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The effect of intra-amygdalar leptin administration on anxiety, depression and learning behaviors in rats

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Abstract

Leptin is released by adipose tissue. Leptin can cross the blood–brain barrier and bind to receptors on neurons in brain areas to exert its biological function when released into circulation. This study aimed to determine the influences of intra-amygdalar administration of high and low doses of leptin on anxiety, depression, learning behaviors of rats. In the experimental protocol I, intra-amygdalar injection of high and low doses of leptin (0.1 and 1µg/ kg) and saline were administered 30 min before the behavioral tests. Then, the animals were exposed to open field, elevated plus maze, Porsolt and Morris water maze tests for measuring of behaviors. In experimental protocol 2, the cerebrospinal fluids of all groups of experimental protocol 1 were collected by microdialysis method and then were analyzed by HPLC. The effect of the low dose of leptin was significant on the open field. The effect of the high and low dose of leptin was significant on the elevated plus maze test. The effect of the low dose of leptin was significant on mobility in the center of the Porsolt. A high dose of leptin group had spent less time around the platform than controls in the Morris water maze test. HPLC analysis showed that the amount of serotonin and glutamate in the amygdala region increased after low dose leptin administration. Intra-amygdalar injection of low doses of leptin may decrease anxiety and depression-like behavior in rats by increasing serotonin and glutamate levels in the amygdala.

Keywords: leptin, behavior, amygdala, anxiety, depression

1. Introduction

Leptin is one of the most important adipokines released by adipose tissue (Havel, 2000). When leptin is released into the circulation, it binds to receptors on neurons in brain regions, crossing the blood-brain barrier to perform its biological function (Zhang et al., 1994). Leptin receptors in the central nervous system are widely present especially in the hippocampus, amygdala, and hypothalamus (Krishnan and Nestler, 2010; Patterson, 2011).

Recent studies have shown the effects of leptin on neuronal function. It affects the function of the central nervous system, such as anxiety and depression (Liu et al., 2011; Liu et al., 2015). High levels of leptin and receptors have been detected in many areas of the brain. Evidence that the leptin receptor is present in limbic regions suggests that leptin has a possible role in controlling emotional processes in the limbic region of the brain (Liu et al., 2015).

In rats, hippocampal administration of leptin has been found to significantly reduce depression and affect hippocampal gene expression. Leptin has an antidepressant effect, and this effect has been noted in many studies (Banks et al., 2000; Liu et al., 2011). Leptin acts on the HPA (hypothalamo-pituitary axis) axis (Heiman et al, 1997; Liu et al., 2011). The antidepressant effect of leptin may be associated with the normalization of HPA axis hyperactivity (Banks et al., 2000; De Kloet et al., 2005). HPA axis disorder is the most important factor in the pathogenesis of depression, which causes an increase in corticosteroids in serum. In animal models, leptin has been found to reduce plasma corticosterone (Reseland et al., 2005). Therefore, leptin weakens the HPA axis at various levels (Heiman et al., 1997; Yu et al., 1997; Liu et al., 2011).

Leptin increases serotonin and reverses the increased corticosterone, so that HPA axis hyperactivity is regulated by leptin, which promotes the antidepressant effect of leptin (Hastings, 2002). Leptin has an antidepressant effect in rodents (Lu et al., 2007). Decreased serum leptin levels were found in individuals with major depressive disorder compared to healthy controls (Jow et al., 2006). Other studies in women with MDD found significantly increased plasma leptin levels (Rubin et al., 2002; Esel et al., 2005; Zeman et al., 2009). Similarly, it has been reported in some studies that leptin levels increase or do not change in various ways with antidepressant

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therapy (Kraus et al., 2002). Finally, some studies have suggested that leptin could potentially be a biological marker for depression (Pasco et al., 2008). Hence, this study aims to investigate the effects of intraamygdalar administration of leptin at high and low doses on anxiety, depression, and learning behaviors in rats.

2. Materials and methods

2.1. Animals

All experimental animals have been treated based on the guiding principles approved by the animal Ethical committee of Abant Izzet Baysal University as well as all the treatments comply with recommendations on the Declaration of Helsinki (Registration number 2012/04). The experiment was performed on male Wistar-Albino rats with weighing between 270-300 grams. Animals were housed under a 12 L / 12 D light-dark cycle (light on 07:00h) and room temperature of 22-25 C°. Food and water were ad libitum.

2.2. Experimental protocol

In the first study, rats were selected and randomly divided into the three experimental groups [control-male (i.c.v saline, 2 µl / min., n=10), high dose leptin administration (i.c.v, 1 µg / kg, n=10), and low dose leptin administration (i.c.v, 0.1 µg / kg, n=10)] for 5 min. The leptin and saline treatment were applied thirty minutes before the behavioral tests. All groups were tested by the open field and elevated plus maze tests for measuring anxiety-like behaviors; force swimming test was performed for measuring depression-like behaviors and Morris water maze test was performed for measuring the learning and memory performance. In the control group, animals were applied with the same amount of saline to obtain the same stress conditions as was the case in the experiment groups. In the second study, the cerebrospinal fluids (CSFs) of all groups of experimental protocol 1 were collected by a means of the micro dialysis method. A cannula (CMA 12 elite micro dialysis cannula; CMA Micro dialysis AB, Sweden) was implanted aseptically into the amygdala region (coordinates interior+0.6 mm; at the midline and ventral−7.8 mm; relative to bregma). Following the thirty-minute i.c.v treatments, collecting 7 tubes of CSF from each animal that took thirty minutes per tube and a total of 2.5 hours of time per animals' CSFs were collected. Following the completion of CSF collections, the tubes were saved at -20°C. Then all of the collected CSFs were analyzed using the HPLC method. The Agilent 1100 series HPLC-FLD system (Germany) was used.

