Hyperglycemia and neuronal damage in cerebral ischemia and beyond

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Abstract

The aggravation of cerebral ischemic neuronal damage by preischemic hyperglycemia has been lauded as the proof that lactic acidosis is a major detrimental factor in such damage. This phenomenon has steered clinicians to attenuate blood glucose levels as a means of brain protection against the risk of ischemia during cardiopulmonary bypass surgery and neurosurgical procedures. Researchers who use in vivo models of ischemia have repeatedly confirmed this paradoxical phenomenon where the only energy substrate capable of supporting ATP formation in the absence of oxygen must be avoided. Consequently, lactate, too, acquired a bad reputation among clinicians and researchers alike. The only investigators to defend glucose as a possible neuroprotectant have been those who use in vitro models of ischemia/hypoxia, where the higher the glucose concentration preischemia, the lower the degree of neuronal damage postischemia. Nevertheless, after almost three decades since the inception of the lactic acidosis hypothesis of cerebral ischemia, no direct proof has been presented in its support. Recent studies in our laboratory provide evidence that refutes the lactic acidosis hypothesis of cerebral ischemia and offer a different explanation for the glucose paradox of cerebral ischemia in which hyperglycemia-induced increase in the release of the stress hormone corticosterone, not lactic acidosis, is responsible for the aggravation of the ischmic damage. These studies led us to explore the role of stress hormones in diabetic hyperglycemia and neuropathy.

Introduction

The seminal paper by Myers and Yamaguchi [1] on the aggravation of ischemic brain damage by preischemic hyperglycemia has initiated an on-going debate among both scientists and clinicians on the pros and cons of glucose administration prior to surgical procedures and especially prior to neurosurgical procedures.
Hypoglycemia-related acidosis is a major mediator of ischemic brain damage, correlating a decrease in both extra- and intracellular pH with the increase in damage. To support their argument, the authors claim that normoglycemic animals with superimposed hypercapnia showed aggravation of ischemic damage, though not identical to the aggravation produced by hyperglycemia. Moreover, they demonstrate that despite the shorter period of calcium “overload” observed in hyperglycemic animals in comparison with normoglycemic ones, the damage in the former was greater which, by itself, is a paradox, unless one assumes that extra glucose allows for longer glycolytic maintenance of ion homeostasis during ischemia. In addition, the authors minimized the relevance of *in vitro* findings that indicate acidosis to protect neuronal tissue against hypoxic/excitotoxic damage, possibly via desensitization of the NMDA receptor [19-21]. All of the studies on hyperglycemia-aggravated ischemic brain damage cited above relied on experiments in which hyperglycemia was induced by glucose administration shortly (10-45 min) before ischemia. The importance of this fact must not be overlooked and will be elaborated on later. Evidently, the ability of mild acidosis to protect brain tissue in vitro against hypoxic and/or excitotoxic neuronal damage did not help in refuting the lactic acidosis hypothesis of cerebral ischemia. Even the demonstration that brain tissue in vitro can function normally when lactate is the sole energy substrate [22] did not shake the wide acceptance of this hypothesis has enjoyed. However, these studies have succeeded in raising the awareness of investigators to the fact that lactic acid is probably not a toxic metabolic end product.

