# Insulin Neuroprotection and the Mechanisms

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## Abstract

**Objective:** To analyze the mechanism of neuroprotection of insulin and which blood glucose range was benefit for insulin exerting neuroprotective action.
**Data Sources:** The study is based on the data from PubMed.
**Study Selection:** Articles were selected with the search terms "insulin", "blood glucose", "neuroprotection", "brain", "glycogen", "cerebral ischemia", "neuronal necrosis", "glutamate", "γ-aminobutyric acid".
**Results:** Insulin has neuroprotection. The mechanisms include the regulation of neurotransmitter, promoting glycogen synthesis, and inhibition of neuronal necrosis and apoptosis. Insulin could play its role in neuroprotection by avoiding hypoglycemia and hyperglycemia.
**Conclusions:** Intermittent and long-term infusion insulin may be a benefit for patients with ischemic brain damage at blood glucose 6–9 mmol/L.

**Key words:** Blood Glucose; Brain Ischemia; Insulin; Neuroprotection

## Introduction

Insulin is the only endocrinology hormone to decrease blood glucose. In addition, insulin serves as a growth factor, modulating mitogenesis, growth, and differentiation.[1] Animal studies showed insulin attenuate brain damage independent of its hypoglycemic effect.[2,3] Insulin has neuroprotection itself. Insulin will cause hypoglycemia when used to treat ischemic brain damage by venous infusion. Hypoglycemia can make brain damage worse. How could insulin fully play its role of the neuroprotective effect? It is important which blood glucose range is benefit for insulin exerting neuroprotective action. The goal of this paper is to discuss the mechanisms of insulin neuroprotection and optimal blood glucose range while insulin administered.

## Effect of Insulin on the Neurotransmitter

Glutamate receptor family influences fast excitatory synaptic transmission, whereas γ-aminobutyric acid (GABA) receptors mediate fast inhibitory synaptic transmission. The delicate signaling balance that permits synaptic communication is profoundly shifted in the direction of over-excitation by transient cerebral ischemia, which sets in a series of changes that ultimately lead to neuronal cell death.[4] Insulin may decrease glutamate levels, and increase GABA levels.

### Excitatory neurotransmitter

Glutamate is the predominant excitatory neurotransmitter of the central nervous system (CNS).[5] Excess release of glutamate and other excitatory amino acids can lead to excitotoxic lesions in the brain while the ischemia, epilepsy, and neurodegenerative diseases.[5,6] Glutamate concentrations in plasma are 50–100 μmol/L in human, in whole brain, they are 10,000–12,000 μmol/L but only 0.5–2.0 μmol/L in extracellular fluid.[7,8] The large gradient between brain cell and extracellular fluid maintained by $Na^+$-dependent glutamate transporters.[7] If the oxygen supply is insufficient and the $Na^+$ gradient is dissipated, glutamate is released from both astrocytes and neurons.[7]

Following brain injury or ischemia, neurotoxic amino acids are elevated for about 30 min in rodents.[3] Patients have sustained global posttraumatic ischemic brain damage or ischemic stroke, the levels of glutamate in the brain increased 50–70 times for up to 4 days.[8] Excitatory amino acid release appears to be longer and stronger in ischemically damaged human tissue than in animal models.[8]

Cultured murine cortical neurons exposed to low concentrations of glutamate receptor, were produced neuronal cell body swelling over the next 2 h. By 8 h after exposure onset, the neuronal cell membranes and

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cytoplasmic organelles were disrupted.[9] Insulin partially protected against glutamate decreased cell viability in cultured neuronal cells.[10,11] Fanne *et al*. found, insulin decreased glutamate levels in the cerebral spinal fluid of rats, decreased infarct size and improved neuro-scoring.[3] Insulin exerts a neuroprotective effect that correlates with their capacity to reduce glutamate, rather than by modifying glucose levels.[3]

