INTRODUCTION

Dementia represents a variety of symptoms and signs, including a reduction in memory, thinking and communication skills with a gradual loss in the ability to fulfill activities of daily living. The commonest forms are Alzheimer’s disease (AD) and vascular dementia (VaD). So, we aimed to investigate the effect of sitagliptin in improving the working and reference memories in diabetic rats. Thirty six male Sprague-Dawley rats divided equally (n=12) into three groups: control, type 2 DM and type 2 DM treated with DPP-4 inhibitor (sitagliptin) for one month (10 mg/kg) orally. Working memory and reference memory were assessed by using the holeboard memory test. In all rats, serum glucose, insulin, adiponectin, total cholesterol (TC), TG, low (LDL) and high (HDL) density lipoprotein with calculation of the homeostasis model of assessment-insulin resistance index (HOMA-IR) and atherogenic index. The hypothalamus was separated for determination of the acetylcholine level and adiponectin receptors 1 (Adipo R1) m-RNA expression. Type 2 diabetic rats exhibited a significant decrease in both working and reference memories, with increased glucose, insulin and HOMA-IR. The adiponectin level, acetylcholine content of the hypothalamus and Adipo R1 m-RNA expression were significantly reduced. Treatment with sitagliptin significantly improved the working and reference memories with significant reduction in the glucose, insulin and HOMA-IR. Moreover, sitagliptin increased significantly the acetylcholine content of the hypothalamus and Adipo R1 expression. In conclusion, sitagliptin might improve the cognitive function of the diabetic rats and the hypothalamic acetylcholine level possibly through increased AdipoR1 expression.

Key words: working memory, acetylcholine, adiponectin, adiponectin receptors 1, sitagliptin, diabetes mellitus type 2, glucose, insulin, dementia
adjustment of glucose and fatty acids metabolism in peripheral tissues such as muscle and liver (10). Adiponectin is well-defined as an anti-diabetic and anti-atherogenic adipokine (11). It acts through two receptors: Adipor1 and Adipor2 due to its metabolic effects (12, 13). However, Adipor1 is more abundantly expressed in muscles while Adipor2 predominates in the liver (12). Recently, they have also been described in rodent and human hypothalamus (14-16). Adiponectin is protective in human neuroblastoma cells with amyloid B neurotoxicity. Adiponectin has been reported to be neuroprotective against 1-methyl-4-phenyl-piridinium ion (MPP) induced cytotoxicity (17), against acetaldehyde-induced apoptosis, in a mouse model of epilepsy against kainic acid-induced excitotoxicity (18) and the rat model of stroke against cerebral ischemia-reperfusion injury (19). A recent study reported that intense expression of AdipoR1 was noted in the hypothalamus and the nucleus basalis of Meynert in the basal forebrain which is frequently affected in AD (20).

In the present study, the holeboard food search task has been used in rats to analyze their learning ability and different types of memory, IC working and reference memory. Vawter and Van Ree (21) confirmed that the performance in the holeboard memory test is sensitive to the degree of food deficiency. A higher level of food deprivation resulted in a superior performance of the animals, but the processes implicated in learning and memory were less affected. The data obtained by them indicated that both external and internal characteristics can influence the results of the hole board food search task, and thus the calculated scores for learning and memory (21).

However, there is no single report in literature investigating the possible protective effect of sitagliptin on the cognitive function of the diabetic rats. The aim of the present work is to investigate the effect of type 2 diabetes mellitus and incretins treatment on the working memory and reference memory through the holeboard apparatus in a 4'4 array. The testing area of the holeboard apparatus consists of 16 holes in a 4'4 array. The holes are 20 cm apart and are filled with plastic cups (2.5 cm deep, 3 cm in diameter) for sweetened cereal food pellets. Some were spilled on the floor to eliminate the impact of smell (olfactory stimuli) on the search for food. The rats had to collect pellets in specified periods of time.