**The Role of Lactate in Cerebral Ischemia**

Intriguingly, in vivo studies, for the most part, could reproduce the preischemic hyperglycemia-aggravating effect on the recovery of neuronal function postischemia, supposedly, via intensification of acidosis; all that in vitro studies could demonstrate with increased glucose was an improvement of the postischemic/hypoxic outcome and a possible benefit from acidosis. For many, a leap from the premise that lactic acid is not detrimental in cerebral ischemia to the claim that it is a crucial, maybe even an obligatory, aerobic energy substrate for neural recovery postischemia, has been too great to make. Obviously, there are not many studies published that specifically aim at testing lactate’s role as an energy substrate for neural recovery postischemia. Nevertheless, published studies provide more than incidental data to support such a role for lactate. Neuronal function of rat hippocampal slices exposed to a short hypoxic period in the presence of low glucose concentration (3 mM) was greatly improved when glucose was replaced with an equicaloric concentration (6 mM) of lactate [15]. Additional studies confirmed this finding [23] and demonstrated that lactate, formed during the hypoxic period is an obligatory aerobic energy substrate immediately post hypoxia [24,25]. These later studies made use of the monocarboxylate transporter inhibitor a-cyano-4-hydroxycinnamate (4-CIN) to manipulate the transport of lactate from the extracellular milieu into neurons, depriving these cells of the monocarboxylate. When this very approach was applied in vivo, similar results were obtained. The inhibitor, 4-CIN, was found to cross the blood-brain barrier and to aggravate the postischemic neuronal damage by inhibiting the transport of lactate formed during ischemia, presumably in glia, into neurons [26,27]. A study of astrocytes isolated from stroke-prone spontaneously hypotensive rats showed a reduced lactate production during hypoxia and reoxygenation [28]. Phillis and coworkers [29], using a rat cerebral cortex model of ischemia, showed that lactate supplementation reduced excitatory amino acid release and fueled recovery of function postischemia. Moreover, these investigators demonstrated that inhibition of the monocarboxylate transporter greatly enhanced lactate accumulation and increased the release of the excitatory amino acid aspartate during the ischemic period [30]. Bliss and Sapolsky [31] studied the regulation of energy substrate utilization in hippocampal cultures through interactions among glucose, lactate and adenosine. They concluded that their results support our notion [15,24,26] that after ischemia/hypoxia neurons are biased toward lactate over glucose utilization. Such bias is inevitable since ATP pools at the end of the ischemic period are all but disappeared and thus glucose phosphorylation, a prerequisite for aerobic glycolytic formation of pyruvate upon reperfusion/reoxygenation, is unlikely. In contrast, the formation of pyruvate from lactate does not require energy investment and can produce 17 moles of ATP for each mole of lactate (pyruvate) that enters the tricarboxylic acid cycle. It is thus clear from the existing literature that the lactic acidosis hypothesis does not satisfactorily explain the phenomenon of the glucose paradox of cerebral ischemia.

**Hyperglycemia and the Stress Hormone Corticosterone**

In vitro systems, by their nature, suffer from the absence of systemic influences. Where brain energy metabolism is concerned, the absence of the adrenal gland and its various hormones is perhaps more relevant than the absence of other systemic components. Insulin or insulin-
like factor, glucagon, and glucocorticoids (GCs), all are excluded from most of the in vitro systems employed in the study of brain energy metabolism.

Many publications have documented the involvement of endogenous GCs in the development and progression of ischemic damage. The cellular mechanisms by which these stress hormones may exert their effects are under study as well (see below). Although, GC medications are used extensively in treating patients under neurological and neurointensive care, there are few situations in which these medications have been proven to benefit neurologic function on long-term outcome. This is not to say that GCs are of no benefit in cases such as spinal cord injury and in the treatment of edema secondary to brain tumor or injury. However, frequently, the administration of dexamethasone, a synthetic steroid, exacerbates neurologic damage and induces hyperglycemia [32]. Sapolsky’s studies in the mid-1980s have demonstrated the GC-induced exacerbation of ischemic damage (see below). Nevertheless, the hyperglycemia that accompanies steroid administration helped to advance the speculation that not the steroid itself, but rather the increased in blood glucose level exacerbates the ischemic damage, i.e., lactic acidosis is blamed for the observed GC–induced aggravation of ischemic neuronal damage. Obviously, with this explanation, it is most appealing to administer insulin to treat the resultant hyperglycemia [32]. Nevertheless, insulin treatment did not alleviate all the dexamethasone-aggravated histopathology. In a follow up study [33], work from the same laboratory continued the attempt of explaining dexamethasone effect via the induction of hyperglycemia. The investigators ignored the fact that in most cases, steroids are administered postischemia where hyperglycemia has been shown not to worsen the postischemic outcome [34-36]. GC-aggravated ischemic damage in vitro has been demonstrated recently where hyperglycemia and acidosis were irrelevant issues [37]. Moreover, many studies by Sapolsky and coworkers (see below) demonstrated GCs to aggravate neuronal insults other than ischemia.

The elucidation of the mechanism(s) of action of GCs has been a major focus of Sapolsky’s laboratory. These compounds have been shown to inhibit glucose transport and glutamate uptake in hippocampal astrocytes [38], accelerate ATP loss following metabolic insults in neuronal culture [39] and, thus, exacerbate insult-induced declines in energy metabolism [40]. These findings were summarized in several outstanding reviews [41-43]. Others have shown dexamethasone to induce insulin resistance in healthy humans by decreasing glucose oxidation independent of glucose transport [44,45]. Adachi and his coworkers [46] showed that dexamethasone aggravated ischemia-induced neuronal damage through the facilitation of the onset of anoxic depolarization and an increase in intracellular Ca2+.