### Inhibitory neurotransmitter

γ-aminobutyric acid is the main inhibitory neurotransmitter in the CNS.[12] GABA acts at one of two types of receptor, $GABA_A$ and $GABA_B$. $GABA_A$ that controls chloride entry into the cell and stabilize the membrane potential below firing threshold.[13,14] Most of the fast inhibitory synaptic transmission within the CNS is mediated by $GABA_A$ receptors.[15] The impairment of GABAergic synaptic transmission in brain ischemia is partly due to a down-regulation of synaptic $GABA_A$ receptors, which may contribute to the ongoing neuronal excitability and possibly to neuronal death.[6]

When rats hippocampal neurons treated with insulin for 10 min, the expression of $GABA_A$ receptors increased about 20%.[15] Oxygen-glucose deprivation (OGD) was used as an *in vitro* model of cerebral ischemia.[16] Insulin protected cultured neurons against OGD by enhancing the presence of $GABA_A$ receptors on the cell surface.[4]

Brain extracellular GABA levels increased in gerbils treated with insulin whatever the blood glucose level of gerbils was high, low, or normal.[17] This effect is independent of insulin's hypoglycemic effect.

On the basis of cell and animal studies, insulin might regulate neurotransmitters to exert neuroprotective action during euglycemia.

## INSULIN INCREASES GLYCOGEN STORES

Brain glycogen is located almost entirely in astrocytes, which are distributed throughout the brain.[18] In the human brain, there are at least as many astrocytes as neurons. Normal human astrocytes express insulin receptor.[19] Astrocytes take up glucose and store energy as glycogen, this energy storage is important to support neurons with energy.[19] Under physiological conditions, glycogen provides energy to sustain glutamatergic neurotransmission in the presence of glucose.[20] Brain glycogen may provide energy to allow continuous uptake of excitotoxic glutamate from the synaptic cleft and to provide lactate as a fuel for neurons during hypoglycemia.[21] The amount of glycogen would be expected to fuel brain metabolism for several minutes if it were the only energy supply available in the brain. However, the normal level of glycogen should be able to support brain metabolism for about 100 min of hypoglycemia.[18,21,22] Brain glycogen is consumed very gradually during hypoglycemia.[22] But once glycogen content is depleted, it cannot be replenished.[23]

Although the functional role of glycogen in the brain has yet to be completely unraveled, it has repeatedly been demonstrated that glycogen can sustain neuronal activity during energy deprivation.[20,23-26] Increased astrocyte glycogen stores may extend neuronal survival and axonal function during periods of OGD or hypoglycemia. Glycogen content is regulated by numerous factors, including glucose, insulin, noradrenalin, and so on.[22-24,27] On the basis of cell culture, insulin-stimulated glycogen synthesis in normal human astrocytes and rats astrocytes.[19,28] Insulin could enhance the ability of astrocytes to store glycogen prior to ischemia; this would increase the amount of glucose available to the cells during ATP depletion.[29] In the animal study, glucose or insulin increased brain glycogen content.[18,30] There was a significant synergistic effect of glucose and insulin in increasing brain glycogen concentration when administered together with physiologic plasma glucose concentration.[18] Rats were subjected to insulin-induced moderate hypoglycemia and when the level of brain glucose approached zero, brain glycogen content began to decrease gradually, demonstrating utilization of glycogen. When plasma and brain glucose concentrations were restored, glycogen increased and the concentration exceeded the prehypoglycemic level by several fold. The data suggested that brain glycogen rebound (super-compensate) after a single episode of hypoglycemia.[21] Mice brain glycogen concentrations were elevated by 25% after acute or recurrent hypoglycemia.[31]

Human brain glycogen content is about 3.5 μmol/g that is three- to four-fold higher than free glucose at euglycemia.[27] In astrocytes, glycogen is continuously synthesized and degraded.[32] Turnover rate of brain glycogen is 0.16 $\mu mol \cdot g^{-1} \cdot h^{-1}$, implying that a complete turnover required 3–5 days.[27] The moderate level of hyperglycemia 6.0 ± 0.4 mmol/L and hyperinsulinemia in nine healthy volunteers study did not appear to promote significant amounts of glycogen accumulation. But hyperglycemia more often has been defined as blood glucose ≥7.2 mmol/L.[33] In the human study, volunteers did not accept insulin, and did not have the test about insulin concentration in blood. It needs further study whether glucose and insulin promote glycogenesis in human.

