis for glucose entry into the brain across the blood–brain-barrier and also into individual brain cells. A high molecular weight isoform of glucose transporter-1 (55 kDa), which is heavily glycosylated, is the carrier protein for glucose entry at the blood–brain barrier. Other carrier proteins are present in the membranes of different cell types within the brain. Astroglial cells appear to rich in a lower molecular weight (i.e., less glycosylated) glucose transporter-1, which is probably widely distributed in other cells within the brain. Glucose transporter-3 and glucose transporter-8 are glucose transport proteins that exist primarily in neuronal cells and have an apparently complementary distribution (Reagan et al., 2002). Glucose transporter-5 is highly concentrated within microglial cells of the brain (Vannucci et al., 1998). Its inefficiency as a glucose transporter makes this isoform an unlikely source of significant glucose availability to the brain (Shepherd et al., 1992).

Specialized roles for glucose transporter-4 and glucose transporter-2 in relation to insulin-like growth factor-1 (IGF-1) and glucose sensing have been postulated based upon their discrete, selective distribution in some neuronal populations and their biochemical associations in brain (Ngarmukos et al., 2001; Leloup et al., 1994; Leloup et al., 1996; Leloup et al., 1998; Cheng et al., 2000). Glucose transporter-4 expression in brain may be regulated by combined high insulin and glucose levels in some rodent models (Vannucci et al., 1998).

In addition to these long recognized and better-characterized glucose transporter-1–5 isoforms, there are several recently cloned isoforms. Their nomenclature has changed from that originally used (see review by Joost and Thorens, 2001). These include glucose transporter-x1 (now called glucose transporter-8), glucose transporter-9 (now called glucose transporter-6), glucose transporter-10, glucose transporter-11 and glucose transporter-12. The role of glucose transporter-8, which is a hormonally regulated neuronal transporter, is most clearly relevant to diabetes and the brain (Reagan et al., 2000). Glucose transporter-8 appears to be susceptible to regulation by uncontrolled diabetes and stress (Reagan et al., 2000). Glucose transporter-9 may have limited expression in brain in addition to some of the other newly recognized isoforms. Whether they will turn out to be an important in brain cellular transport and altered by diabetes or hypoglycemia needs additional research to clarify.

2.5. Blood–brain barrier and brain cellular transport regulation

Several studies have reported that blood–brain transport of glucose may be downregulated in uncontrolled diabetes (Gjedde and Crone, 1981; McCall et al., 1982, see review in the work of Mooradian, 1997). Conversely, upregulation of blood–brain barrier transport in chronic hypoglycemia has been described (McCall et al., 1986; Pelligrino, 1990; Uehara et al., 1997; Simpson et al., 1999, see review in the work of McCall, 1998). Reduced efficiency of transport in uncontrolled diabetes remains controversial (Simpson et al., 1999), although a number of studies using differing techniques have found this. Unrecognized physiological differences in the models as well as different methods to measure transport might be important to the discrepancies between these studies. Findings of upregulation of glucose transport are consistent in most animal models, but questions remain because of the absence of confirmation in human studies. Additional studies may yield more definitive answers.

2.6. Brain intolerance to hypoglycemia

The primary products of oxidative energy within the brain are high-energy phosphate compounds, such as creatine phosphate and adenosine triphosphate. The metabolic processes in the brain presumably supported by energy generation relate to synaptic transmission and its energy-dependent restoration of ionic equilibrium and neurotransmitter reuptake (axons, dendrites and synapses). A significant proportion of fuel use by the brain occurs in the neuronal perikarya, however. When glucose deprivation occurs, as with acute insulin hypoglycemia in humans, several investigators have reported neuronal dysfunction with abnormal latency of evoked potentials (e.g., P300) after visual or auditory stimuli occurs at blood glucose values of ~ 3 mmol/l (54 mg/dl) in non-diabetic adults