However, despite this wealth of data, not many investigators were persuaded to look at the deleterious effects of GCs independently from their known ability to induce hyperglycemia. Multiple studies have been published on stress-induced release of GCs and their effect on disorders such as cerebral ischemia. An association between a pronounced systemic stress response with markedly elevated plasma cortisol and increased mortality or morbidity was first reported in 1977 [47]. Two years earlier, the steroid prednisone was shown to inhibit insulin secretion and to elevate blood glucose levels [48]. Later, corticosterone (CT), the rodent equivalent of human cortisol, was shown to depress the release of glucose-induced insulin [49]. Others have concluded that hyperglycemia in acute stroke patients probably represents a stress response [50,51]. A retrospective study that examined the effect of steroid treatment on the outcome of 458 consecutive patients admitted after out-of-hospital cardiac arrest has concluded that there is no role for steroids in the treatment of global brain ischemia [52].

Although it has been shown that GCs are elevated in situ by insults such as cerebral ischemia, no studies were specifically designed to test whether or not glucose loading induces an elevation in blood GCs. Harris et al. [53], who studied energy restriction and aging, found that intraperitoneal glucose challenge in mice elevated CT blood levels 6-fold 30 min after glucose injection. These high levels were back to baseline levels 2 h after glucose injection. Wang et al. [54] showed that a high-carbohydrate diet significantly elevates CT blood levels.

What, if any, is the potential significance of these findings for our general understanding of the ischemic brain and the possible roles that glucose and GCs play in the ensuing ischemic damage? Our laboratory tested whether or not preischemic glucose loading correlates both with blood CT elevation and aggravation of postischemic brain damage. The first indication that preischemic hyperglycemia-aggravated delayed neuronal damage is time-dependent has unveiled itself when rats were loaded with glucose either 15-min or 120-min preischemia [27]. While in both cases hyperglycemic levels of glucose were measured 2 min prior to the ischemic insult, only rats loaded with glucose 15-min preischemia exhibited aggravation of neuronal damage in comparison to control, saline-injected rats. Those loaded with glucose 120-min preischemia exhibited a significant reduction in the degree of delayed neuronal damage compared both to control, saline-injected rats, and 15-min glucose-loaded rats [55]. Moreover, brain lactate levels in these two hyperglycemic groups of rats were significantly and equally...
Higher than the levels measured in control, normoglycemic rats. Additional studies have revealed that exogenously-supplemented CT, when given 15-min preischemia, aggravated the ischemic outcome similarly to glucose supplementation 15 min preischemia, but not when supplemented 120 min preischemia [56]. Moreover, measurements of blood CT concentrations following glucose administration have shown a sharp increase in CT levels 15 to 30 min after a bolus administration of glucose (i.p.) that returned to baseline levels by 120 min post glucose injection [55]. These results confirmed the findings of Harris et al. [53]. Administration of metyrapone, an inhibitor of GC synthesis (chemical adrenalectomy), has been shown to prevent ischemia-induced loss of synaptic function in rat hippocampus and to attenuate the increase in CT blood levels following ischemia [57]. When metyrapone was administered 60 min preischemia to rats loaded with glucose 15-min preischemia, a complete block of the aggravating effect of hyperglycemia was observed [55]. Moreover, these rats exhibited a degree of ischemic damage that was significantly lower than the degree measured in control, normoglycemic rats. Thus, once the glucose-induced increase in CT level is blocked, glucose can exert its beneficial effect even when loaded shortly postischemia, as has been observed with glucose loading 120-min preischemia [55] and also in vitro [58,59]. Furthermore, we have clearly demonstrated that the degree of postischemic neuronal damage in rats preloaded with glucose is correlated with the blood levels of CT just prior to the ischemic insult, but not with the levels of blood glucose [55]. In addition, we found that the specific GC receptor antagonist, mifepristone (RU486) [60], when administered at 40 mg/kg, i.p., 30 min preischemia, completely blocked the aggravating effect of 15-min preischemic glucose administration [55].