In the human brain, glycogen may be super-compensate. Oz *et al*. utilized *in vivo* $^{13}C$ nuclear magnetic resonance spectroscopy to measure brain glycogen metabolism in healthy volunteers. The study indicates mobilization of glucose units from glycogen during moderate hypoglycemia (3.2 ± 0.5 mmol/L). Levels of newly synthesized glycogen measured from 4 to 80 h were significantly higher after hypoglycemia (2.9 ± 0.3 mmol/L) than after euglycemia (5.1 ± 0.1 mmol/L), indicating increased brain glycogen synthesis after moderate hypoglycemia.[34]

Glycogen is metabolized to lactic acid under ischemic conditions, and the resulting acidosis may have deleterious effects that outweigh the beneficial effects of increased energy

reserve.[22] However, some animal studies showed there was no more lactic acid in glycogenolysis. The rats experienced 15 min of transient forebrain ischemia, glycogen content in neocortex doubled after 6 h of recovery. About 50% of accumulated glycogen degraded during the following ischemia (30 min). In spite of this extensive degree of energy failure, degradation of glycogen was slow and incomplete. The amount of lactate formed during the second ischemic insult did not exceed values usually obtained during complete ischemia in animals with normal glycogen contents.[35] The super-compensate glycogen seemed to be degraded slowly when it was necessary. It has been estimated that brain lactate concentration of 16–20 mM/g may produce the morphologic injury. Studies in a rat model of forebrain ischemia (10 min) discovered that lactate concentrations of hyperglycemic diabetic rats were 29 mM/g, and the rats experienced severe functional and histologic injury; in contrast, euglycemic insulin-treated diabetic rats experienced mild to moderate functional and histologic injury, and the brain lactate concentrations were 15–18 mM/g. There was no significant difference between the two groups in total brain carbohydrate consumption during ischemia. The percentage of total brain carbohydrate consumption originated as glycogen is 27% for hyperglycemic diabetic rats and 52% for insulin-treated diabetic rats.[36] So glycogen may be better energy than glucose during ischemia.

On the basis of cell culture and animal model studies, increased glycogen might provide a buffer against the energy crisis in brain injury and ischemia. It is impossible to enhance glycogen storage before stroke or traumatic brain injury (TBI), but it is possible to do so before elective surgery. Patients are fasted overnight before surgery. Hypoglycemia is an early response to fasting within the first 9 h. While fasting more than 9–12 h, decreasing insulin levels initiate the mobilization of stored energy.[37] The glycogen storage may be elevated by infuse glucose and insulin during surgery. Neurosurgery operation and cardiovascular operation may result in cerebral ischemic injury. Insulin may play the neuroprotective effect by the super-compensate glycogen. Future clinical trials are needed to clarify the effects of insulin and glycogen.

## Neuronal Necrosis and Apoptosis

Focal brain ischemia/reperfusion, neuronal cells in the ischemic core died by necrosis, neurons in the penumbra may be apoptosis. Insulin can inhibit neuronal necrosis and apoptosis as neurotrophic factors.[38-41]

The cultured cells of rat cortical experienced 2 h hypoxia (<0.4% $O_2$) and 3 h reperfusion, 50% of the total neurons died by necrosis. When insulin was added to the culture medium throughout hypoxia and reperfusion, the percentage of necrosis neurons reduced to 30–60% at 3 h reperfusion. Insulin-mediated inhibition of neuronal necrosis through protein kinase C pathway.[38,39]

In the animal model of brain ischemia, insulin was administered before ischemia or at the onset of reperfusion. Insulin induces robust PI3-K/Akt activation, inhibits cytochrome c release from mitochondria,[42] results in improved neuronal survival and preservation of learning and memory ability.[40,41] The activation of PI3-K is required for the anti-apoptosis effects of insulin. [43] PI3-K is a key anti-apoptotic effector in the growth factor signaling pathway. Akt, a 57-kDa protein-serine/threonine kinase, serves a key role in mediating anti-apoptotic actions of growth factors on cell.[1] Insulin receptor is tyrosine-specific protein kinase. Stimulation of tyrosine kinase growth factor receptors activates PI3-K, which leads to Akt activation.[1] Precise regulation of Akt is critical for neuronal survival after brain injury.[40] Cytochrome c formed the apoptosome thereby initiating apoptotic cell death once cytochrome c released into the cytosol.[44]