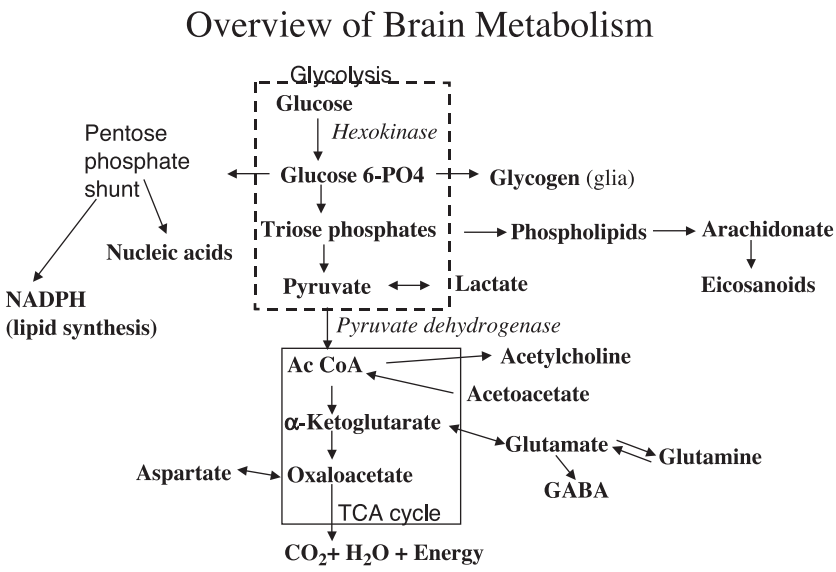

Fig. 1. This schematic illustrates some of the relationships between glucose metabolism and a number of important intermediary metabolites and compounds important for neurochemistry derived from them.

(Mitrakou et al., 1991; Jones et al., 1990, Blackman et al., 1990) (Fig. 1).

3. Brain glycogen

3.1. Glycogen stores in brain

Glycogen is a stored source of glucose present within many tissues. The levels in brain for many years have been estimated to be relatively low when compared to muscle. Clarke and Sokoloff (1999) suggest that estimates for glycogen levels in rat brain are best given at about 3.3 μmol/g. By comparison, muscle and liver glycogen content is estimated to be more than 10-fold greater (Bischof et al., 2001; Iglesias et al., 2002) Glycogen storage is primarily contained within astrocytes; histologic stains for glycogen in the brain usually only reveal significant astrocytic stores.

One physiological implication of these traditionally low glycogen levels in brain has been that stored glycogen amounts would clearly be insufficient to form a substantial reservoir of energy to sustain emergent energy needs by brain. A level of 3 μmol/g is estimated to last no more than about 10 min in the face of fuel deprivation. Thus, glycogen stores are described (Siesjö, 1978; 1988; Clarke and Sokoloff, 1999) as not being relevant to the response to acute hypoglycemia nor adaptations to recurrent hypoglycemia. This view appears to be consistent with the well-described intolerance of human or most other mammalian brains to acute glucose lowering with ensuing subtle or sometimes marked neurological dysfunction.

3.2. Controversy about brain glycogen amount

Recently, however, evidence from several lines of investigation has suggested that glycogen storage in the brain may be substantially greater than previously estimated. One reason for difference in estimates of glycogen levels in the brain may relate to the methods of biochemical assay to minimize rapid postmortem degradation. Another important difference in estimates may be related to the functional role of glycogen and the lability of brain glycogen levels in the brain in relation to neuronal activity (Dienel and Cruz, 2003).

Cruz and Dienel (2002) have reported levels of brain glycogen using the amyloglucosidase procedure in dilute HCl digests of frozen powders or ethanol-insoluble fractions to be much higher than previously reported values including from their own work. In particular, ethanol extract values were substantially higher than previously, even when great care had been taken to minimize in vitro ischemia through use of funnel freezing. They interpret their work as suggesting most prior studies have incomplete prevention of post mortem glycogen degradation.

