Investigation on the expression of IGF-I protein in insulin-resistant rat brain

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ABSTRACT

Background: In insulin-resistant animal models, insulin uptake from the periphery to the brain is impaired. Although brain insulin is not involved in glucose transfer to the neurons, it is required for neuron survival and function, mediated by binding to insulin receptors. Furthermore, an insulin homologue called insulin-like growth factor (IGF-I), which is abundantly expressed in mature neurons and acts in parallel with insulin in the brain, has the ability to bind to the insulin receptor and trigger the signal transduction pathway.

Objectives: Although reduced levels of brain insulin and serum IGF-I have been reported during insulin resistance, no data is available on IGF-I levels in the brain. In this study, we sought to investigate if the expression of IGF-I is also altered in brains of insulin-resistant rats.

Materials and Methods: Wistar rats were given 10% fructose in their drinking water for up to 4 months to induce insulin resistance. The rats were then killed and perfused with PFA 4%; then, their brains were excised, sectioned, and examined for immunoreactivity of IGF-I.

Results: Our results showed an increased intensity of IGF-I in most brain areas of the insulin-resistant rats.

Conclusions: Altogether, an increased expression of IGF-I in the brain could be a compensatory mechanism and substitute for low levels or lack of insulin in the brains of insulin-resistant animals.

ARTICLE INFO

Article Type: Original Article

Article history:
Received: 5 Dec 2010
Revised: 17 Dec 2010
Accepted: 1 Jan 2011

Keywords:
Diabetes
Insulin resistance
Brain
IGF-I

1. Background

Increasing evidence indicates that brain insulin plays important roles in metabolism and food uptake (1, 2). Most brain insulin is derived from the pancreas, through a receptor mediated transfer (3, 4). In insulin-resistant rats, insulin uptake and therefore its level is reduced in brain (3-5). It is generally accepted that insulin resistance
is a result of altered receptor-mediated signal transduction (6). Although brain insulin is not involved in glucose uptake by neurons, it affects neuron survival and function. The effects of insulin and insulin-like growth factor (IGF-I) in the brain are receptor mediated (3, 4). Mutant mice for insulin receptors are obese, nontolerant to glucose, and insulin resistant (7, 8). Unlike insulin which is either not synthesized or present at low levels, IGF-I is expressed in almost all areas of the adult brain (8). Altogether, with respect to the role of insulin in regulation of energy and metabolism (9) in the brain and high energy demands for formation and function of neurons and their synapses, a homologue and ubiquitous substitute for insulin is required. This substitute should fulfill autocrine and paracrine actions of insulin in energy consumption (10) as well as low levels of insulin in insulin resistance (11).

2. Objectives

In this study, we have therefore sought to examine if brain IGF-I is altered in insulin-resistant rats and can be regarded as a substitute for insulin.

3. Materials and methods

3.1. Induction of insulin resistance

Male Wistar rats (200–250 gr) were purchased from the Pasteur institute. The animals were weighed, placed in separate cages, and divided in to control and experimental groups (n = 8). The experimental rats were fed with fructose in their drinking water (10%), and the control rats were given normal water. Both groups were fed with normal chow. To determine the insulin resistance, the levels of glucose, triglycerides, and insulin were measured every 2 weeks. Following 4 months of fructose treatment, there was a significant increase in the serum level of triglycerides (P < 0.05) but a decrease in the level of insulin (P < 0.05).

3.2. Measurements of serum glucose, triglyceride, and insulin levels

Parsazmun kit was used to measure the levels of glucose and triglycerides at 496 nm, and DRG diagnostic kit for that of insulin at 450 nm. The regression equation was obtained from the absorbance values against different concentrations of the standard samples. Using the obtained equation, concentrations of the samples were calculated.

3.3. Perfusion

Using 2% chloral hydrate, the animals were deeply anesthetized and perfused by buffer phosphate followed by glutaraldehyde/paraformaldehyde (500 ml). The skulls were opened, the brains were taken out and postfixed in the same fixative for another 24 hours. For cryosectioning, brains had to be kept for 2 weeks in sucrose (30%) with formalin (10%), and for long-term preservation, sucrose (30%) containing sodium azide (0.05%) at 4°C was used.

3.4. Immunohistochemistry

Prior to cryosectioning, the brains were soaked in OCT compound, sectioned at 40 nm and mounted on poly-L-lysine-coated slides. After being completely dried, sections were soaked in PBS and incubated in sodium borohydride (0.1%) followed by periodic acid (0.1%) for 10 minutes to reduce the background. For signal intensification, sections were then incubated in citrate buffer (in a 96°C water bath) for 40 minutes. After blocking in skimmed milk for 2 hours, sections were incubated with IGF-I antibody (cell signaling, USA) overnight at 4°C. Following incubations with biotinylated secondary antibody for 2 hours at room temperature, HRP-streptavidin for 1 hour at room temperature, the reaction was developed and visualized by DAB chromogen.

