Effects of Insulin on Blood, Plasma, and Brain Glucose in Hyperglycemic Diabetic Rats

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This study, in biologically bred hyperglycemic diabetic rats, examined the effect of an intravenous insulin infusion (1.5 units \( \cdot \) hr\(^{-1} \)) on blood, plasma, and brain glucose concentrations to determine their relationship during decreasing blood and plasma glucose levels. The data were compared to saline-treated diabetic rats and saline-treated nondiabetic littersmates. The volume and duration of the treatment infusion were similar in all groups. Insulin infusion in diabetic rats produced the expected reduction in blood and plasma glucose, and normoglycemia was produced within 78±37 minutes (mean±SD). However, once normoglycemia was achieved, brain glucose was still significantly greater by 44% than in nondiabetic rats \( \left( p=0.015 \right) \). Moreover, the ratio of brain to plasma glucose was more than 50% greater in diabetic than nondiabetic rats, irrespective of whether or not they received insulin \( \left( p<0.01 \right) \). We conclude that measurement of blood or plasma glucose in diabetic subjects will tend to underestimate the amount of glucose in the brain and that this relationship is not influenced by acute insulin therapy. \cite{Stroke199122:505-509}

Hyperglycemia and increases in brain glucose will exacerbate cerebral injury during and after a period of global ischemia. An increase in brain glucose also may contribute to the worsened neurologic outcome in untreated diabetics after resuscitation from cardiac arrest. The effect of insulin treatment (resulting in normoglycemia) on brain glucose and its effect on postischemic neurologic injury has not been reported. Previous studies in this laboratory investigated the effects of insulin treatment on plasma and brain glucose concentrations in streptozocin-induced diabetic rats. These studies found that when insulin was used to achieve normoglycemia over a 2-hour period in diabetic rats, normoglycemic diabetic rats had a greater amount of glucose \( (>25\%) \) in the brain than did normoglycemic, nondiabetic rats. Our data suggested that this finding is due to an alteration in the manner in which the diabetic brain handles glucose and is not due to an insulin effect or a hysteresis effect.

Streptozocin has been used as a means of inducing diabetes resembling human juvenile-onset diabetes in various animal models through its propensity to lower the nicotinamide adenine dinucleotide and its consequent histopathologic alteration of the insulin-producing islet beta cells of the pancreas. Because of the mechanism of action of streptozocin, it is possible that the drug also may affect organs other than the pancreas. Thus, our previously reported data describing alterations in brain glucose metabolism in streptozocin-induced diabetic rats may have been due to one or both of the following factors: 1) a diabetes-induced alteration in brain glucose metabolism, or 2) a direct effect of streptozocin on the brain that was independent of the diabetic state. The purpose of the present study, in biologically bred diabetic rats, was to resolve this issue by determining whether acute restoration of blood or plasma glucose to normoglycemic values would result in restoration of normal brain glucose values using a diabetic animal model other than that induced by streptozocin.

Materials and Methods

This protocol was reviewed and approved by the Institutional Animal Care and Use Committee. We used 20 biologically bred Wistar rats \( (12 \text{ were spontaneously diabetic, and eight were nondiabetic littersmates}) \) weighing 230–390 g. Rats were obtained from Animal Resources Division, Health and Welfare, Ottawa, Canada. The diabetic animals were given 1 unit daily of subcutaneous protamine zinc insulin \( \text{(Eli Lilly & Co., Indianapolis, Ind.)} \) to prevent ketosis. The insulin treatment was discontinued 96 hours before the study. All rats were fasted 10–12 hours before the study but had free access to water.

The rats were weighed and then anesthetized in an induction box with 3% halothane in oxygen. After the induction of anesthesia, tracheal intubation was performed via a tracheostomy, and the rats were me-
mechanically ventilated with a rodent ventilator (model 7025, Ugo Basile, Varese, Italy). Pancuronium (0.5 mg i.m.) was given to provide muscle paralysis, and thereafter, anesthesia was maintained with 1.3% inspired halothane (i.e., a surgical anesthetic dose) in nitrogen and oxygen for the remainder of the study. The inspired oxygen and halothane concentrations were measured with a RASCAL laser-type gas analyzer (Albion Instruments, Salt Lake City, Utah). Temperature was measured using a rectal thermistor (model 73A, Yellow Springs Instruments Co., Yellow Springs, Ohio) and maintained near 37°C with a heating lamp and pad. The femoral artery was cannulated with a polyethylene catheter (PE-50) for blood sampling and measurement of mean arterial pressure. The femoral vein was cannulated (PE-50) for the administration of intravenous fluids and drugs.

Arterial blood gases were measured using electrodes at 37°C (Instrumentation Laboratories, Inc., Lexington, Mass.). Blood and plasma glucose were measured using a Yellow Springs Model 23A glucose analyzer. This device has a detection range of 0–500 mg·dl⁻¹ (0–28 μmol·ml⁻¹) and a sensitivity of 1 mg·dl⁻¹ (0.05 μmol·ml⁻¹).