Another important observation related to in vivo glycogen lability is that levels of resting glycogen were ~ 12 μmol/g in rats not subject to behavorial and sensory stimulation. Thus, brief sensory stimulation appears to accelerate glycogen breakdown. These authors have confirmed that the glycogen levels that they measured were biologically active and responsive to both sensory stimulation and pharmacological manipulations. Most of the brain glycogen was found to be biologically available and breaks down to glucose, glucose-phosphate and lactate. This work suggests that the importance of brain glycogen stores in astrocytes needs re-evaluation. In addition, it seems clear from this

work and that of others that neurotransmitter release associated with sensory stimulation may increase glycogen breakdown (Dienel et al., 2002).

Another technique has confirmed that brain glycogen levels may be higher than prior estimates. The use of high energy focused microwave (which rapidly prevents post mortem glycogen degradation) to assess brain metabolism and glycogen confirms the results of Cruz and Dienel. The highest glycogen levels reported tend to be using 10 W microwave fixation (Kong et al., 2002). Lower energy levels appear to underestimate brain glycogen content presumably because lower microwave energy does not completely prevent glycogenolysis.

In separate investigations using ^{13}C-MR spectroscopy, Choi et al. (1999), Choi and Gruetter (2003), Gruetter (2002), Gruetter (2003), Oz et al., 2003 have also found their estimates of brain glycogen to be greater than traditional estimates. This laboratory has reported that values may be as high as 10 μmol/g. With much higher levels estimated by magnetic resonance spectroscopy and other methods, the importance of glycogen as a store for brain glucose consumption may be different than previously construed.

3.3. Potential importance of brain glycogen to hypoglycemia tolerance

In a recent review, Gruetter (2003) argues that in the presence of hypoglycemia, the recently estimated substantial glycogen stores present in astrocytic cells may provide a buffer allowing brain function to proceed relatively unimpaired by providing available glucose. Choi et al. (2003) and Seaquist and Gruetter (2002) have also found that brain glycogen content is sensitive to the effect of insulin hypoglycemia. After hypoglycemia, a period of supercompensation with elevated glycogen levels of >10 μmol/g occurs within 6–8 h after hypoglycemia (Choi et al., 2003).

Supercompensation of glycogen levels has also been described after sleep deprivation (Kong et al., 2002). In the work by Choi et al. (2001), the plasma glucose at which brain glucose estimates approach 0 and increases in cerebral blood flow occur to compensate for lack of fuel, is about 2.1 mmol/l. Why this value is lower than clinical neurophysiology studies suggest cerebral dysfunction occurs (e.g., latency of waveforms for sensory relay to the brainstem are prolonged) is not clear. It may be that their estimates, which are largely based on reading in an area of occipital cortex, are different significantly for other areas of the brain, potentially including specific hypoglycemia sensor regions such as the ventromedial hypothalamus (Borg et al., 1999, 2003).

3.4. Brain metabolism and insulin

These data on glycogen responsiveness to insulin hypoglycemia might appear to contradict the long held view that the brain is largely insulin insensitive (Bachelard, 1981). It seems clear from work in both animals and humans that the brain is not sensitive to insulin acutely in the same manner that muscle is. Thus, transport of glucose into the brain and oxidative energy metabolism is not acutely (i.e., within minutes) responsive to insulin action in the brain as it is in muscle or fat.

Indeed, Seaquist et al. (2001) using ^{1}(H) magnetic resonance spectroscopy methods have confirmed older data (Bachelard, 1981) and found that insulin has no acute effect upon glucose transport or glucose metabolism in humans. The estimate for brain glucose concentrations in their studies is somewhat higher than others have estimated using different means ($\sim$ 5.5 μmol/g) but they convincingly show using glucose clamp technology combined with magnetic resonance spectroscopy that an increase in plasma insulin from 16 to 668 pmol/l has no impact on brain glucose levels. Similarly, they have also found that short-term hyperglycemia does not appear to affect kinetics of glucose transport over a short period of time using a glucose clamp. Interestingly, work from Bingham et al. (2002) has recently suggested that basal insulin levels may influence brain glucose uptake in a regionally specific manner with the greatest effect upon cortical regions and least effect upon cerebellum.