4. Results

4.1. Triglyceride but not glucose is increased in insulin resistant rats

Glucose and triglyceride measurements in serum were performed in both treated and control groups every month. The average level of serum glucose did not change significantly after 4 months (Figure 1; 391 ± 70 mg/dl in the fructose-fed rats compared with 140 ± 120 mg/dl in the control rats). The level of triglyceride, however, increased significantly after 4 months (Figure 2; 48 ± 8.5 mg/dl in the fructose-fed rats compared with 26 ± 2.2 mg/dl in the control rats; P < 0.05).

4.2. Insulin in increased in insulin resistant rats

Serum level of insulin was assayed by using a rat-insulin Elisa kit (DRG diagnostics). The results showed significant increase in the level of insulin in fructose-fed animals (Figure 3; 0.88 ± 0.1 mg/dl in fructose fed rats compared to 0.25 ± 0.2 mg/dl in control rats; P < 0.02).
4.3. Immunoreactivity of IGF-I is increased in insulin-resistant brains

The intensity of IGF-I immunoreactivity in different brain areas in the fructose-fed rats was compared with that in the control rats and categorized as low (+), moderate (++), and intense (+++). Analysis of the comparisons was performed by Mann-Whitney and Kruskal-Wallis statistical methods (Table 1). The results show that IGF-I ir in different areas of the brain, such as the brain stem, cerebellum, hippocampus, and thalamus increased significantly ($P < 0.05$) in the fructose-fed rats (Figure 4: a–g) compared to the control rats (Figure 4: b–h).

5. Discussion

5.1. Fructose diet induces insulin resistance

Fructose syrups, as the most commonly used sweetener in food products, result in increased uptake of daily carbohydrates and therefore increase the chance of insulin resistance and metabolic disturbances (12, 13). Based on both animal and human studies (12-15), we used fructose 10% in drinking water of adult rats to induce an animal model for insulin resistance. Our measurements of triglyceride and insulin levels indicated that the levels of both increased significantly after 4 months. This time point should be considered as a critical point to reduce both the time and efforts in triglyceride and insulin measurements. The latency of 4 months also has been documented elsewhere (14, 16). Moroz and colleagues who have used high fat diet in obese mice, introduced measurements of insulin concentration as a reliable indicator which increased with a latency of 16 weeks (16).

5.2. Increased expression of IGF-I in insulin-resistant brain as a compensatory mechanism

Reports have shown that despite the essential role of brain insulin in controlling peripheral metabolism, food intake, and body weight, it is not involved in glucose uptake by neurons. In mutant mice lacking insulin receptors in the brain, studies found that control of food intake and metabolism was disturbed and resulted in insulin resistance (1, 2). Insulin resistance which disturbs insulin transfer from the liver to the brain, impairs neuron function and survival and therefore memory and learning (16). IGF-I as a homologue for insulin has been shown to act in parallel with insulin (9). In this study therefore, we sought to examine if IGF-I level has also been changed in insulin resistant brain. According to our findings, the level of IGF-I significantly increased in different brain areas of rats fed with fructose in their drinking water 10% for at least 4 months. Similarly, Moroz and colleagues...
Figure 4. Photomicrographs of IGF-I immunostained sections from the fructose-fed rats (a = Purkinje cells of cerebellum, c = brain stem, e = hippocampus, g = thalamus) and the control rats (b = Purkinje cells of cerebellum, d = brain stem, f = hippocampus, h = thalamus). A significant increase was observed in the IGF-I ir in different areas of the insulin-resistant brains. 1 cm bar = 90 μm.
16) have also shown that in mice fed with high fat food, IGF-I mRNA is significantly increased in brain. Since IGF-I acts through insulin receptors and triggers the intracellular pathways, one may conclude that the increased brain IGF-I compensates for the reduced insulin levels in insulin-resistant brain. Furthermore, the increased IGF-I may also compensate for the reduced level of IGF-I in serum and peripheral tissues in diabetes type II(17, 18). Indeed, the regulatory feedback mechanism driven by peripheral IGF-I to control brain IGF-I level has also been documented by Torres alleman (19). Altogether, IGF-I signaling could be regarded as a complementary pathway for insulin in the brain to prevent disruption in metabolism and survival of neurons in the brain. In patients with diabetes type III or Alzheimer’s disease, the IGF-I signaling pathway is impaired even at the early stages of the disease (20, 21), resulting in dramatic changes in the brain. Future studies on signaling molecules activated in response to the increased levels of IGF-I in insulin-resistant brains would lead to a better understanding of the complex dynamic of insulin resistance.

Financial support

This project was supported by a grant from the National Research Institute of Genetic Engineering and Biotechnology of Iran.

Conflict of interest

There are no conflicts of interest.

Acknowledgments

Technical assistance in generating the animal model was kindly provided by the animal facility in the Department of Clinical Biochemistry, Hospital for Sick Kids, University of Toronto, Toronto, Canada.

References