Topical Insulin and Accumulation of Excitotoxic and Other Amino Acids in Ischemic Rat Cerebral Cortex (44510)

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Abstract. **Insulin plays a neuroprotectant role in the brain and spinal cord during ischemia. However, studies have shown insulin to increase the sensitivity of cultured cortical cells to glutamate toxicity. The present study looked at the relationship between topically administered insulin (1 mIU insulin/ml and 100 mIU insulin/ml) during a four-vessel model of global ischemia and the accumulation of amino acids, especially glutamate, from the ischemic rat cerebral cortex. The lower dose of insulin was found to attenuate the release of excitotoxic and other amino acids from the cortex in ischemia/reperfusion. This may occur because insulin increases glucose availability to glial cells resulting in maintenance of glycolysis and ionic pumps that can reduce glutamate release and maintain uptake during ischemia/reperfusion. The higher dose of insulin, which significantly increased the amount of aspartate, glutamate, taurine, and GABA during reperfusion, may act to stimulate the amount of glycogen stored in astrocytes, reducing the availability of glucose for metabolic purposes.**

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Insulin has been shown to reduce ischemic necrosis and improve neurologic function in global and focal animal models of cerebral ischemia when given prior to (1–3) or after (4, 5) the onset of ischemia. Insulin given prior to spinal cord ischemia in rabbit (6) and rat (7) models has also been shown to improve recovery of evoked potentials and neurologic function. Insulin injected directly into the ventricular system reduced the effects of ischemic neuronal damage (8). The exact mechanism by which insulin acts as a neuroprotectant is still unknown. Insulin attenuates neuronal necrosis in the cortex, striatum, and hippocampus during global ischemia independent of its ability to lower blood glucose levels (9). This point is important in hyperglycemia which causes systemic insulin release, and is associated with lactic acidosis and more extensive ischemic neuronal injury (10).

Insulin has also been shown to stimulate the synthesis of DNA, RNA, and proteins in C6 glioma cells through a hormone-receptor interaction (11) and increase DNA and RNA synthesis by 50% in cultured cortical neurons (12). Sullivan *et al.* (13) also found that insulin can increase protein synthesis during reperfusion by dephosphorylation of eukaryotic initiation factor 2α (eIF2α). In addition to acting as a potential growth factor, insulin can also alter metabolism. Insulin stimulates the enzyme pyruvate dehydrogenase, which can fuel the production of ATP and allow for continued cellular functioning during ischemia/reperfusion (14). However, insulin also stimulates the enzyme glycogen synthase (14), which can lead to the storage of glycogen in astrocytes reducing the amount of glucose available for glycolysis.

Schafer and Erdo (12, 15), contrary to the above, demonstrated that insulin increased cultured cortical cell vulnerability to the excitotoxic effects of glutamate. They found that chronic exposure to insulin, but not insulin-like growth factor or β-fibroblast growth factor, increased the toxicity of

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glutamate possibly through increasing the expression of one or more excitotoxic amino acid receptors.

The present study looked at the relationship between insulin, topically applied prior to global ischemia/reperfusion, and the efflux of amino acids, especially glutamate, from the normoglycemic rat cerebral cortex. Insulin was applied topically to minimize systemic side effects such as hypoglycemia.

Materials and Methods

Twenty-one male Sprague-Dawley rats were prepared using the four-vessel occlusion model and cortical cup technique that has been described previously (16). The artificial CSF consisted of Na^+ 155.8 mEq/l; K^+ 2.95 mEq/l; Ca^{2+} 2.5 mEq/l; Mg^{2+} 1.85 mEq/l; Cl^- 141.1 mEq/l; $HCO3^-$ 22 mEq/l, and urea 40.2 mg/dl. There were 150 μl of the artificial CSF placed in the cortical cups and collected for each 10-min collection period.

Three sets of experiments were performed: i) artificial CSF (components listed above) placed topically (four 10-min basal collections) on the rat cerebral cortex undergoing 20 min of ischemia (two 10-min collections) and 40 min of reperfusion (four 10-min collections); ii) artificial CSF with 1 mIU insulin/ml applied topically following the collection of two basal 10-min collections and present in all subsequent superfusate applications that included two preischemic collections, two ischemic collections, and four post-ischemic collections; and iii) artificial CSF with 100 mIU insulin/ml added topically sampled in the manner described for the 1 mIU insulin/ml experiments. Eleven rats were used in Exp 1, and five rats were used each in Exps 2 and 3.

After the first two basal collections in each experiment (10 min each), the cortex was incubated with insulin for 15 min, with replenishment of the superfusates at 5-min intervals, and then for the remaining eight collections. A total of 0.03125 μg and 3.125 μg of insulin was placed in contact with the cortex prior to ischemia during the experiments in the 1 mIU insulin/ml and the 100 mIU insulin/ml groups, respectively.

