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Antidepressant effects of insulin in streptozotocin induced diabetic mice: Modulation of brain serotonin system

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HIGHLIGHTS

- STZ induced diabetes resulted in depression in mice.
- STZ induced diabetes exhibited decreased serotonin levels in the brain.
- Insulin treatment produced an antidepressant effect in STZ induced diabetic mice.
- Insulin significantly increased the brain serotonin concentrations.
- Thus, insulin could be acting through modulation of the brain serotonin system.

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ABSTRACT

Diabetes is a persistent metabolic disorder, which often leads to depression as a result of the impaired neurotransmitter function. Insulin is believed to have antidepressant effects in depression associated with diabetes; however, the mechanism underlying the postulated effect is poorly understood. In the present study, it is hypothesized that insulin mediates an antidepressant effect in streptozotocin (STZ) induced diabetes in mice through modulation of the serotonin system in the brain. Therefore, the current study investigated the antidepressant effect of insulin in STZ induced diabetes in mice and insulin mediated modulation in the brain serotonin system. In addition, the possible pathways that lead to altered serotonin levels as a result of insulin administration were examined. Experimentally, Swiss albino mice of either sex were rendered diabetic by a single intraperitoneal (i.p.) injection of STZ. After one week, diabetic mice received a single dose of either insulin or saline or escitalopram for 14 days. Thereafter, behavioral studies were conducted to test the behavioral despair effects using forced swim test (FST) and tail suspension test (TST), followed by biochemical estimations of serotonin concentrations and monoamine oxidase (MAO) activity in the whole brain content. The results demonstrated that, STZ treated diabetic mice exhibited an increased duration of immobility in FST and TST as compared to non-diabetic mice, while insulin treatment significantly reversed the effect. Biochemical assays revealed that administration of insulin attenuated STZ treated diabetes induced neurochemical alterations as indicated by elevated serotonin levels and decreased MAO-A and MAO-B activities in the brain. Collectively, the data indicate that insulin exhibits antidepressant effects in depression associated with STZ induced diabetes in mice through the elevation of the brain serotonin levels.

1. Introduction

Diabetes is a chronic metabolic disorder characterized by hyperglycemia as a result of impaired insulin regulatory mechanism, which often results in neuropsychological complications such as depression. Studies have reported the prevalence rates of depression of 24–30% in diabetic individuals [1,2] with significantly increased risk of morbidity and mortality [3,4]. Pharmacological reports manifest that the altered physiological processes such as elevated glucose oxidation, insulin deficiency and/or sensitivity and adaptive neurocellular responses as a consequence of diabetes may cause depression [5,6].

Insulin as a peptide hormone and neuromodulator has been known to have diverse functions in the brain [7,8]. Previous studies have demonstrated the role of insulin in improving cognitive functions including learning and memory, and in attenuating the neuronal damage associated with neurodegenerative diseases [9,10]. In addition, a growing body of evidence suggests the activity of insulin in depression comorbid with diabetes. Behavioral studies have shown that treatment with insulin produces antidepressant effects in streptozotocin (STZ) induced

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diabetes such as a decreased duration of immobility in mouse tail suspension test (TST) and rat forced swim test (FST) [11,12]. Furthermore, insulin increases reward behavior in STZ diabetic mice subjected to an intracranial self stimulation testing paradigm [11]. While insulin deficiency leads to depression like symptoms in STZ-diabetic rats [13]. However, the neurochemical mechanism underlying these effects is poorly understood.

The monoamine serotonin is a well established neurotransmitter involved in the pathophysiology of depression. The classic monoamine hypothesis states that monoamine imbalance or more specifically neurotransmitter deficiency in certain areas in the brain develops depression [14,15]. Decreased serotonin levels, resulting in altered neurobehavioral functions has been evident both in diabetic animal models and humans suffering from diabetes [16–20]. Interestingly, insulin has been known to modulate the serotonin system in the brain. Literary data give evidence that the administration of insulin causes a sequential increase in the concentration of tryptophan (a serotonin precursor) and serotonin in the rat brain [21,22]. While the destruction of insulin secreting beta cells in rat pancreas has shown a significant decrease in central tryptophan levels and increased serotonin metabolism [23], indicating lower levels of serotonin in the brain, which is reversed by the administration of insulin. Furthermore, the presence of insulin receptors in the brain stem, olfactory bulb, hypothalamus and limbic system, and a high synaptic density in hippocampal cultured neurons of the rat brain demonstrate the modulatory effect of insulin in the signal transduction pathway involved in the regulation of mood and behavior [24]. Accordingly, it is hypothesized that the antidepressant effect of insulin may be associated with increased serotonin levels in the brain. Therefore, the present study, by using the validated behavioral models and biochemical assays such as estimation of serotonin levels and MAO activity in the whole brain content, investigated the antidepressant effect of insulin and whether the brain serotonin activity plays any role in the postulated effect of the peptide in STZ induced diabetic mice.