The neuroprotection of insulin is limited. Mouse hippocampal slice exposed to 45 min OGD. 1 μM insulin pre-treatment for 24 h reduced OGD induced injury in slice through activating PI3-K/Akt signaling.[16] When slices cultured with insulin and mitogen-activated protein kinase (MAPK) inhibitor for 24 h followed by 45 min OGD, neuronal injury further decreased. These suggested insulin/MAPK pathway might act to antagonize insulin/PI3-K/Akt and contribute to the neuronal relative vulnerability to OGD. In another study of mouse cortical culture, insulin effectively attenuated neuronal apoptosis within 20 h, continued exposure to insulin for 48 h resulted in widespread neuronal necrosis.[45] Insulin can be both neuroprotective and neurotoxic in the same cell system but by way of different signaling.[45] These results suggest that insulin is not proper for continuous and long period usage.

## Insulin Resistance

Patients experiencing acute ischemic stroke may develop hyperglycemia, even without diabetes. Animal studies suggest that stress-induced hyperglycemia is associated with insulin resistance.

Acute ischemic stroke is associated with the hepatic insulin resistance in male rats, then unsuppressed hepatic glucose output may become a significant contributor to fasting hyperglycemia.[46] For mice, insulin sensitivity and insulin-secreting capacity were decreased on day 1 after middle cerebral artery occlusion.[47] The neuronal damage observed on day 3 was completely suppressed by administration of intermediate-acting insulin 3 times/d during the first 48 h.[47] In the gerbil model of cerebral ischemia, insulin-secretion was lower, and the apoptosis of pancreatic β-cell was higher in ischemic gerbils than sham operation group during 28 d after ischemia.[48]

On the basis of animal studies, there is short of insulin absolutely or relatively after brain ischemia. Interruption using a small dose of insulin may be better than continuous infusing insulin for neurons. It may be so in human, but that is short of clinical study.

## Brain Glucose and Blood Glucose

Gentile *et al*. found glycemic control (<7.2 mmol/L) was associated with a 4.6-fold decrease in mortality risk as compared with the case of stroke patients with persistently hyperglycemia.[33] However, some clinical studies showed intensive insulin therapy (IIT) (4.4–6.7 mmol/L) group and conventional insulin therapy group had similar mortality and neurological outcome.[49-52] Next discuss why strict control blood glucose did not improve outcome. First, both groups used insulin that mediated neuroprotection. Many animal studies demonstrate that administration of insulin reduces the brain injury associated with ischemia/reperfusion.[53,54] Second, IIT increases the risk of hypoglycemic episodes.[49-51] Both hyper and hypoglycemia have been associated with poor outcome in TBI, stroke, and brain hemorrhage.[55,56] Last, hyper and hypoglycemia must be avoided to prevent aggravation of underlying brain damage. The optimal blood glucose concentrations remain debatable.[57] IIT may be not as good as enough.

With regard to the CNS, brain glucose levels are two- to three-fold lower than the plasma glucose levels.[40] Brain glucose was influenced by arterial blood glucose. Brain glucose to blood glucose ratio was significantly increased at low blood glucose below 5 mmol/L followed by a significant decrease in arterial blood glucose of more than 5 mmol/L, reaching lowest brain to blood glucose ratio levels at blood glucose above 8 mmol/L.[58] The blood-brain barrier can regulate glucose transport depending on the pathophysiological state of the CNS.[59]