It is appropriate here to expand somewhat on the timing issue of glucose administration and its relevancy to the action of steroid hormones such as CT. These hormones are known to affect cell function through intracellular receptors that regulate transcriptional (genomic) activity [62-64]. However, evidence is accumulating to show that CT (and cortisol) has also an acute effect on cell surface which, among other effects, alters ion permeability [65-68]. While the typical GC action has a time course of hours, the non-genomic actions are exerted in minutes. The fact that the glucose-induced exacerbation of ischemic neuronal damage occurs when the sugar is administered shortly (15 min) preischemia, but not when administered two hours preischemia, suggests that the effect of CT, if involved in the production of the glucose paradox, is a non-genomic one.

The Corticosterone Hypothesis of Cerebral Ischemia

Based on the above results and considerations we formulated the “corticosterone hypothesis” to explain the glucose paradox of cerebral ischemia [55]. This hypothesis postulates that induction of hyperglycemia via bolus glucose administration brings about a short-lived, sharp elevation in CT blood levels. If cerebral ischemia occurs while CT blood levels are still elevated (15-60 min after glucose loading), an exacerbation of the postischemic neuronal damage ensues. Blockade of CT action, either via inhibition of its synthesis with metyrapone or by antagonizing its receptor with mifepristone, prevents the hyperglycemia-aggravated ischemic damage. The CT hypothesis can account for the differences observed between in vivo and in vitro studies where the effect of elevated glucose on ischemic/hypoxic tissue is concerned. While in vivo the systemic effect of glucose involves the short-lived elevation in CT blood levels, such an effect is absent in the in vitro environment, hence, the consistent reporting of glucose’s neuroprotective effect in vitro. In addition, the CT hypothesis can account for the neuroprotective effect of glucose in vivo where either metyrapone or mifepristone is used or when enough time is allowed to lapse between glucose administration and the return of CT blood to baseline levels (~ 60-90 min post glucose loading).

Hyperglycemia, Corticosterone and Diabetes Mellitus

Could the elucidation of the glucose paradox of cerebral ischemia be applied to other conditions where hyperglycemia is believed to play a detrimental role? One such major condition, of course, is diabetes mellitus. In both Type I and Type II diabetes, elevated blood glucose over a span of years is believed to be responsible for the known degenerative complications of the disease. Consequently, the prime objective in the treatment of diabetes mellitus is to lower blood glucose levels. As no other mechanism has been offered to explain the degenerative complications of diabetes, most studies over the past century have concentrated on glucose itself as the culprit. The discovery that glucose can form a stable conjugate with the α-amino group of the b chains of hemoglobin, known as hemoglobin A1c, led investigators to hypothesize that the late complications of diabetes may arise from the presence of hemoglobin A1c [68]. Another postulate on the possible cause of these complications has been the production of sorbitol from excess glucose by aldol reduc-
tase [68].

Diabetes has been recognized as a risk factor in stroke. Atherosclerosis is a major cause of cerebral ischemia in diabetics and hyperglycemia is believed to be the main factor that augments the extent of ischemic brain damage [69]. Clearly, the corticosterone hypothesis of cerebral ischemia is applicable in the diabetic situation as it is in the non-diabetic one. However, stroke in diabetes is just a heighten risk, not a sure complication. The chronic complications of diabetes, such as retinopathy, nephropathy and neuropathy, both of the central and peripheral nervous systems are the hallmark of the diabetic condition and, as of today, are believed to be the direct outcome of chronic elevation of blood glucose.

The focus on hyperglycemic blood glucose levels both as the indicator of diabetes and the cause of its long-term complications has discouraged investigators from searching for alternative monitors for diabetes and/or causes of its complications. Thus, there is relatively small number of studies that were published over the past six decades where other factors than glucose were examined as possible participants in the etiology of the disease. Nevertheless, during the 1950s and the 1960s, several investigators focused their attention on the role of the adrenal function in the complications of diabetes [70-74]. Unfortunately, either because of lack of sensitive methods for the detection of small quantities of plasma glucocorticoids in those years or due to insufficient evidence to connect adrenal hyper-function with diabetic complications, the interest in this direction has waned.

Ongoing studies in our laboratory are aimed at understanding the relationships between plasma corticosterone, glucose, lactate, insulin and neuropathy in diabetic mice.

References


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