The experimental design

The animals were then divided randomly into two groups. Normal rats (n=12) were fed standard rat chow diet and remained untreated for the duration of the study. Experimental rats (n=24) were fed a diet enriched in fat (25% fat, 15% protein, 51% starch and 5% fiber). After 1 month on their respective diets, experimental rats were injected intraperitoneally with streptozotocin (25 mg /kg body weight) and normal rats were injected with vehicle (0.05 mol/ L citric acid, pH 4.5). Both the low dose of streptozotocin and the high-fat diet are essential elements of the model designed to induce type 2 diabetes with insulin resistance. After 1 month, rats were fasted overnight and given 20% glucose (3 g/kg body weight). Blood samples were taken from the tail vein to measure glucose and insulin levels at 2 and 12 hours after glucose administration (Fig. 2). The diabetic rats were subdivided into two groups: type 2 diabetes mellitus treated with sitagliptin in a dose of 10 mg/kg daily for a month (22). Sitagliptin was dissolved in orange juice and administered to rats in a dose of 10 mg/kg by orogastric gavage, with an appropriate feeding needle as a volume of 5 ml/kg at 9 p.m.

With the end of the treatment duration, the rats were exposed to the holeboard memory test to measure speed of learning and memory capacity in rats. The experimental procedures were undertaken according to EU directives and local ethical regulations. The rats were housed in standard cages with liquid and food available ad libitum, under an artificial reversed 12-h light-dark cycle with light off at 7 a.m. The psychometric tests and pharmacological treatments were carried out in the dark phase of the cycle.

Psychometric assessment (holeboard memory test)

To measure the speed of learning and memory capacity in rats, the holeboard memory test was used (22). The testing area of the holeboard apparatus contains 16 holes in a 4'4 array. The holes are 20 cm apart and are filled with plastic cups (2.5 cm deep, 3 cm in diameter) for sweetened cereal food pellets. Some were spilled on the floor to eliminate the impact of smell (olfactory stimuli) on the search for food. The rats had to collect pellets in specified periods of time.

The test consists of 2 phases: habituation and training. During the habituation and training phases, food was restricted for the rats to enhance their motivation to search for it (water was available ad libitum).

The habituation phase was for 4 consecutive days. Each hole in the board was baited with 50 mg of cereals. The trial started when the rat entered the testing area, and it was stopped after 10 min or earlier, i.e. the time necessary to collect all food pellets. The monitoring included the time and the number of visits and revisits to the baited holes. Rats which found at least 14 of 16 pellets on the 4th day qualified for the training phase (Fig. 1A).

Training phase started 3 days after habituation and continued for 7 consecutive days with a pause on the 5th and 6th day. The rats were trained to collect pellets form 4 holes: A1, B3, C2 and D4 (Fig. 1B). During one training session, four trials were carried out for each animal. The maximum time given to collect 4 food pellets was 5 min. There were intervals of about 1 min between the trials for cleaning and baiting A1, B3, C2 and D4 holes with pellets. Apart from the time of performance, the registration included latency time (time between the beginning of the trial and the first hole visit) and the number of visits and revisits to the baited and empty holes. Based on these parameters, two distinct memory functions - working and reference memory - were evaluated.

Working memory ratio (WM) was presented as a percentage of all visits to the baited set of holes that had been supplied with food (calculated as the number of food rewarded visits divided by the number of visits and revisits to the baited set of holes). Reference memory ratio (RM) was expressed by the number of visits to the baited set of holes as a percentage of the total number of visits to all holes (calculated as the number of visits and revisits to the baited set of holes divided by the number of visits and revisits to all holes). The parameters were evaluated based on results of the 7th day of training. Training on the holeboard apparatus gradually reduced latency, and the total time needed to complete the trial and markedly improved working (WM) and reference (RM) memories.
Blood and brain samples collection

The rats were euthanized after the 7th training day. The blood samples were drawn by non-heparinized capillary tubes from the retroorbital plexus of the eye from anesthetized rats. The rat was anesthetized with diethyl ether, and the tube was pointed at the inner corner of the eye, beside the eyeball. The tube was then slid a few millimeters forward, gently but firmly, along the side of the orbit to the ophthalmic venous plexus (orbital sinus), allowing blood to enter the capillary tube. The eye was compressed for 30 s until bleeding stopped. These blood samples were collected without anticoagulant, left for 10 min, then centrifuged for 10 min at 4000 r/min to obtain serum, which was stored at −20°C until further biochemical analysis. The brain was quickly removed from the skull; under the dissecting microscope, first the cerebellum was cut off. Then, the olfactory pulp was dissected followed by removal of the frontal cortex by cutting at the anterior end of the corpus callosum. Another cut was performed at the tip of the anterior fornix and anterior commissure fibers. This was followed by removal of the brain stem and midbrain to get good exposure of the hypothalamus. Tissue samples were immediately frozen in liquid nitrogen and were kept at −70°C until assayed.