Nonetheless, despite the absence of an acute effect of insulin on glucose transport and metabolism, several different aspects of brain metabolism from neurotransmitter reuptake (Figlewicz et al., 1996) to glycogen metabolism (Choi et al., 2003) clearly do respond to insulin action. Moreover, there is a substantial body of evidence to suggest that appetite control in also influenced by insulin action within the brain and that insulin action as a satiety factor in the brain is mediated at least partly by neuropeptide Y (Sipols et al., 1995; Sindelar et al., 2002; Niswender and Schwartz, 2003). Insulin effects on the hypothalamus in particular may use the PI3-kinase pathway and overlap with the role of leptin in control of body adiposity (Niswender et al., 2003). Moreover, even the insulin responsive glucose transporter, glucose transporter-4, is well described in a number of studies (Ngarmukos et al., 2001; Leloup et al., 1996; Cheng et al., 2000) as being present in discrete areas of the brain and in small amounts within the microvascular endothelial cells of the brain (McCall et al., 1997).

4. Role of oxidative glucose metabolism

4.1. The traditional view

The metabolism of glucose in the brain is thought to be primarily oxidative (Clarke and Sokoloff, 1999). The cerebral metabolic rates for oxygen are almost exactly six times higher than the cerebral metabolic rate for glucose. This means that complete oxidation of glucose normally occurs and the respiratory quotient (RQ) of the

brain is about 1. Thus, the overall energy metabolism in the brain would be given (Shulman and Hyder, 2001) as the following equation:

$$C_6H_{12}O_6 + 6O_2 \rightarrow\rightarrow 6CO_2 + 6H_2O$$

4.2. The astrocyte-neuron lactate shuttle

The full oxidation of glucose to carbon dioxide and water however may not always occur (Shulman and Hyder, 2001; Shulman et al., 2001). There remains a lot of interest in and controversy about the importance of an alternative pathway of metabolism in the brain (Pellerin et al., 2002). This has been referred to as the Astrocyte-Neuron Lactate Shuttle hypothesis or ANLSH. It has also been called the glycogen shunt as it invokes an important role for astrocytic stores of glycogen. The notion of this shuttle originally comes out of observations from imaging studies in which Fox and Raichle (1986) and Fox et al. (1988) found that with visual or somatosensory stimulation the change in cerebral blood flow and cerebral metabolic rate for glucose rose by about 50% while that for oxygen rose only about 5%. The conclusion reached was that stimulation of brain activity was supported metabolically by glycolysis. Since there is sufficient oxygen in the brain tissue, this is so-called aerobic glycolysis. A further important observation is that cerebral glucose oxidative metabolism is linked to glutamate transmitter release and the glutamate/glutamine cycle within brain (Pellerin et al., 2002). An important role for glycogen as a source for glycolysis within the astrocyte is postulated as a part of this hypothesis.

Chih and Roberts (2003) have recently reviewed the ANLSH critically. In a companion commentary, it has been defended by two of its major proponents, Pellerin and Magistretti (2003). In short, the hypothesis proposes that with brain activation (as for example may occur as a result of somatosensory stimulation), the release of glutamate, an important excitatory neurotransmitter, occurs. With glutamate release into the synaptic cleft, the astrocytes remove the synaptic glutamate by an energy- and sodium-dependent process using high affinity glutamate transporters (monocarboxylic acid transporters or MCT). Activation of the enzymes glutamine synthetase and Na^+-K^+ATPase occurs in astrocytes; as a result, there is an increase in both intra-astrocytic glutamate and sodium.

