Intranasal administration of insulin lowers amyloid-ß levels in rat model of diabetes

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Alzheimer’s disease (AD) is a neurodegenerative disorder characterized by abnormal accumulation of amyloid β (Aß) peptide in brain regions subserving memory and other cognitive functions. Hyperglycemia and perturbed insulin signaling have been proposed as pathogenic factors contributing to AD. The aim of the present study is to validate the use of streptozotocin (STZ) injected rats as an experimental model of AD. Using this model, the effect of intranasal administration of insulin on reduction of Aß levels was measured. The current findings strengthen the case for insulin as therapy for AD afflicted individuals with or without diabetes.

Keywords: Alzheimer’s disease, Amyloid β, Diabetes, Insulin, Streptozotocin

Alzheimer’s disease (AD) is a neurodegenerative disorder with progressive decline in cognitive functions caused by selective dysfunction of neurons in the neocortex, hippocampus and basal forebrain. The pathological hallmarks of AD include extracellular senile plaques that contain the amyloidogenic, 40-42 amino acid long amyloid β (Aß) peptide generated by proteolytic processing of a larger precursor protein. The second feature of AD is the accumulation of intracellular neurofibrillary tangles consisting of hyperphosphorylated tau arranged in the form of paired helical filaments.

Increasing amount of evidence from clinical studies implicates diabetes mellitus, both Type 1 and Type 2, as risk factors for AD. Disturbance of the insulin signaling pathway is emerging as a common feature of both AD and diabetes. Jolivalt et al. have demonstrated the presence of learning deficits associated with increased glycogen synthase kinase 3 activity, increased tau phosphorylation and increased Aß protein levels, which are characteristic of AD, in the brain of a mouse model of Type 1 diabetes. Similar changes were also shown in a rat model of spontaneous Type 1 diabetes. By a novel approach, Jolivalt et al. characterized a mouse model of combined insulin-deficient diabetes and AD and found that diabetes exaggerates defects associated with AD in the brain of these transgenic mice.

In the present study, hyperglycemia has been induced in rats and the influence of intranasal insulin administration on modulation of hippocampal Aß levels investigated, as a plausible therapeutic approach to AD.

Materials and Methods

Materials—Streptozotocin, insulin (human, recombinant), phenylmethanesulphonyl fluoride (PMSF), Coomassie Brilliant Blue R-250 were purchased from Sigma-Aldrich, Bangalore. ELISA kit for estimation of rat Aß was purchased from Uscn Life Science Inc, Wuhan, P.R. China. All other reagents used were of analytical grade and obtained locally.

Animal studies—Six-month-old female Sprague-Dawley rats recruited for the study were housed three per cage with water and food ad libitum. A 12 : 12 h L : D cycle was maintained. All the protocols used in this study were approved by Institutional Animal Ethics Committee.

Diabetes was induced in the rats by a single injection of streptozotocin (STZ) in 0.1 M citrate buffer, pH 4.4 (35 mg/ kg body weight) through ip route. To confirm the hyperglycemic status, test bleeds were collected 3 days after STZ injection and at the time of conclusion of the study by cardiac puncture and the glucose levels monitored by glucose oxidase/ peroxidase (GOD/POD) method. After
4 weeks of STZ injection, insulin (human, recombinant) (5 IU/rat/day) was administered through intranasal route for 6 consecutive days. After 24 h, CSF was collected by the procedure described elsewhere. Briefly, rats were anaesthetized with 5% halothane. The rat head was flexed at approx 45° such that the appearance of a rhomb between occipital protuberances and the spine of the atlas becomes visible. 23G needle was inserted into the cisterna magna for CSF collection without making any incision at this region. Around 100-120 µl of CSF could be tapped by this method, which was free of red blood cell contamination. CSF glucose levels were estimated by GOD/POD method.

**ELISA method for estimation of Aβ**—The rats were sacrificed by cervical dislocation and the brain removed. Hippocampi were dissected and homogenized in ice-cold 50 mM Tris-HCl buffer, pH 7.4 containing 150 mM NaCl, 2 mM EDTA, 1 mM PMSF and 0.5% Triton X-100 followed by sonication (10 sec × 2 cycles). Homogenates were then centrifuged at 13, 000 g for 20 min. The clear extracts obtained were taken for quantitative measurement of Aβ by sandwich ELISA method as per manufacturer’s instructions. The sensitivity of the assay was 5.9 pg/mL and the detection range was 7.8 – 500 pg/mL.