Chemicals

Streptozotocin is (Trade name Zanosar) was purchased from Sigma chemical company (St. Louis, Mo, USA). The drug was dissolved in 0.1 M sodium citrate (pH 4.5). Sitagliptin was a gift from Merck (Rahway, NJ, USA).

Determination of hypothalamic acetylcholine

The hypothalamic homogenates were centrifuged, and the supernatant was stored at −20°C. The hypothalamic content of acetylcholine was measured by ELISA kits catalog number E0912r in duplicate.

Determination of serum glucose

Serum glucose was determined colorimetrically using a Randox reagent kit (Sigma-Aldrich), according to the method of Trinder (24).

Determination of serum adiponectin

Quantitative determination of serum adiponectin was performed using the mouse/rat adiponectin ELISA kit (B-Bridge international, Inc.; Cat# K1002-1), according to the manufacturer’s instructions.

Determination of serum insulin

Determination of serum insulin was performed using an ELISA kit (Diagnostic Systems Labs., Inc.)

Calculation of HOMA index

Homeostasis model of assessment - insulin resistance index (HOMA-IR): HOMA of IR was calculated by using the following equation: HOMA = fasting glucose (mmol L-1) × fasting insulin (mU L-1)/22.5. Typically, a HOMA value >2 is used to identify significant IR. Typically, a HOMA value >2 is used to identify significant IR (25).

Assessment of plasma lipids

Serum TG concentrations were measured by the peroxidase method using a commercial kit (SPINREACT, Spain). Serum TC measurement was performed using the cholesterol oxidase method where the pink color of quioneimine was measured at 500 nm. The used kit was supplied by SPINREACT, Spain. Serum HDL cholesterol was determined enzymatically after precipitation of apoB-containing lipoproteins by the phosphotungstic acid-Mg method with the kit supplied by SPINREACT, Spain. Serum LDL-Cholesterol was estimated with a commercial kit (SPINREACT, Spain), with a two-step technique (26). First, chylomicrons, VLDL and HDL were eliminated. Second, LDL was specifically measured through the action of peroxidase with the formation of pinkish color quinine. The intensity of the color formed is proportional to the LDL concentration in the sample.

Calculation of the atherogenic index (AI)

Atherogenic index (AI) = Log(TG/HDL_C) and “the zone of atherogenic risk”. Triglycerides and HDL-cholesterol in AI

![Fig. 1. (A) The experimental protocol for psychometric test. P – pause, Tr – training, d – day; see text for details. (B) Testing area of holeboard apparatus - position of baited holes during training phase (T) Modified after Lannert and Hoyer, 1998.](image-url)
reflect the balance between the atherogenic and protective lipoproteins.

**RNA isolation and cDNA synthesis**

Hypothalamus tissues were collected from rats. Total RNA was extracted from hypothalamus homogenate using GStract™ RNA Isolation kit II (SA-40005, Maxim BioTech, Inc. San Francisco, USA) guanidium thiocyanate method. The purity and concentration of RNA was quantified by spectrophotometry. Reverse transcription reaction was performed using oligo (dT) primers (USA). The 25 µl cDNA synthesis reaction consisted of Reverse transcription reaction was performed using oligo (dT) primers (USA). The 25 µl cDNA synthesis reaction consisted of 2.5 µl (5x) buffer with MgCl₂, 2.5 µl (2.5 mM) dNTPs (Pharmacia Biotech), 1 µl (10 pmol) Oligo d(T) primer (Pharmacia Biotech), 2.5 µl RNA (2 mg/ml) and 0.5 unit reverse transcriptase enzyme (Qiagen, US). The mixture was incubated at 37°C for 1 hour. PCR amplification was performed in a thermal cycler (Applied Biosystems (ABI), USA) programmed at 42°C for 1 hour, 72°C for 10 min (enzyme inactivation) and the product was stored at 4°C until used.