2. Methods

2.1. Animals

Swiss Albino mice (3 months of age, of either sex) were obtained from Hisar Agricultural University, Haryana, India. Mice were housed in cages in a group of 5–6 and were maintained in standard laboratory conditions with an alternating light and dark cycle of 12 h each, temperature of 23 ± 4 °C and humidity conditions of 62 ± 5% RH in the housing unit. Mice had free access to food (standard pellet chow feed) and filtered water ad libitum except for the blood glucose monitoring, in which mice were fasted for 18–20 h prior to the blood collection. The protocol for the experiment is shown in Table 1. Behavioral testing was done during the light cycle. Mice were treated according to the guidelines of the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA, Registration number 417/01/a/CPCSEA, New Delhi), Government of India and all experiments were conducted in adherence to the approved protocol of the Institutional Animal Ethics Committee (IAEC) of the Birla Institute of Technology & Science, Pilani, India (Protocol number IAEC/RES/14/11, August-2011).

2.2. Drugs

Insulin glargine (Lantus) was purchased from Sanofi-Aventis Pharma Ltd. (Germany). STZ and serotonin were purchased from Sigma-Aldrich, USA. Escitalopram (ESC) was obtained from the Ranbaxy Research Laboratories, India as a gift sample.

2.3. Induction of diabetes and glucose monitoring

To induce diabetes, mice were given a single intraperitoneal (i.p.) injection of 200 mg/kg STZ prepared in 5 M sodium citrate, pH 4.5, or vehicle without STZ. Fasted blood glucose levels were measured periodically, from the 3rd day of STZ injection, using a portable Freestyle glucometer (Akkiscan, Zee^{+} Glucose Meter, Nepro Care, India). Blood was obtained via tail snip. Mice with fasting blood glucose values of 200 mg/dl or above were included in the diabetic groups. Glucose levels were then measured on a weekly basis, in the morning between 0800 and 1000, until the completion of the study.

2.3.1. Insulin treatment

Long-acting insulin glargine (1–2 IU/kg, i.p.) was delivered to mice once a day from the 7th day of STZ injection and continued till the 20th day (i.e. treatment given for 14 days). Mice in the vehicle groups received saline once daily for 14 days.

2.4. Experiment 1: Behavioral assays

Behavioral testing began after 24 h of last insulin treatment. There were five experimental groups with 6 mice randomly divided in each within diabetic and non-diabetic group: non-diabetic control (NC); diabetic control (DC); diabetic mice given insulin at 1 IU/kg (D-Ins 1); diabetic mice given insulin at the dose of 2 IU/kg (D-Ins 2); and diabetic mice given ESC at the dose of 10 mg/kg (D-ESC 10) [25].

2.4.1. Forced swim test

FST was carried out as described elsewhere with slight modifications [26]. Mice were dropped individually into a plexiglass cylinder (height: 30 cm, diameter: 22.5 cm) filled with water to a depth of 15 cm and maintained at 23–25 °C. In this test, after an initial vigorous activity of 2 min, mice acquired an immobile posture which was characterized by motionless floating in the water and making only those movements necessary to keep the head above the water. The duration of immobility (s), was recorded during the last 4 min of the 6 min test. The mice were subjected to a 15 min training session under similar conditions, 24 h before the test.

2.4.2. Tail suspension test

Mice were individually suspended by the tail to a horizontal bar (distance from the floor was 50 cm) using scotch tape (distance from tip of tail was approximately 1 cm). Typically, mice exhibited several escape-oriented behaviors interspersed with temporally increasing

Table 1
Simplistic representation of study protocol.

Day	0	1	3	8–21	22	26
Treatment	Initial body weight (g) and fasted blood glucose (mg/dl) measurements	STZ/ vehicle dosing	Fasted blood glucose (mg/dl) monitoring	Insulin/ ESC dosing	Behavioral assays	Neurochemical assays

(→) Represents the continuation of the treatment during the study period.