**Statistical analysis**—The data are expressed as mean ± S D and differences between groups were analyzed by one-way ANOVA using GraphPad Version 3 (Prizm; GraphPad Software Inc, San Diego, California, USA).

**Results and Discussion**

Epidemiological studies have shown that patients with diabetes exhibit an increased risk of developing AD compared with healthy individuals. Insulin deficiency associated with type 1 diabetes attenuates long term potentiation and leads to cognitive deficits observed in patients with AD. Suggested biological mechanisms that link the development of AD with diabetes include abnormal protein processing, impairment in insulin signaling, dysregulated glucose metabolism, mitochondrial dysfunction, hypercholesterolemia, and the activation of inflammatory pathways common to both diseases. Placement of insulin implants subcutaneously partially prevented behavioural and biochemical changes observed in the type 1 diabetic mouse model suggesting that insulin deficiency plays a pathogenic role in the development of AD-like features in the brain.

Induction of diabetes—In the present study, STZ induced diabetic rats were used as a model system to study the benefits of intranasal insulin treatment on modulation of brain Aβ levels. Towards this, adult female Sprague-Dawley rats were injected with a single dose of STZ (35 mg/kg, ip). Measurement of glucose levels in the serum and CSF collected 3 days after STZ injection confirmed the hyperglycemic status in these animals. The elevated glucose levels in the CSF and sera remained high till the point of sacrifice (5 weeks). Throughout the duration of the experiments, STZ-injected animals did not show any significant change in the total body weight over the controls (Table 1).

Intranasal administration of insulin—The choice of intranasal route to reach the CNS used in the present study is based on the merits this method offers over the invasive procedures like intracerebroventricular injections to overcome the blood-brain barrier, in terms of the ease and cost. With intranasal administration, insulin-like peptides reach the brain within minutes via extracellular bulk flow transport along the olfactory and trigeminal perivascular channels, as well as through axonal transport pathways. Several classes of therapeutics have successfully been intranasally delivered to the CNS including IGF-1, cytokines and several small molecules. It is very well known that intranasal insulin improves memory in normal adults and patients with AD. In the present communication a direct correlation between intranasal insulin administration and reduction in Aβ levels in the hippocampi of normal and STZ-induced diabetic rats has been presented. Towards this, after 4 weeks of onset of hyperglycemia, to the treatment groups, insulin (5 IU/day) was given intranasally for 6 consecutive days. Measurement of blood glucose levels revealed that intranasal insulin in the CNS did not significantly influence the peripheral levels.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Initial body weight (g)</th>
<th>Final body weight (g)</th>
<th>Final serum glucose (mg/ dL)</th>
<th>Final CSF glucose (mg/ dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>184±5.47</td>
<td>194±6.45</td>
<td>87±17.2</td>
<td>32.5±12.17</td>
</tr>
<tr>
<td>STZ-injected</td>
<td>202±16.76</td>
<td>175±18.52</td>
<td>499±112.4</td>
<td>178.6±35.51</td>
</tr>
</tbody>
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hyperglycemic status in the STZ-injected rats (data not shown). Total protein profile of the hippocampal extracts as visualized by SDS-PAGE did not show any significant change across the different groups (Fig. 1A). Quantitative measurement of Aβ levels by ELISA (Fig. 1B) showed comparable amounts in the control and STZ-injected rats. Interestingly, intranasal insulin treatment significantly (P<0.001) reduced the levels of Aβ in both the groups (Fig. 1B). These results are in accordance with the previous reports on the influence of intranasal insulin in improving cognition and modulating the peripheral Aβ levels in early AD volunteers. The present findings provide direct evidence that intranasal treatment with insulin lowers Aβ levels in the brain. Particularly, the reduction in Aβ levels in the control animals strengthens the case of insulin as therapy for AD. This method of intranasal delivery can revolutionize the treatment of AD and other neurodegenerative disorders.

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References