Real time PCR and quantitative estimation of adiponectin R1 mRNA For qRT-PCR, a set of primers: sense; 5'-AGGAGTTTCGATAAAAGGTTCTG-3', antisense; 5'-ACATATTGGTCTGAGCATGGT-3') were designed from the published cDNA sequences of the rat AdipoR1 gene which amplified a 243 bp product. The reaction was carried out using Rotor-Gene 6000 system (Qiagen, USA) and consisted of 12.5 µl of 2X QuantitechSYBR® Green RT Mix (Fermentas, Germany), 1.0 µl of 25 pm/µl AdipoR1 primers, 2 µl cDNA (100 ng) and 9.25 µl of RNase free water. Samples were spun well before loading in the Rotor’s wells. The real time PCR program was performed as follows: initial denaturation at 95°C for 10 min.; 40 cycles of 95°C for 15 s; annealing at 60°C for 30 s and extension at 72°C for 30 s. Data acquisition performed during the extension step. This reaction was performed using Rotor-Gene 6000 system (Qiagen, USA). Negative controls were included in each set of PCR assays where cDNA was substituted with water as a control for contamination from exogenous sources. In addition, RT was omitted in some samples as a negative control for amplification of genomic DNA. Housekeeping gene, GDPH (glyceraldehyde 3-phosphate dehydrogenase) was amplified under similar conditions: sense; 5'-ATTGATACATCTGGGCACCA-3'; antisense reverse primer 5'-GAGATACACTTCAACTTTGACCT-3' for comparative quantification analysis using Rotor-Gene 6000 Series Software (Qiagen, USA). PCR products were electrophoresed on 1% agarose gel and visualized by ethidium bromide staining under UV light. The fold increase in the mRNA expression in all groups was calculated. Comparative quantification analysis was done using Rotor-Gene 6000 Series Software based on the following equation:

\[
\text{Ratio target gene expression (experimental/control)} = \frac{\text{Fold change in target gene expression (expt/control)}}{\text{Fold change in reference gene expression (expt/control)}}
\]

**Statistical analysis**

The results were expressed as means ± S.D. Statistical analysis was performed by using GraphPad Prism 6.0 (GraphPad Software Inc., San Diego, CA). The efficacy of training in the holeboard apparatus was assessed by the Two-way ANOVA, followed by the Tukey multiple comparison test. The biochemical data were analyzed by One-way ANOVA, followed by the Tukey multiple comparison test. P value of 0.05 or less was considered significant.

**RESULTS**

From **Table 1**, the serum glucose, insulin and HOMA-IR significantly increased in the diabetic rats as compared with the control group (p<0.05) and in response to sitagliptin treatment of the diabetic rats there were a significant reduction of the glucose, insulin and HOMA-IR (p<0.05) when compared with the diabetic rats. The lipid profile of the diabetic rats showed a significant increase in the cholesterol, triglycerides and LDL-cholesterol showed a significant increase, with a significant reduction in the HDL-cholesterol (p<0.05) as compared with the control group. The diabetic rats showed an increased risk of atherosclerosis as detected by the significant increase in the atherogenic index (p<0.05). In contrary, treatment of the diabetic rats with sitagliptin significantly lowered the circulating cholesterol, triglycerides and LDL-cholesterol (p<0.05) with a significant increase in the HDL-cholesterol (p<0.05). Also, the atherogenic index significantly decreased in response to sitagliptin treatment of the diabetic rats.

After feeding rats with high fat diet for one month and streptozotocin (25 mg /kg body weight) injection for induction of type 2 DM the serum glucose and insulin levels after 2 and 12 hours from i.p. injection of glucose showed that the plasma glucose after 2 h was 267.6±29.4 mg/dl and decreased to 151.8±17.2 mg/dl after 12 hours. Additionally, serum insulin after 2 hours was 4.89±0.41 ng/ml and decreased after 12 hours to 3.56±0.57 ng/ml (Fig. 2).

