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Exenatide Treatment Exerts Anxiolytic- and Antidepressant-Like Effects and Reverses Neuropathy in a Mouse Model of Type-2 Diabetes

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Background: Comorbid neurobehavioral disturbances and type-2 diabetes mellitus (T2DM) warrant immediate research attention. Exenatide, which is a potent and selective agonist for the glucagon-like peptide-1 (GLP-1), is used in the treatment of T2DM. Exenatide displays a multitude of effects in the central nervous system. The aim of this study was to investigate the anxiolytic- and antidepressant-like effects and analgesic effects of exenatide in a type-2 diabetic mouse model.

Material/Methods: Modified elevated plus-maze test for anxiolytic-like, forced swimming test for depression-like behavior and hot-plate test for neuropathy were used as behavioral tasks. Behavioral parameters were investigated in a streptozocin – (100 mg/kg, i.p.) and nicotinamide – (240 mg/kg, i.p.) induced type-2 diabetic mouse model. Exenatide (0.1 µg/kg, s.c., twice daily) was administered for 2 weeks. Vehicle (control), diabetic, and exenatide-treated diabetic mice were tested.

Results: Our results confirm that exenatide exerts anxiolytic- and antidepressant-like effects and might be effective in diabetic neuropathy in a diabetic mouse model.

Conclusions: Exenatide may be a good candidate as a treatment option for depression, anxiety, and neuropathy in patients with type-2 diabetes.

MeSH Keywords: **Anti-Anxiety Agents • Behavior, Animal • Depression • Diabetes Mellitus, Type 2 • Glucagon-Like Peptide 1**

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Background

Type-2 diabetes (T2DM) is a chronic disease that affects the quality of life. Comorbid neurobehavioral deficit in T2DM patients in clinical practice has become an important research area in recent years [1]. The prevalence of anxiety and depression symptoms in patients with diabetes is considerably higher than in general population samples [2]. As we are learning more about the relationship between the gastrointestinal system and the brain, the importance of the link between T2DM and neurobehavioral disturbances becomes increasingly important.

Exenatide, the first incretin mimetic, is a synthetic version of a naturally occurring peptide, exendin-4, that is found in the parotid gland of the Central American Gila monster lizard, *Holo derma suspectum*. Exenatide shares 53% homology with native GLP-1 and has been approved for the treatment of T2DM [3].

Glucagon-like peptide-1 receptor agonists are incretin-based therapies used for the treatment of T2DM [4].

GLP-1 is produced in the central nervous system [5]. Exendin-4 (a potent and selective agonist for the GLP-1 receptor) displays many effects in the central nervous system. A few clinical reports have shown the relationship between the diabetes and central nervous system complications that are most prominent in elderly T2DM patients [1].

The extent of changes in emotional well-being in patients starting on exenatide therapy in comparison with new insulin starters were investigated and it has been reported that “well-being” generally tends to improve in exenatide-treated patients. Also, it has been concluded that exenatide could be used as an adjunctive therapy for depression in the context of diabetes [6].

In an experimental study, it has been found that exendin-4 therapy improves cognitive functions and ameliorates impaired hippocampal synaptic plasticity in dietary-induced obesity in mice fed a high-fat diet [7].

In this study, our aim was to investigate the potential effects of chronic exenatide administration in a mouse model of T2DM on different behavioral tasks: the modified elevated plus-maze for anxiolytic-like behavior, in the forced swimming test for depression-like symptoms, and on the hotplate test for neuropathy.

Material and Methods

Animals

Male inbred BALB/cByJ mice, (Uludag University, Bursa, Turkey) weighing 35–45 g were housed 4 to 5 per cage

(L30×W20×H12.5 cm) in an animal colony facility for 2 weeks. The animals were maintained at a constant room temperature (22±2°C) under a 12-h light/dark cycle (light onset at 07:00 h). Tap water and food pellets were provided ad libitum. All animals were naive to the tests. Each mouse was tested individually and only once. Experiments were conducted between 10:00 and 14:00 h. All procedures complied with the European Community Council Directive of 24 November 1986, and the Ethics Committee of Kocaeli University granted ethics approval (HAYDEK 13/8-2011).