In one cerebral microdialysis (CDM) study for 20 patients (subarachoid hemorrhage, intracerebral hemorrhage, TBI, brain infarction), systemic glucose levels were categorized into two ranges: Tight 4.4–6.7 mmol/L and intermediate 6.8–10.0 mmol/L. Tight systemic glucose control significantly reduced cerebral extracellular glucose and increased prevalence of brain energy crisis, which in turn correlates with increased mortality. Brain energy crisis was defined as the combination of CMD glucose values <0.7 mmol/L with a lactate/pyruvate >40. At the extremes of systemic glucose concentrations (i.e., <4.4 mmol/L and >10 mmol/L), the highest lactate/pyruvate were observed.[56] CDM glucose was lower in nonsurvivours than in survivors (0.46 ± 0.23 mmol/L vs. 1.04 ± 0.56 mmol/L, $P < 0.05$).[56] In another CDM study for TBI patients, IIT (5–6.7 mmol/L) resulted in a significant reduction in microdialysis glucose and an increase in microdialysis glutamate and lactate/pyruvate without conveying a functional outcome advantage.[55]

The problem is which blood glucose range is proper to use insulin. Insulin had an ameliorative effect on the outcome of ischemic insult in gerbils when injected daily without hypoglycemia.[53] Rats were treated with insulin after brain ischemia. The nadir for infarction size lay in the 6–7 mmol/L blood glucose ranges, and the increased infarction size occurred in blood glucose of 2–3 mmol/L.[60]

The retrospective analysis of 20 TBI patients suggested that insulin administration at arterial blood glucose <5 mmol/L should be avoided because it was associated with decreased interstitial brain glucose, increased interstitial brain lactate levels, and elevated lactate-to-glucose ratios. The lactate-to-glucose ratio is not only a marker of anaerobic glycolysis but also could be used as a surrogate marker for underlying mitochondrial dysfunction. At arterial blood glucose levels >7 mmol/L, insulin administration is encouraged to increase extracellular glucose concentrations, decrease interstitial brain lactate levels, and decrease lactate-to-glucose ratios.[57] In addition, neuronal damage did not directly proportion to the degree of hyperglycemia, but it seems to have a threshold value around 9.44 mmol/L.[51] On the basis of the available data, the blood glucose levels appear to be optimal at 6–9 mmol/L that may be benefit for insulin neuroprotection.

## Conclusion

Most patients with acute stroke have only moderate hyperglycemia, and glucose concentrations fall spontaneously with the early introduction of a saline infusion.[61] Rapid changes in blood glucose related to excessive or insufficient insulin administration may contribute to secondary brain damage.[57] Adequate insulin therapy might provide intensive insulin doses with concurrent glucose infusion, to avoid both hypoglycemic episodes and insulin deficiency.

Glucose potassium insulin (GKI) infusions had been used to ischemic stroke patients. Acute stroke patients were randomly assigned to receive GKI or saline as a continuous intravenous infusion for 24 h. GKI infusion can be safely administered to acute stroke patients with mild to moderate hyperglycemia.[62] But there was no significant difference in mortality and neurological outcome.[61-63] Two reasons should be considered. First, the course of treatment was short. Animal studies showed apoptotic cells were detectable a few weeks after central nerve system injury.[64] Patients often have comorbidity of diabetes and/or hypertension. The etiology of cerebral ischemia may not be removed immediately. So injury of central nerve system would be more serious, and the course of GKI treatment should be prolonged. Second, in cortical neurons culture, insulin activated Akt and downstream effectors within 15 min of treatment and lasted for 2 h.[40] Twenty-four hours insulin stimulation mediated blunting of Akt and its downstream effectors.[40] If it do so in human, GKI is not proper for continuous infusion. Intermittent and long-term infusion insulin may be a benefit for patients with ischemic brain damage at blood glucose 6–9 mmol/L.

Experimental evidence shows insulin has a neuroprotective effect. Insulin should be used to ischemic stroke patients no matter the blood glucose is high, low or normal. It is good that insulin treatment as early as possible. It is harmful when insulin used improperly. Clinical trials are the much-needed step to studying the level of blood glucose, insulin dose, and insulin treating period. Intermittent and long-term GKI or

insulin infusion to treat ischemic brain damage may be a benefit to patients.

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