**Table 1**: serum glucose, insulin, HOMA-IR, cholesterol, triglycerides, LDL-cholesterol, HDL-cholesterol and atherogenic index in the experimental groups.

<table>
<thead>
<tr>
<th></th>
<th>Control (n=12)</th>
<th>Type 2 D.M. (n=12)</th>
<th>Type 2 D.M. M+sitagliptin (n=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dl)</td>
<td>74.50 ± 11.91</td>
<td>281.5 ± 22.62*</td>
<td>163.0 ± 14.24*</td>
</tr>
<tr>
<td>Insulin (ng/ml)</td>
<td>3.405 ± 0.3207</td>
<td>6.09 ± 0.644*</td>
<td>4.092 ± 0.119*</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>0.6303 ± 0.1454</td>
<td>4.22 ± 0.459*</td>
<td>1.645 ± 0.131*</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>85.7 ± 8.945</td>
<td>215.2 ± 12.4*</td>
<td>115.4 ± 9.452*</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>89.8 ± 7.945</td>
<td>189 ± 10.865*</td>
<td>127.3 ± 6.45*</td>
</tr>
<tr>
<td>LDL-cholesterol</td>
<td>29.7 ± 3.419</td>
<td>53.5 ± 6.154*</td>
<td>40.5 ± 5.12*</td>
</tr>
<tr>
<td>HDL-cholesterol</td>
<td>48.8 ± 3.846</td>
<td>22.4 ± 3.125*</td>
<td>30.7 ± 7.592*</td>
</tr>
<tr>
<td>Atherogenic index of plasma</td>
<td>-0.113 ± 0.034</td>
<td>0.504 ± 0.046*</td>
<td>0.132 ± 0.027*</td>
</tr>
</tbody>
</table>

* p<0.05 versus the control group; $ p<0.05 versus the diabetic group.
Fig. 2. Plasma glucose and insulin level 2 and 12 hours after i.p. injection of glucose in streptozotocin treated rats.

Fig. 3. (A) Effect of sitagliptin treatment on adiponectin level in type 2 diabetic rats, (B) on hypothalamic acetylcholine level. (C) Pearson correlation of the serum adiponectin and central acetylcholine levels (r=0.8449) (n=36) (p<0.001). (D) Pearson correlation of the central acetylcholine levels and adiponectin R1 receptors (r=0.5884) (n=36) (p=0.002). Data were expressed as mean ±S.D. of 12 rats; *: p<0.05 versus the control group; $: p<0.05 versus the diabetic group.
Fig. 3A showed that the serum adiponectin level was significantly reduced in the diabetic rats as compared with the control group (p<0.05). On the other hand, treatment of the diabetic rats with sitagliptin significantly increased the circulating adiponectin level (p<0.05). Also, Fig. 3B showed that the hypothalamic acetylcholine decreased significantly in the diabetic rats (p<0.05) and it increased significantly (p<0.05) in response to treatment. Fig. 3C showed that the significant (p<0.001) positive correlation between serum adiponectin level and hypothalamic acetylcholine level (r=0.9449) (n=36), Fig. 3D showed that the significant (p<0.001) positive correlation between central acetylcholine levels and adiponectin R1 receptors (r=0.9488) (n=36).

Fig. 4 showed that the AdipoR 1 mRNA expression was significantly decreased (p<0.05) in the diabetic rats by 63% as compared with the control group. While treatment of diabetic rats with sitagliptin significantly (p<0.05) increased the AdipoR1 expression by 55% versus the diabetic rats.

From Fig. 5A, the time needed to collect the pellets from the holes of the holeboard memory showed a significant decrease (p<0.05) on the 7th day as compared with the training sessions of the 1st, 2nd, 3rd and 4th days in the three experimental groups. In comparison with the control rats, the diabetic rats needed a significantly longer time (p<0.05) by 96%. Interestingly treatment of the diabetic rats with sitagliptin significantly (p<0.05) shortened the time to collect the food pellets on the 7th day from 161.3±11.96 seconds to 110.9±7.103 s. The F value for the interaction was 7.037.