After a 10-min centrifugation (1200*g*), perfusate samples were injected directly into the HPLC system without further processing. HPLC analyses of perfusate amino acid content was conducted within a few hours using previously published procedures (16).

Peripheral blood samples were analyzed for glucose prior to and after 35 min of topical insulin placement. All animal use procedures were in strict accordance with the NIH *Guide for the Care and Use of Laboratory Animals* and were approved by the University Animal Care Committee.

Statistical differences between amino acid levels measured between experimental groups 2 and 3 (1 mIU insulin/ml and 100 mIU insulin/ml) were analyzed by one-way ANOVA with contrast to the control group (0 IU insulin/ml).

Results

All animals had suppression of their electrocorticogram (ECoG) after the onset of ischemia with minimal recovery after 40 min of reperfusion. There were no significant differences in blood glucose levels before or after the topical placement of the 1 mIU insulin/ml or 100 mIU insulin/ml. Prior to insulin treatment, average blood glucose was 71.10 ± 1.05 mg/100 ml. Post 1 mIU insulin/ml average blood glucose was 70.40 ± 0.87 mg/100 ml, and post 100 mIU insulin/ml was 69.20 ± 0.68 mg/100 ml.

During ischemia/reperfusion, a significant decrease in aspartate occurred in the 1 mIU insulin/ml group compared with the control group, and during reperfusion a significant increase occurred in the 100 mIU insulin/ml animals compared with the controls (Fig. 1). During ischemia/reperfusion, glutamate was significantly decreased in the 1 mIU insulin/ml group and, during reperfusion, glutamate was increased in the 100 mIU insulin/ml group compared with the control. During reperfusion, there were significant decreases in phosphoethanolamine levels in both insulin-treated groups. During reperfusion, there was a significant decrease in taurine levels in the 1 mIU insulin/ml group and a significant increase in taurine levels with the higher dose

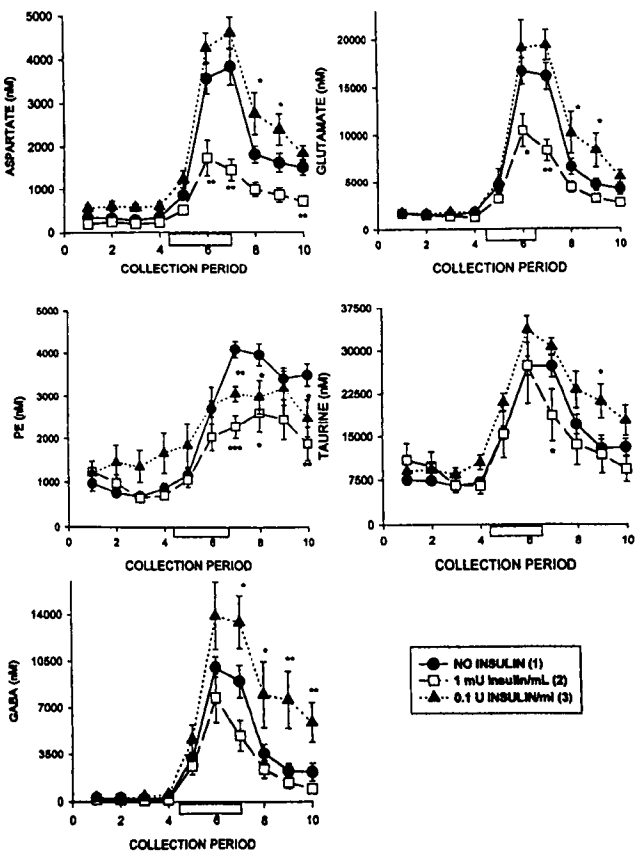

Figure 1. Comparison of three different levels of topical insulin, 0 IU/ml, 1 mIU/ml, and 0.1 IU/ml (100 mIU insulin/ml), on amino acid accumulation in rat cortical perfusate. Statistics used were one-way ANOVA with contrast to the control group. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. The clear bar under the collection period represents the ischemic period.