With the activation of Na^+-K^+ATPase, there is a reduction of astrocytic ATP. The astrocyte regenerates the ATP through glycolysis. The generation of lactate from astrocytes is hypothesized to be exported using the astrocytic monocarboxylic acid transporters (MCT1) and lactate then enters neurons through a different MCT (MCT2) into the neuron (Debernardi et al., 2003; Rafiki et al., 2003). According to the ANLSH, the neuron then oxidatively metabolizes lactate (as well as glucose) to support the increase brain activation and neuronal firing that started the process off. The restoration of cellular sodium distribution is thought to be an especially important aspect of this energy metabolism.

It is important to realize that the ANLSH may be largely dependent upon the release of specific neurotransmitters. While evidence exists for this pathway as important in response to the excitatory neurotransmitter glutamate (Chatton et al., 2000), even its proponents find no evidence for its importance with the common inhibitory neurotransmitter, gamma amino butyric acid, also known as GABA (Chatton et al., 2003).

4.3. Controversy about the ANSLH

In a recent critique (Chih and Roberts, 2003) of the ANLSH, three major differences between ANLSH and the conventional theory of oxidative glucose metabolism are noted. First, these theories differ in the site (astrocyte vs. neuron) and mode (glycolysis vs. oxidative metabolism) of glucose utilization. Second, the site of lactate production (astrocyte) and its use (neurons) differ in the ANLSH compared to the traditional view of neuronal oxidative glucose metabolism. Third, the timing and role of lactate use are also different. In the conventional view, neurons (to a very large degree) and astroglia are primarily generating energy through oxidative glucose metabolism. The conventional view assumes glycolytic production of lactate is relatively proportionate with oxidative metabolism and at most transiently exceeds oxidative glucose metabolism. Another big difference between the conventional theory and the ANLSH model is that glycolytic lactate utilization is thought in the conventional theory to be significant primarily when lactate production is relatively low. One can understand some of the objections about the ANLSH raised by Chih and Roberts (2003), as this theory seems inherently inefficient to cause this shuttling of glucose to lactate and then lactate from astrocytes to neurons. Nonetheless, there is considerable support for its presence and for lactate utilization by neurons both in vivo and in vitro. Whether diabetes mellitus and/or hypoglycemia affect this fuel shuttle is not yet known but may deserve further investigation especially in light of the increased importance of glycogen in the brain.

5. Effects of short-term changes in plasma glucose on brain

5.1. Hypoglycemia

Hypoglycemia of brief duration and moderate severity (far short of that needed to cause coma or an isoelectric electroencephalogram) may produce several negative effects on emotion, cognition and motor function (Gonder-Frederick et al., 1997). Early descriptions of hypoglycemia symptoms include sensory and motor aphasia, loss of fine motor control in the hands, dysarthria, confusion, disorientation

and even delirium (Fletcher and Campbell, 1922). Early controlled studies of mild to moderate hypoglycemia in those with Type 1 diabetes mellitus showed slowed reaction times to cognitive testing but little change in testing accuracy (Holmes et al., 1983). Hyperglycemia also may impair reaction time although less than hypoglycemia (Cox et al., 2002). Pramming et al. (1986) studied the effects of hypoglycemia on several psychomotor tasks and reported that complex tasks were more affected than simple tasks like finger tapping. Prompting of individuals with hypoglycemia may be required during testing (Deary, 1993). Testing done without such prompting may falsely reduce the accuracy of test results.

In experimental hypoglycemia, visual-evoked potentials and auditory-evoked potentials may be significantly delayed. P300 latency from visual or auditory stimuli become prolonged during moderate hypoglycemia (2.6 mM; 47 mg/dl) (e.g., see Blackman et al., 1990). Importantly, these evoked potentials do not return to normal for 45–75 min after restoration of normal blood glucose concentration. This delay suggests a metabolic "stunning" of brain function persists after hypoglycemia; the metabolic basis for this stunning is not known. In this study, another type of evoked potential (P140; a measure of sensory processing) was normal during hypoglycemia. The authors concluded that neither sensory nor motor processing per se was abnormal as a result of hypoglycemia. They instead concluded that there was an abnormality in decision making.