From Fig. 5B, the time to visit the first hole (latency) is significantly shortened (p<0.05) in the three groups with the best time on the 4th and 7th days. In the diabetic rats, the latency is significantly (p<0.05) prolonged than the control rats from 2.583±0.3601 seconds to 19.83±2.858 seconds. While the treatment of the diabetic rats, latency was significantly (p<0.05) shortened from 19.83±2.858 seconds to 5.550±1.111 seconds. The F value for the interaction was 7.77.

From Fig. 5C, working memory increased significantly (p<0.05) from the 1st day of training in the three groups in response to repeated training to reach the best on the 7th day. In the diabetic rats, working memory decreased significantly (p<0.05) by 23% as compared with the control rats. While treatment of the diabetic rats significantly (p<0.05) increased the working memory from 50.67±7.528 % to 63.5±2.168 %. The F value for the interaction was 10.59.

From Fig. 5D, reference memory increased significantly (p<0.05) in the experimental groups. In comparison between the diabetic rats versus the control, reference memory decreased significantly (p<0.05) to 43.33±3.266 % from 81.00±2.608 %. In response to sitagliptin treatment of the diabetic rats, reference memory increased significantly (p<0.05) by 69.76%. The F value for the interaction was 10.3.

**DISCUSSION**

Results of the present study revealed that diabetic rats showed a significant increase in fasting blood glucose, LDL, total serum cholesterol, and TGs levels, with a significant decrease in HDL. Furthermore, there was a significant increase in fasting insulin and HOMA-IR, with a significant decrease in adiponectin level when compared with control rats, indicating the development of insulin resistance, which is the main criterion in type 2 DM. These data are consistent with previous studies in animal models of high-fat feeding (27, 28) (Table 1).
Fig. 5A and 5B. (A) Time 5 min or less (the time needed to collect all food pellets). (B) Latency: the time that elapsed between trial start and visit to the first hole parameter in the holeboard memory test. Data were expressed as mean ±S.D. of 12 rats. *: p<0.05 versus the control group; $: p<0.05 versus the diabetic group; a: p<0.05 versus the first day of training; b: p<0.05 versus the second day of training; c: p<0.05 versus the third day of training; d: p<0.05 versus the fourth day of training.
Fig. 5C and 5D. (C) Calculated working memory number of food rewarded visits divided by number of visits and revisits to the baited set of holes `100. (D) Calculated reference memory number of visits and revisits to the baited set of holes divided by number of visits and revisits to all holes `100 in the experimental groups on the 1st, 2nd, 4th and 7th days of training. Data were expressed as mean ±S.D. of 12 rats. *: p<0.05 versus the control group; $: p<0.05 versus the diabetic group; a: p<0.05 versus the first day of training; b: p<0.05 versus the second day of training; c: p<0.05 versus the third day of training; d: p<0.05 versus the fourth day of training.
In the current study, the diabetic rats needed longer time than the control rats to collect the food pellets in the holeboard memory test. Also, the time needed to start the collection of the food pellets was significantly prolonged than that of the control. Also, diabetic rats showed a significant reduction of the hypothalamic content of acetylcholine with decreased both working memory and reference memory. There are several mechanisms that explain cognitive impairment in diabetics such as cerebral microvascular disease which appeared also likely to be multifactorial. Chronic hyperglycemia produces structural and functional abnormalities in small blood vessels. Hypertension is also highly prevalent in people with type 2 diabetes and is associated with microangiopathy (29). Hyperinsulinemia may share in the pathogenesis of vascular dementia. Individuals with pre-diabetic states (e.g. impaired glucose tolerance or fasting hyperglycemia) or early type 2 diabetes typically have elevated circulating plasma insulin concentrations because of peripheral insulin resistance, which is in turn related to central obesity. Insulin receptors are found in high concentrations within the limbic system of the brain. In epidemiological studies of non-diabetic adults, hyperinsulinemia has been associated with poorer cognitive performance and an increased risk of AD (29).