Table I. Amino Acid Levels in Rat Cortical Perfusates in the Three Experimental Groups during Basal Conditions, Ischemia, and Reperfusion

Amino acids	Basal levels (n*m*)			Ischemia levels (n*M*)			Reperfusion levels (n*m*)		
	Control	1 mIU insulin/ml	0.1 IU insulin/ml	Control	1 mIU insulin/ml	0.1 IU insulin/ml	Control	1 mIU insulin/ml	0.1 IU insulin/ml
Aspartate	322 ± 27	317 ± 20	476 ± 82	2183 ± 215	1103 ± 244[a]	2723 ± 253	2148 ± 207	989 ± 142[a]	2865 ± 291[a]
Glutamate	1666 ± 204	1483 ± 303	1763 ± 258	10623 ± 949	6819 ± 944[a]	12106 ± 2084	7839 ± 727	4649 ± 504	10842 ± 1420
Serine	12353 ± 1023	11533 ± 1924	13942 ± 1102	11913 ± 774	11791 ± 1836	14420 ± 779	13693 ± 1084	12115 ± 1838	15688 ± 923
Glutamine	26213 ± 1634	27178 ± 5083	31248 ± 2244	25944 ± 1497	27791 ± 5737	34159 ± 1866	24806 ± 1365	24893 ± 4511	31147 ± 2538
Glycine	23649 ± 2139	20454 ± 3581	26762 ± 2817	20800 ± 1563	20311 ± 3393	25499 ± 2402	26277 ± 2330	24720 ± 3786	32001 ± 3041
PE	823 ± 106	899 ± 138	1143 ± 350	1933 ± 155	1553 ± 229	2317 ± 464	3704 ± 163	2313 ± 370[a]	2931 ± 301[a]
Taurine	7100 ± 704	8499 ± 1924	9294 ± 994	21406 ± 1327	21342 ± 5250	27268 ± 1791	17374 ± 1429	13305 ± 3280	23086 ± 2324
Alanine	19722 ± 1716	17639 ± 3108	23256 ± 2133	15989 ± 1254	15519 ± 2658	22552 ± 1710	26447 ± 2346	21755 ± 3269	3262 ± 2939
GABA	252 ± 71	162 ± 45	354 ± 111	6627 ± 590	5161 ± 1237	9279 ± 1792	4221 ± 675	2389 ± 585	8730 ± 1887[a]

[a] $P < 0.05$ using one-way ANOVA with contrast to the control group. Basal, ischemia, and reperfusion levels are the average ± SEM of two, two and four collections, respectively.

of insulin compared with the control group. During reperfusion, the levels of GABA in the 100 mIU insulin/ml group were significantly increased. Levels of all amino acids during basal conditions, ischemia, and reperfusion are presented in Table I.

Discussion

This study demonstrates that insulin (1 mIU insulin/ml) placed topically on the rat cerebral cortex prior to ischemia is able to attenuate the efflux of amino acids, including aspartate, glutamate, phosphoethanolamine, and taurine, from the rat cerebral cortex during ischemia/reperfusion. This may be directly related to insulin's ability to stimulate glycolysis and pyruvate dehydrogenase. The increased energy available to the brain could help astrocytes continue their uptake of glutamate from the extracellular space (17). The resulting increases in ATP and lactate, which can serve as an energy source for neurons postischemia (18), would maintain ion pumps resulting in a decrease in amino acid efflux into the aCSF.

Insulin, in addition to stimulating glycolysis, also stimulates glycogen synthase (14). 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. Insulin could also have a direct influence on cells by acting as a growth factor and stimulating protein synthesis (11, 13). These are all mechanisms by which insulin could result in a decrease in glutamate efflux into the aCSF from the ischemic rat cerebral cortex and play a neuroprotectant role.

The fact that the higher dose of insulin increased the amount of aspartate, glutamate, taurine, and GABA during reperfusion may reflect the resulting acidosis caused by the even higher rate of glycolysis and formation of lactate that could lead to increased neuronal damage. Insulin has also been shown to cause increased sensitivity to glutamate in cultured cortical neurons, possibly due to an upregulation of the excitotoxic receptors at a dose of 5 μg/ml (12, 15). Our high dose of insulin for the total experiment including preincubation, ischemia, and reperfusion was comparable to that used in these studies and could explain the increased efflux into the aCSF of several of the amino acids during reperfusion due to the buildup of insulin to this neurotoxic dose.

Conclusion

Topically placed insulin at a dose of 1 mIU insulin/ml reduces the amount of aspartate and glutamate accumulation from the rat cerebral cortex during ischemia/reperfusion. This could reflect the ability of insulin to stimulate glycolysis and protein synthesis that could enable the cell to maintain its energy sources and ion pumps during ischemia/reperfusion.

Topically placed insulin at a dose of 100 mIU insulin/ml increased the accumulation of excitotoxic and other amino acids during reperfusion. This could be due to the buildup of insulin that could then act to upregulate excitotoxic amino acid receptors and render the cells more susceptible to the effects of glutamate. Insulin at this dose could also cause acidosis due to the increased stimulation of glycolysis and lactic acid formation.

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