If this interpretation about hypoglycemia impairing decision making is correct, it implies an increased risk for patients who have syndromes of either hypoglycemia unawareness or defective hypoglycemic counterregulation. These syndromes appear to represent an acquired form of hypoglycemia associated autonomic failure related to some aspect of recurrent moderate hypoglycemia primarily found in Type 1 diabetes mellitus but also seen in longstanding insulin treatment of Type 2 diabetes mellitus (Cryer, 2002). If complex cognitive function and decision making are impaired by moderate hypoglycemia these defects may alter the ability to self treat and recover. The persistence of cognitive performance problems is greater with hypoglycemia experienced by those with hypoglycemia unawareness (Gold et al., 1995).

5.2. Hyperglycemia

Hyperglycemia of relatively short duration may affect cognitive function. Davis et al. (1996) examined the effects of transient hyperglycemia (20–30 mM; 360–540 mg/dl) in children with Type 1 diabetes and found a reduction in performance intelligence quotient with a mean of nearly a 10% decline in percentile score. Findings contrary to Davis et al. however came from Draelos et al. (1995) in a study of short duration type 1 diabetes mellitus in adults with a wide range of glycemic control and graded hypoglycemia to hyperglycemia. These investigators reported that only with hypoglycemia did cognitive function worsen. Moreover, they found that glucose values of 21 mM did not impair cognitive performance.

Pennebaker et al. (1981) reported that acute hyperglycemia in 30 hospitalized diabetic patients was reliably associated with mood disturbances and/or physical symptoms more than half the time. This observation might have bearing on cognitive performance. Based on self-monitoring and cognitive assessment involving a handheld computer, Kovatchev et al. (2001) have found that graded hyperglycemia is associated with deterioration in verbal fluency and speed of performing mental subtractions in treated insulin dependent diabetic subjects. An unknown in these studies of hyperglycemia and hypoglycemia with brain function is the extent to which abnormal glucose and energy metabolism are an important correlate or perhaps a determinant of the abnormal brain function.

5.3. Effects of hyperglycemia and hypoglycemia on brain microvasculature

The brain microvasculature is less affected by diabetes than retinal vessels (McCall, 1992a,b). Moreover, there is no clear syndrome of vascular leakage of lipoprotein or hemorrhage in the brain with poor diabetes control as there is seen in diabetic retinopathy. Despite this, microvessels in the brain are similar to those of the retina. Their cellular composition is similar and includes endothelial cells, smooth muscle cells and pericytes. One notable difference between retinal and brain microvasculature is a reduced number of pericytes, a type of cell especially vulnerable to adverse metabolic impact of hyperglycemia (Sussman et al., 1988). Pericytes also may exert important influences upon microvascular flow.

Pathological abnormalities do exist within the brain microvasculature. Loss of pericytes can occur in brain capillaries (Mukai et al., 1980). Other pathological changes in the microvasculature of the brain, including thickening of basement membranes, occur in longstanding diabetes (Reske-Nielsen et al., 1965; Johnson et al., 1982).

5.4. Microvascular metabolism in diabetes and hypoglycemia

Metabolic abnormalities in vitro of the cerebral microvessels occur in animal models with uncontrolled diabetes and in recurrent hypoglycemia (McCall, 1992a,b). Hingorani and Brecher et al. (1987) first observed a reciprocal relationship between glucose and fatty acid oxidation by isolated rabbit brain microvessels, suggestive of the glucose-fatty acid cycle (Randle et al., 1963). Moreover, in uncontrolled diabetes, a depression of glucose oxidation in the absence of an effect upon microvascular fatty acid oxidation was found. In other studies, glucose oxidation was reduced by more than 50% and glycolytic flux was also

reduced in brain microvessels from rats with uncontrolled diabetes (McCall et al., 1984, 1988). Fuel metabolism was normalized in the brain microvessels by insulin treatment for 48 h or by starvation (presumably both by returning glucose levels to normal). Uncontrolled diabetes appears therefore to reduce glucose metabolism in both the brain microvessels and in the brain parenchyma (Ruderman et al., 1974; Jakobsen et al., 1990).