The performance of the experimental rats might be affected by the degree of food deprivation. The deprivation for all the groups was the same. In agreement with our data, Moreira et al. (30) concluded that type 2 diabetic Goto-Kakizaki rats showed cognitive impairment induced by the progression of diabetes at 4 months age. They studied the operant behavior and positional discrimination in a lever-press task (LPT) for food in parallel with the evaluation of the exploratory activity and habituation to a novel environment in both GK and control Wistar rats. Holeboard memory test was previously used by Stasiak et al. (31), who it to assess the working and reference memory in a rat model of vascular dementia.

From the present data, the hypothalamic acetylcholine was significantly reduced in the diabetic rats when compared with the control rats (Fig. 3). These data are consistent with a previous study performed by Malouf and Birks (32) who concluded that the deficit in cholinergic neurotransmission, a characteristic of Alzheimer’s disease, has also been postulated to contribute to the cognitive disturbances of vascular disease of the brain in humans (32). Reduced acetylcholine production in diabetics has been reported by Irie et al. (33) who found the neuronal damage caused from increased oxidative stress and advanced glycation end products (amino-sugar compounds that are increased in hyper-glycemia and contribute to oxidative stress) leads to reduced acetylcholine production (33).

So far, several studies have documented that insulin resistance is linked to lack of adiponectin (34). They have proposed that obesity leads to hyperinsulinemia and reduction in adiponectin levels. This lack of adiponectin effect contributes to insulin resistance and type 2 DM. Also, Spiegelman’s group reported that adiponectin expression reduced in obese diabetic murine model db/db mice (35). Plasma levels of adiponectin have also been reported to be significantly reduced in obese/diabetic mice and humans (36, 37). Moreover, plasma adiponectin levels have been shown to be decreased in patients with cardiovascular diseases (38, 39), hypertension (40), or metabolic syndrome (41). Thus, reductions in plasma adiponectin levels are commonly observed in a variety of states often associated with insulin resistance.

In the present study, treating diabetic rats with sitagliptin produced a significant improvement of the metabolic dysfunctions (Table 1). The effects of sitagliptin on metabolic disturbances and insulin resistance in diabetic rats are consistent with Ahren et al. (42) who demonstrated that after 12 weeks of oral administration with DPP-4 inhibitor, vildagliptin in patients with type 2 diabetes, long-term glycemic control, post-prandial plasma glucose, as well as fasting plasma glucose were significantly reduced (40). Previous clinical trials also indicated that a DPP-4 inhibitor such as vildagliptin improved critical B-cell function (43) and increased estimated insulin secretory rate after meal ingestion (44). Also, sitagliptin increased the adiponectin level suggests that adiponectin production may be one of the mechanisms of the insulin sensitizing action of sitagliptin in type 2 DM (Fig. 3). Our results were consistent with Lim et al. (45) who reported that treatment with sitagliptin induced increasing pattern of plasma concentration of adiponectin (45). This was also documented by Suzuki et al. who presented that sitagliptin treatment in Japanese type 2 diabetes patients caused increase of adiponectin (46).

The main aim of the present research is to examine the potential role of Adipo R1 in improving the working and reference memories. The data obtained from this study showed that type 2 DM rats exhibited a significant decrease in the expression of Adipo R1 receptors as compared with the control rats. In agreement with our data, a previous study reported that Adipo R1 expression were decreased in the diabetic rats as reviewed by Al-Hashim et al. (47) who documented that type 2 diabetes mellitus reduced the expression of Adipo R1 in the soleus muscle of Sprague-Dawley rats (47). While, treatment of the diabetic rats with sitagliptin increases the expression of the hypothalamic Adipo R1 mRNA significantly, as compared with the diabetic rats. Additionally, data showed that the acetylcholine content of the hypothalamus increased significantly in the treated group versus the diabetic rats. Post analysis showed a strong significant direct correlation between hypothalamic Adipo R1 mRNA expression and the acetylcholine level in the hypothalamus. Also, hypothalamic acetylcholine is directly correlated with serum adiponectin level. Furthermore, both working and reference memories disturbances are improved in the treated diabetic rats versus non-treated.