Experimental procedure

Type-2 diabetes mellitus was induced in overnight-fasted BALB/c mice by a single intraperitoneal injection of 100 mg/kg of streptozotocin (STZ), 15 min after the intraperitoneal administration of 240 mg/kg nicotinamide (NA) (1, 4). After the treatment of NA/STZ, non-fasting glucose level was monitored. Exenatide was administered subcutaneously (0.1 µg/kg) twice daily (n=15), initiated on Day 13 and continued for 2 weeks after the diabetic model procedure.

Vehicle (control), T2DM, and exenatide-treated diabetic BALB/c mice were tested. STZ (Sigma, St. Louis, MO, USA) was dissolved in cold 50 mM-citric acid buffer (pH 4.5) and NA (Sigma) was dissolved in saline. Exenatide was purchased from Byetta® (Lilly Ltd. Sti. Istanbul, Turkey).

Behavioral studies

Locomotor activity test

Because compounds altering motor activity may give false-positive or false-negative effects in behavioral tasks, spontaneous locomotor activity of mice was evaluated by monitoring the activity of the animals in locomotor activity cages. Locomotor activity (MAY, NAC-4500, locomotor activity cabinet system) was measured with a computerized system (40×40 cm box). The “speed” of animals was measured on the locomotor activity test. An animal was placed in the center of the test box. During a 5-min period, the speed (cm/s-mean) of the animals was recorded using Ethovision-XT software (Noldus).

Modified elevated plus-maze test

Anxiety-related behavior was measured by the modified elevated plus-maze (mEPM) test. Experiments were conducted in a dimly lit, semi-soundproof room that was illuminated with a table lamp (80 lux). The maze was made of wood and consisted of 2 open arms (29 cm long×5 cm wide) and 2 closed arms (29×5 cm with 15-cm high walls) forming a square cross, with a 5-cm square centerpiece. To avoid falls, the open arms were surrounded by a short (1-cm) Plexiglas edge. The maze was

elevated 40 cm above the floor. The open arms and central platform were painted white and the closed arms were painted black. Each mouse was placed at the center of the maze, facing 1 of the open arms, and was allowed to explore the maze. During a 5-min test period, the number of entries into either the open or the closed arms of the maze (defined as the entry of all 4 limbs into the arms) and the time spent on the open arms were recorded. The open-arm activity was evaluated as follows: 1) the time spent in the open arms relative to the total time spent in the plus-maze (300 s), expressed as a percentage and 2) the number of entries into the open arms relative to the total number of entries into both the open and closed arms, expressed as a percentage. These values were accepted as the indices of anxiety in rats. Any animal that fell off the maze was excluded from the experiment. If the values for both measured parameters changed in the same direction compared to the control values (ie, if both the time spent in open arms and the number of open arm entries were increased or if both were decreased) and the change in 1 of the parameters was statistically significant, then an effect on anxiety was considered to have occurred. In the current study, the time spent in the open arms and the number of open arm entries always seemed to change in the same direction.

Forced swimming test

FST, the most widely used behavioral test for the screening of antidepressant drugs, was performed following the procedure described [8,9]. Briefly, the mice were dropped individually into plexiglas cylinders (height 25 cm, diameter 10 cm) containing 10 cm of water maintained at 23–25°C and left there for 6 min. The duration of immobility was recorded during the last 4 min of the 6-min testing period. The absence of hind leg movement was recorded as immobility by stopwatch cumulation by a single observer who was aware of the treatments during the exposures.

Hotplate test

The hotplate test was used to measure the pain reaction latencies. Animals were placed into a square glass container maintained on a hotplate at 55±0.1°C. The first passes to lick the hind paws or jumping was recorded and served as an index of pain reaction. An end-point time of 60 sec was used.

Statistical analyses

We evaluated 3 groups: the vehicle (control) group, the T2DM group, and the exenatide-treated T2DM group. “Time spent (%) in open arms” and “entry (%) to open arms” were evaluated via one-way analysis of variance (ANOVA) with a post-hoc Tukey test. One-way analysis of variance (ANOVA) with a post-hoc Tukey test was used to measure the hotplate test and the

Figure 1. Speed data of vehicle, diabetic, and exenatide-treated diabetic groups. There was no significant difference between the speed of vehicle, diabetic, and exenatide-treated diabetic mice in the locomotor activity test (H: 6.79, $p=0.03$, Kruskal-Wallis test)

FST results. The Kruskal-Wallis test was used for analyses of locomotor activity data. Data are expressed as the mean values ±SEM. Differences were considered to be statistically significant when $p<0.05$.