The rat was placed in the prone position with its head in a stereotactic headframe. The skin of the scalp was reflected, exposing the calvarium, and a funnel was secured to the exposed bone. The bottom of the funnel was filled with a sheet of paraffin to prevent heat and moisture loss. Thereafter, 10-minute stabilization periods were allowed, after which the anesthetic agent, ventilation, and oxygenation were adjusted until the changes resulted in data within the preestablished protocol criteria: PAO₂, 36–40 mm Hg; Pao₂, 125–175 mm Hg; mean arterial pressure, greater than 60 mm Hg; temperature, 36.5–37.5°C; and inspired halothane concentration, 1.2–1.4%. Furthermore, we preestablished that to be included in the study, diabetic rats had to have a blood glucose >200 mg·dl⁻¹ and nondiabetic rats a blood glucose of >60 but <120 mg·dl⁻¹ before any intervention. All study animals were allowed at least two 10-minute stabilization periods after the completion of the surgical preparation, but before the start of the study.

Rats were divided into three groups according to the presence of diabetes-induced hyperglycemia and fluid infusion: 1) nondiabetic rats treated with saline (ND-S; n=8), 2) diabetic rats treated with saline (D-S; n=6), and 3) diabetic rats treated with insulin (D-I; n=6). All rats received an infusion of 0.9% NaCl solution at 2 ml·hr⁻¹. In the D-I group, regular porcine/bovine insulin (Eli Lilly & Co.) was added to the saline solution to provide a delivery of insulin at 1.5 units·hr⁻¹. In previous studies using streptozocin-induced diabetic rats, it was determined that this concentration and rate that an insulin infusion for approximately 2 hours would result in normoglycemia. After stabilization in six diabetic rats, an insulin infusion was started, and 50-μl blood samples were drawn approximately every 15 minutes for determination of blood glucose until the diabetic rats were normoglycemic (i.e., blood glucose was <120 but >60 mg·dl⁻¹). During the study period, physiologic variables other than glucose were monitored to ensure that they remained within predetermined ranges.

Immediately after the attainment of normoglycemia, blood and plasma samples were taken, physiologic variables were recorded, and the brains were rapidly frozen in situ. The latter was performed by removing the insulation covering the exposed calvarium and filling the funnel with liquid nitrogen. Liquid nitrogen was used to bathe the brain during removal as well as for transportation to temporary storage in a −76°C freezer. Later, the brains were moved to a −25°C environment, the venous sinuses and meninges were dissected away, the hemispheres were separated from each other, and the cortex was dissected from the remainder of the cerebrum. Brain glucose concentrations in the cortex were measured using a previously described enzymatic fluorometric technique. This technique has a sensitivity of 0.2 μmol·g⁻¹.

Studies in six D-S rats and eight ND-S rats were conducted similarly to the D-I rats except saline was infused without insulin. Again, physiologic variables were measured, and blood and plasma samples were obtained concomitant with brain harvesting. The duration of saline infusion in the D-S and ND-S groups was matched to the duration in the D-I groups to eliminate the influence of time on data analysis.

Data between groups were compared using Bonferroni’s correction of unpaired t tests. A corrected value of p<0.05–3=p<0.0167 was considered significant. The Pearson product–moment correlation coefficient was used to determine the relationship between plasma and brain glucose. All data are presented as mean±SD.

Results

Groups were well matched for systemic physiologic variables except glucose. Both blood and plasma glucose were measured in all rats. Because the correlation coefficient for the relationship between blood and plasma glucose was 0.97 (p<0.0001 by t test), we have elected to report primarily plasma glucose data.

At the time of brain harvesting, plasma glucose in the ND-S group was 7.64±1.19 μmol·ml⁻¹ (139±22 mg·dl⁻¹), and brain glucose was 1.70±0.42 μmol·g⁻¹. As expected, the D-S group had significantly greater plasma glucose (23.58±3.65 μmol·ml⁻¹, 429±67 mg·dl⁻¹) and brain glucose (7.62±1.74 μmol·g⁻¹) than did the ND-S group (p<0.0001 for both). Furthermore, the brain-to-plasma glucose ratio (Gp/Gh) was 50% greater in D-S rats (0.33±0.09) compared with ND-S rats (0.22±0.03; p<0.01).

In the D-I rats, the plasma glucose decreased to normoglycemic levels (6.66±0.97 μmol·ml⁻¹, 121±18 mg·dl⁻¹) by 78±37 minutes (Table 1). Although the brain glucose in the D-I group (2.45±0.59 μmol·g⁻¹) was significantly less than that of the D-S
was not due to a hysteresis between brain and plasma glucose due to hysteresis, or a direct effect of insulin on the brain. Some studies suggest that brain glucose accumulation in hyperglycemia is in agreement with data from another recent study (Weglinski and Lanier)9 recently demonstrated in biologically bred diabetic rats. The previous study9 revealed that insulin treatment in streptozocin-treated diabetic rats was similar in both saline- and insulin-infused diabetic rats (0.33±0.09 versus 0.36±0.05; p=0.46). Both values were significantly different from the ratio in nondiabetic rats (0.22±0.03; p<0.01). These findings were similar to those in our recent study using streptozocin-treated diabetic rats. The findings of glucose accumulation independent of a hysteresis effect in our present and previous studies is in agreement with data from another recent study by Pelligrino et al.20 They reported that insulin therapy in streptozocin-induced diabetic rats was associated with accumulation of glucose in the brain, regardless of whether normoglycemia was attained over periods of 0.5–24 hours of insulin therapy.