The physiological importance of the central adiponectin action is reinforced by the fact that low molecular forms of adiponectin can cross the brain blood barrier (BBB) and are also found in human cerebrospinal fluid (48, 49) and exerts several neuroprotective effects (17-20). As previously known, the hypothalamus is a brain region that plays a key role in memory formation and mood regulation. One unique feature of the hypothalamus is its capacity for neurogenesis. Hypothalamic disorders have been implicated in the pathophysiology of mood disorders and deficits in learning and memory (50). Thus, possibly the mechanism that explained the development of the cognitive function in the treated group could be attributed to the significant increase in the level of acetylcholine in the hypothalamus. Zhang et al. (50) reported that adiponectin via activation of Adipo R1 stimulates the proliferation of the adult hippocampal neurons through stimulation of the p38MAPK/GSK-3β-catenin signaling cascade. Furthermore, Zhang and his colleagues reported that apoptosis and differentiation of adult hNSCs into neuronal or glial lineage were not affected by adiponectin (50). With increased adiponectin level and through stimulation of Adipo R1, adiponectin has been shown to activate AMPK and p38MAPK signaling pathways in different cell lines and tissue (34) Adiponectin stimulates phosphorylation of GSK3β on Ser389, a key inhibitory site, and results in nuclear accumulation of the primary substrate of GSK3β, β-catenin.

Improvement of memory in the diabetic rats could be partially caused by GLP-1. GLP-1 exerts various proliferative, neogenic and anti-apoptotic effects on neuronal cells. GLP-1R agonist treatment of PC12 cells stimulates neurite outgrowth, enhances nerve growth factor induced differentiation and improves cell survival after nerve growth factor withdrawal (51).
GLP-1R agonists prevent glutamate-induced apoptosis in cultured rat hippocampal neurons and restore cholinergic marker activity in the basal forebrain of ibotenic acid-treated rats, a rodent model of neurodegeneration (52). Furthermore, GLP-1R-activated pathways seem to be necessary for learning and memory. The GLP-1R agonist administration is associated with enhanced learning in rats, an effect that can be blocked by the co-administration of exendin (9, 42). In contrast, GLP-1RK/K mice exhibit deficits in learning that can be overcome by hippocampal Glp1r gene transfer (52). GLP-1RK/K mice are also more susceptible to kainate-induced seizures and hippocampal neuronal degeneration, whereas GLP-1R agonist treatment prevents kainate-induced apoptosis in wild type animals (53). These observations have led to the suggestion that GLP-1R agonists may potentially be useful for the treatment of neurological disorders, including the neuropathy that results as a secondary complication of diabetes (54). In contrast, endogenous GLP-1 has also been implicated in the pathogenesis of b-amyloid protein-induced neurotoxicity as a continuous coinfusion of a GLP-1R antagonist with b-amyloid protein prevented memory impairment and hippocampal apoptosis in rats. Additionally, the results produced by Abbas et al. (55) demonstrated that the murine GLP-1R plays an important role in the control of synaptic plasticity and some forms of memory formation in animal models of Alzheimer’s disease.

There are several factors that influence the function as well as working and reference memories such as human opioid which protects enkephalins from degradation by human neutral endopeptidase and aminopeptidase-N and inhibits pain perception in various behavioral rodent models of pain via endogenous enkephalin-related activation of opioidergic pathways (56). Also, the memory and learning could be disturbed in A.D., Va.D. (31) as well as liver cirrhosis (57).

This study showed that increased Adipo R1 mRNA expression in the hypothalamic neurons is response to sitaglitin treatment in type 2 diabetic rats. Possibly, Adipo R1 might be the cause of increased the acetylcholine level in the hypothalamus with the enhancement of working and reference memories. Further research is needed to explore the different effects of adiponectin on the hypothalamus.

Conclusion

Treatment of the diabetic rats with sitaglitin caused increase of adiponectin level and AdipoR1 mRNA expression, that possibly stimulates the formation of acetylcholine in the hypothalamic neurons. Increased acetylcholine level in the hypothalamus possibly improved working and reference memory.

Conflict of interest: None declared.

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