Results

The speed of vehicle, diabetic mice, and exenatide-treated diabetic mice on the locomotor activity test

In Figure 1, the speed of vehicle, diabetic, and exenatide-treated diabetic mice are shown. There was no significant difference between the “speed” of vehicle, diabetic, and exenatide-treated diabetic mice in the locomotor activity test (H: 6.79, $p=0.03$, Kruskal-Wallis test) (Figure 1). Compounds or disease altering motor activity may give false-positive or false-negative results in mEPM, FST, or hotplate tests. Locomotor activity of mice was evaluated by monitoring the activity of the animals in locomotor activity cages. According to speed data, neither T2DM itself nor the selected dose of exenatide altered locomotor activity of mice.

The time spent (%) in open arms of vehicle, diabetic, and exenatide-treated diabetic mice in the mEPM test

In Figure 2, the time spent (%) in open arms of vehicle, diabetic, and exenatide-treated diabetic mice in the mEPM test is shown. In the mEPM test, “time spent (%) in open arms” of diabetic mice was significantly decreased compared to vehicle group ($p<0.001$). Exenatide treatment to diabetic mice reversed the diminished time spent (%) in open arms ($p<0.05$) in diabetic mice ($p<0.05$) (Figure 2). T2DM animals show anxiety-like behavior in the mEPM test. Exenatide administration to T2DM mice reversed this effect, suggesting that exenatide has anxiolytic-like effect.

Figure 2. Percent time spent in open arms of vehicle, diabetic, and exenatide-treated diabetic groups in the mEPM test. The time spent (%) in open arms was significantly less compared to vehicle ($p<0.001$). Exenatide treatment of diabetic mice reversed the diminished time spent in open arms (%) ($p<0.05$) $F(2.34)=10.33$, $p=0.0003$, vehicle vs. diabetic *, diabetic vs. (diabetic+exenatide) **.

Figure 3. Entry (%) to open arms of vehicle, diabetic, and exenatide-treated diabetic groups on the mEPM test. "Entry (%) to open arms" of diabetic mice was significantly less compared to vehicle ($p<0.001$). Exenatide treatment of diabetic mice reversed the diminished total number of entries (%) of diabetic mice ($p<0.001$) to open arms ($p<0.05$). $F(2.34)=9.37$, $p=0.0006$, vehicle vs. diabetic *, diabetic vs. (diabetic+exenatide) **.

The entry (%) to open arms of vehicle, diabetic, and exenatide-treated diabetic mice in the m EPM test

In Figure 3, the entry (%) to open arms of vehicle, diabetic, and exenatide-treated diabetic mice in the m EPM test is shown. In the mEPM test, "entry (%) to open arms" of diabetic mice was significantly decreased compared to vehicle ($p<0.001$). Exenatide treatment to diabetic mice reversed the diminished total number of entries (%) of diabetic mice ($p<0.001$) to open

Figure 4. Immobility time (s) of vehicle, diabetic, and exenatide-treated diabetic groups in the forced swimming test. $F(2.37)=4.36$, $p=0.01$, vehicle vs. (diabetic+exenatide) *, diabetic vs. (diabetic+exenatide) ***.

Figure 5. Latency of hind paw licking of vehicle, diabetic, and exenatide-treated diabetic groups on the hotplate test. The latency to lick the hind paws significantly increased in the diabetic group ($p<0.01$). The latency to lick the hind paws was significantly reversed by exenatide administration ($p<0.05$), $F(2.37)=6.82$, $p=0.003$, vehicle vs. diabetic *, diabetic vs. (diabetic+exenatide) **.

arms ($p<0.05$) (Figure 3). T2DM animals show anxiety-like behavior in the mEPM test. Exenatide administration to T2DM mice reversed this effect. Exenatide showed anxiolytic-like effect in T2DM mice in the mEPM test.

The immobility time (s) of vehicle, diabetic, and exenatide-treated diabetic mice in the FST

In Figure 4, the immobility time (s) of vehicle, diabetic, and exenatide-treated diabetic mice in the FST is shown. In the FST, there was no significant change in the immobility time of T2DM mice. Exenatide treatment to T2DM mice significantly

decreased the immobility time ($p<0.05$) (Figure 4). Exenatide showed an anxiolytic-like effect in T2DM mice in the mEPM test.