Diabetes is commonly overtreated through inappropriate (i.e., non-physiological) or excessive doses of exogenous insulin. In chronic hypoglycemia (produced in rats by subcutaneous grafting of insulin producing tumors), the glucose metabolism of isolated brain microvessels was accelerated (McCall et al., 1988). A reciprocal relationship between oxidation of glucose and that of beta-hydroxybutyrate (the primary ketone body; a short chain fatty acid fuel) was observed in microvessels from brain of chronically hypoglycemic rats. By contrast, in microvessels from diabetic rats, beta-hydroxybutyrate oxidation was increased as glucose oxidation declined. Shorter-term hypoglycemia produced by insulin pumps or insulin injection produced no consistent change in metabolism of isolated brain microvessels. No changes in the ATP levels or ATP/ADP ratios were found in brain microvessels from diabetic or insulinoma engrafted rats. The consequences of these changes in microvessel energy metabolism and substrate utilization are unknown. They could in theory enhance oxidative stress by the microvessels.

Mooradian and colleauges (Mooradian, 1988, 1997; Mooradian and Smith, 1992; Mooradian and Scarpace, 1992; Mooradian et al., 1994a,b) have observed a number of other abnormalities in brain microvessels in uncontrolled diabetes. Lipid peroxidation byproducts such as conjugated dienes were found to be increased after 5 weeks of streptozotocin induced diabetes mellitus (Mooradian and Smith, 1992). Such oxidative stress changes could be related to a switch from glucose oxidative metabolism to greater metabolism of fatty acids and ketone bodies in the brain microvasculature. Beta receptor number is not changed but post receptor activity as indicated by isoproterenol induced adenylate cyclase activity is affected in cerebral microvessels from rats with uncontrolled diabetes mellitus (Mooradian and Scarpace, 1992). Reagan et al. (2002) has found evidence of lipid peroxidation (4-hydroxy-2-nonenal) byproducts that may link to and potentially modify the intrinsic activity of the neural glucose transporter, glucose transporter-3.

5.5. Effects of profound hypoglycemia upon neurochemistry

Acutely depriving the brain of adequate glucose affects the concentrations of many compounds indirectly linked to glucose metabolism in the brain (McCall, 1992a,b, 1993, 1997, 1998, 2002; McCall and Figlewicz, 1997). Included among these are arachidonate, acetylcholine, aspartate and glutamate, and of course, if sufficiently profound, lack of cerebral energy generation (McCall, 1993). Partial depletion of high-energy phosphate compounds (ATP and creatine phosphate) occurs. Cerebral oxygen consumption declines due to substrate lack. Cerebral metabolic rates for glucose are reduced to a greater extent than oxygen consumption. Levels of citric acid cycle intermediates are depressed. Lipolysis is accelerated with a resultant increase in polyunsaturated free fatty acids, particularly arachidonic acid (Agardh et al., 1980, 1981; Agardh and Siesjö, 1981).

Profound hypoglycemia also decreases cerebral protein synthesis and reduces incorporation of amino acids into protein in a regionally specific manner. Areas most affected in the brain are those most damaged by hypoglycemia. In rat models of profound hypoglycemia, which produce an isoelectric electroencephalogram, several additional neurochemical effects occur (Siesjö, 1988).