In contrast to the absence of a hysteresis effect between brain and plasma glucose in the above studies of insulin treatment in diabetic hyperglycemic rats, Weglinski and Lanier16 recently demonstrated in nondiabetic rats that, after glucose infusions, a hysteresis can occur between brain and plasma glucose. In their studies, the hysteresis resulted in a significant increase in the brain-to-blood glucose ratio (i.e., by as much as 23%) as blood glucose declined. How-ever, in the Weglinski and Lanier study, blood glucose declined by 359 mg • dl”1 over a 90-minute period, a rate that was about threefold greater than the rate of decline in blood glucose observed in diabetics, as much as 23% as blood glucose declined. How-ever, in the Weglinski and Lanier study, blood glucose declined by 359 mg • dl”1 over a 90-minute period, a rate that was about threefold greater than the rate of decline in blood glucose observed in diabetics.

In the present study, GlBP/Glp was similar in both saline- and insulin-infused diabetic rats (0.33±0.09 versus 0.36±0.05; p=0.46). Both values were significantly different from the ratio in nondiabetic rats (0.22±0.03; p<0.01). These findings were similar to those in our recent study using streptozocin-treated diabetic rats. The previous study9 revealed that insulin infusion did not affect GlBP/Glp over a 2-hour period, even though plasma glucose decreased from 24.99±1.61 µmol • ml”1 to 10.15±3.41 µmol • ml”1. In that study, there also was no effect of saline treatment on GlBP/Glp in diabetic or nondiabetic rats. The findings of glucose accumulation independent of a hysteresis effect in our present and previous studies is in agreement with data from another recent study by Pelligrino et al.20 They reported that insulin therapy in streptozocin-induced diabetic rats was associated with accumulation of glucose in the brain, regardless of whether normoglycemia was attained over periods of 0.5–24 hours of insulin therapy.

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Although the mechanism by which the diabetic brain will accumulate more glucose than the nondiabetic brain is not clear, this finding may have important clinical implications. It is known that hyperglycemia will exacerbate ischemic neurologic injury, and based on the previously proposed mechanism (i.e., enhanced lactic acid production during ischemia) and previous data from a primate model of global ischemia, it is tempting to conclude that, for a given degree of brain ischemia, there is a correlation between brain glucose and the severity of the postischemic neurologic injury. By reducing brain glucose in hyperglycemic patients to values found in normoglycemic patients, it may be possible to reduce ischemic brain injury. However, if one monitors blood or plasma glucose during restoration of normoglycemia, and those values underestimate the brain glucose reduction, this in turn may lead to an underestimation of the potential for ischemic neurologic injury. This phenomenon was suggested in a previous study from our laboratory examining brain glucose and ischemic neurologic injury in subjects studied after transient glucose infusions. In a subsequent study we demonstrated that in this setting, blood glucose declined faster than brain glucose, and thus, blood glucose measurements tended to underestimate brain glucose and therefore potentially underestimate postischemic neurologic injury. It is perhaps simplistic to assume that the rapid reduction of blood or plasma glucose before brain ischemia will reduce the risk of brain injury in diabetic subjects to that expected in normoglycemic, nondiabetic subjects for several reasons. First, chronic diabetes produces hemorheologic changes that may adversely affect the ability of the diabetic brain to recover from an ischemic insult. Second, the acute restoration of normoglycemia may induce a cerebral hypermetabolic state. Third, as shown in the present study and a previous study from our laboratory, the acute restoration of normal blood or plasma glucose in chronically diabetic subjects may not result in a restoration of brain glucose to normal values. Taken together, these collective studies suggest that acute restoration of normoglycemia in chronically diabetic subjects may be insufficient to return the risk of ischemic brain injury to a level found in nondiabetic subjects. We hypothesize that the use of insulin to treat diabetes may result in some improvement in global ischemic injury when compared with untreated diabetic subjects; however, the magnitude of this improvement must be further evaluated.

In summary, the present study demonstrated that when insulin was used to achieve normoglycemia in biologically bred diabetic rats, insulin-treated diabetic rats had a greater concentration of glucose in the brain than did normoglycemic, nondiabetic litters. Our data suggest that this finding is produced by an alteration in the manner in which the diabetic brain handles glucose; that is, the diabetic brain appears to retain or store glucose. These findings are consistent with those from our previous study in streptozocin-induced diabetic rats. Thus, the present data discount the direct effect of streptozocin on the brain as the origin of our previous observations. If we assume that the degree of neurologic injury that follows a period of complete cerebral ischemia is proportional to the degree of increased brain glucose, as has been previously reported, we conclude that the measurement of blood or plasma glucose concentrations in acutely insulin-treated, normoglycemic diabetics will underestimate both the brain glucose concentration and the degree of postischemic neurologic injury.

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References


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