The latency to lick the hind paws of vehicle, diabetic, and exenatide-treated diabetic mice in the hotplate test

In Figure 5, the latency to lick the hind paws of vehicle, diabetic, and exenatide-treated diabetic mice on the hotplate test is shown. In the hotplate test, the latency to lick the hind paws significantly increased in the diabetic group ($p<0.01$), which means that T2DM itself may cause impaired pain reaction in mice, possibly due to diabetic neuropathy. The latency to lick the hind paws is significantly reversed by exenatide treatment ($p<0.05$) (Figure 5). The neuropathy due to T2DM was reversed by exenatide treatment to T2DM mice.

Discussion

People with T2DM may experience negative emotional symptoms, depression, and anxiety. Depression is linked to a variety of diabetes complications such as neuropathy. In a recent study, a significant association between depression and diabetic complications was found [10].

To the best of our knowledge this is the first study showing that exenatide (a drug that is used in the treatment of T2DM) has anxiolytic- and antidepressant-like effects and reverses neuropathy in a mouse model of T2DM.

Demonstration of the possible antidepressant effect of exendin-4 in animal models of depression and anxiety tests would be important. Our findings support those of a previous study that showed the glucagon-like peptide-1 agonist, exendin-4, improves reference memory performance and decreases the immobility in the FST [11].

GLP-1 agonist exendin-4 was also tested on alcohol-related animal models. Exendin-4 was found to attenuate alcohol-mediated behaviors in rodents. It has been reported that the GLP-1 receptor may be a potential target for the development of novel treatment strategies for alcohol abuse disorders [12]. The GLP-1 agonist liraglutide, but not sitagliptin, has been found to have an antipsychotic-like effect in a mouse model of psychosis [13].

In our study, the mEPM test was used to examine the anxiolytic-like effect of exenatide on T2DM animals. The results show that, firstly, the time spent (%) in open arms and the total number (%) of entries to open arms of T2DM mice were significantly decreased compared to vehicle mice, which means that T2DM animals show anxiety-like behavior in the mEPM test. Secondly, exenatide treatment of diabetic mice reversed the diminished time spent (%) in open arms and the diminished

total number (%) of entries to open arms in diabetic mice correspondingly, showing that exenatide may have an anxiolytic-like effect in T2DM animals.

To test the antidepressant-like effect of exenatide, the immobility time of mice is measured in the FST. In the FST, the administration of exenatide to diabetic mice significantly decreased the immobility time. Exenatide consequently exerts antidepressant-like effects in the FST.

The potential mechanisms for our behavioral results could be explained with the role of GLP-1 on the nervous system. It is already known that GLP-1 plays an important role in the brain. GLP-1 is expressed in neurons and acts as a neurotransmitter. GLP-1 has growth factor-like properties and protects neurons from neurotoxic influences. GLP-1 also reduces the induction of apoptosis of hippocampal neurons and improves spatial and associative learning [14].

From another point of view, it is also known that neuropeptides modulate feeding via the central nervous system, and GLP-1 is known to be a neuropeptide [15]. Neuropeptides are able to modify catecholamine and serotonin release in the hypothalamus [16], which are related neurotransmitters in depression, anxiety, and pain systems. It has been reported that amygdala dopamine signaling is activated by both food intake and the anorexic brain-gut peptide GLP-1 [17], which is also known to be an important brain area for anxiety behavior.

Glucagon-like peptide 1 (GLP-1) may have direct trophic actions on the nervous system. Although it is known that GLP-1 has neuroprotective effects [18], the possible role of GLP-1 in supporting diabetic sensory neurons is still uncertain.

It has been reported that GLP-1 agonists and insulin in combination might benefit some aspects of established diabetic neuropathy [19].

Lastly, in the hotplate test, the latency to lick the hind paws significantly increased in T2DM mice. This result might indicate that T2DM itself may cause impaired pain reaction that may be due to diabetic-neuropathy. The latency to lick the hind paws is significantly reversed by exenatide treatment. According to our hotplate data, exenatide might be a good candidate for use in diabetic neuropathy, and GLP-1 itself might be an important target for diabetic neuropathy.

The high prevalence of anxiety, depression, and neuropathy and their reciprocal interaction with diabetes [20] highlight the need for experimental studies that will enhance understanding and lead to treatments that improve neuropsychiatric outcomes of people with T2DM.

Conclusions

According to our observations in different behavioral tests (mEPM, FST, and hotplate test), exenatide may be a good candidate for treatment of depression, anxiety, and neuropathy in patients with type-2 diabetes.

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Conflict of interest

All authors have no conflict of interest to declare.

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