With profound hypoglycemia, loss of ionic homeostasis occurs: brain extracellular potassium rises (Wieloch et al., 1984) while extracellular calcium falls (Harris et al., 1984). Intracellular calcium increases are thought to activate a number of degradative enzymes including proteases, nucleases and lipases whose action may lead to mitochondrial damage and swelling (Auer et al., 1985a,b; Kalimo et al., 1985). Extracellular pH rises initially and eventually intracellular pH rises with alkalosis itself helping to minimize the extent of damage (preventing widespread necrosis) perhaps by altering glucose metabolism (Pelligrino and Siesjö, 1981; Pelligrino et al., 1981). Loss of high-energy adenine nucleotides and depletion of the nucleotide pools occurs (Agardh and Siesjö, 1981; Agardh et al., 1980, 1981).

Levels of amino acids and amino acid neurotransmitters, many of which are indirectly derived through metabolic intermediates from either glycolysis or the citric acid cycle, are affected by profound hypoglycemia (Lewis et al., 1974; Norberg and Siesjö, 1976). Decreased concentrations of glutamate, glutamine, gamma-aminobutyric acid (GABA) and alanine occur although it is believed that intrasynaptic concentrations of glutamate may rise. Levels of aspartate and ammonia may rise.

These excitotoxic amino acids (glutamate and aspartate) bind selectively to dendrites and perikarya of neurons and by doing so produce selective neuronal necrosis. Moreover, the energy-dependent reuptake, which astrocytic cells use to help detoxify these excitoxic amino acids, is reduced by reduced energy availability. Profound hypoglycemia causes excitotoxicity mediated through NMDA receptors. This is indicated by the ability of NMDA receptor blocking drugs to ameliorate brain damage from hypoglycemia (Wieloch, 1985). Such insights into NMDA receptor involvement have not led to a practical remedy to prevent or repair severe hypoglycemic damage in susceptible individuals however.

Perhaps more promising is a newly recognized pathway implicated in the development of the neuropathology of profound hypoglycemia, which has been recently described (Suh et al., 2003). DNA damage activates the enzyme poly

(ADP-ribose) polymerase-1, which although normally involved in DNA repair, may perpetuate hypoglycemic pathology when the enzyme is activated by DNA damage. Poly (ADP-ribose) polymerase-1 inhibitors protect against greater than 80% of pathological changes from profound hypoglycemia even when administered after the insult.

6. Summary and conclusions

Glucose metabolism in the brain remains the primary means to generate the large amount of energy required to fuel synaptic action and ionic homeostasis. The brain is intolerant of glucose deprivation for even short intervals. Stored glycogen within astrocytic cells of the brain is probably a more substantial reserve of glucose energy than previously thought. It seems likely that insulin hypoglycemia alters glycogen reserves in the brain with a period of supercompensation. The role of astrocytes in brain fuel metabolism is increasingly thought to play a role in supporting the rapid demand of somatosensory stimulation energy needs. The proposed astrocyte neuronal lactate shuttle hypothesis while somewhat controversial appears likely to play a role in brain energy economy with glutamatergic neurotransmission. One aspect of its importance is that it appears to provide a basis for intercellular communication between astrocytes and neurons based on rates of metabolic demand.

Uncontrolled diabetes mellitus in the extremes affects overall oxidative metabolism of the brain and substitution of fatty fuels such as ketone bodies may occur in ketotic diabetes. Moreover, extreme hyperglycemia may alter cerebral blood flow. The extent to which these observations are important in less severe metabolic disturbances in human diabetes is not clear yet. Subtle or regional changes might occur as there is some evidence of brain dysfunction both with hyperglycemia and with hypoglycemia of moderate degree. The role of the astrocyte and of glycogen stores in tolerance to acute and chronic hypoglycemia needs further study. Multiple metabolic and other abnormalities occur in brain blood vessels with uncertain consequences for vascular function.

Severe hypoglycemia affects the function and biochemistry of the brain dramatically. Much more needs to be understood about how the brain adapts to more chronic and repeated moderate hypoglycemia. The hope is that a greater understanding of how brain metabolic abnormalities tied to hyperglycemia and hypoglycemia occur may lead to targeted remedies to prevent adverse consequences to the brain in people with diabetes mellitus.

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