2.3. Leptin administration

Leptin (Sigma) was first dissolved in some ethanol and then diluted with saline. Low-dose leptin was set to 0.1 µg / kg, and high-dose leptin to 1 µg / kg. Leptin was injected into the intraamygdalar region at a flow rate of 2 µL / min. The same volume of saline was administered to the control groups.

2.4. HPLC measurements

The Agilent 1100 series HPLC-FLD system (Germany) was used. The analytes were separated on a reversed-phase Inertsil ODS-2 (150 x 4.6 mm i.d., 5 µm) analytical column (Hichrom, England), which was protected by Inertsil ODS-2 (10 x 3.2 mm) guard cartridges (Hichrom, England). Chromatographic conditions, a fluorescence HPLC method for the simultaneous detection of serotonin and noradrenaline in microdialysates from rat brain was used. The separation was performed with a C18 reversed-phase column using isocratic elution.

2.5. Behavioral test

The Open Field Test (OFT)

The OFT was performed after the last day of treatments. A single rat was placed in the center of a black, Plexiglas square measuring 80 cm in length × 80 cm in width × 40 cm in height. The subject was allowed to discover this new environment for 15 minutes, during the training session. After the training session, the rat was exposed to the test for 5 min in the test session. During the test session, the time spent in center arena and edges were monitored by a video camera (Gkb CC-28905S, Commat LTD. ŞTİ., Ankara/Turkey) and recorded by a videotaped interfaced with the EthoVision video tracking system (Noldus Ethovision, Version 6, Netherlands; Commat LTD. ŞTİ. Ankara/Turkey). During the test session, the frequency of the entry to the center area, the number of center entries, and time spent in the center area were recorded for min and recorded data were calculated by the program.

The Elevated Plus Maze Test (EPM)

The EPM is the most thoroughly utilized test to evaluate anxiety-like behavioral state. It is especially sensitive to anxiety-reducing drugs, such as anxiolytic agents. The structure of the EPM apparatus has black color and is composed of the two open arms which are crossed by two closed arms of equal size (55×10 cm) with 41 cm high walls. The maze was elevated to a height of 55 cm above floor level. The rats of each group were placed individually in the central area of the EPM to explore for five minutes. The time spent on open arms, time spent on closed arms, and the number of open and closed arms entries were recorded by the EthoVision video tracking system Noldus Ethovision (Gkb CC-28905S, Commat LTD. ŞTİ., Ankara/Turkey).

The Porsolt test

A method of Porsolt was adopted to perform FST, where animals were allowed to swim in a glass cylinder (diameter: 22.5 cm, height: 30 cm, filled with water (23 ± 2ºC) up to 15 cm height of 15 cm) for six min, having the initial two min for adjustment and recording the immobility time. All behaviors of rats were recorded by the EthoVision video tracking system (Noldus Ethovision; Gkb CC-28905S, Commat LTD. ŞTİ., Ankara/Turkey).

The Morris Water Maze test

The water maze is composed of a black circular tank, 1,5 m in diameter and 60 cm in height, filled with water up to 40 cm in height, and four identical areas for the purpose for analysis. A square black escape platform 2 cm below the water level was situated at the center of one of the four quadrant of the

apparatus. Four different colors and shapes of cues have ensured the animal. The apparatus comprises 4 consecutive trials for five days with a 1-h inter-trial interval. The rats were allowed to explore for 90 s. If they failed to find the platform, they were gently guided to it for waiting 10 s. One day after the last training trial, each rat was exposed to the test trial where they were let to explore the hidden platform for 60 sec. When they found the platform, the rats were allowed to stay for 5 seconds to let them observe the cues around the platform. The time spent (by each animal) for finding the platform on the fifth training trial was measured by EthoVision video tracking system- (Noldus Ethovision, Version 6, Netherlands).

2.6. Statistical analysis

Data were examined by a means of two (the presence of treatment: high and low doses of intra-amygdalar leptin injection × saline injection (control (sham) group)) between ANOVA design Values were considered statistically significant at P <0.05. Data was presented as mean ± standard error after back transforming from ANOVA results.

3. Results

3.1. Open field measurements

The main effect of intra-amygdalar injection of the low doses of leptin hormone was significant in the amount of time spent in the center of the open field (TSCOF), F (2, 57) = 3.97, p = 0.02 (Fig. 1a). The main effect of intra-amygdalar injection of the low doses of leptin hormone was significant in the total frequency of zone transition of the open field (FZTOF) of rats. Compared to the rats in the control groups, anxiety-like behavior has decreased in the group injected with low dose of leptin F (2, 57) = 3.22, p = 0.04 (Fig. 1b). The main effect of intra-amygdalar injection of the high and low doses of leptin hormone was not significant on the mobility in the open field (MOF), F (2, 57) = 1,90, p = 0.16. The main effect of intra-amygdalar injection of the high and low doses of leptin hormone was not significant on the velocity in the open field, F (2, 57) = 0.25, p = 0.78.

3.2. Elevated plus maze measurements

The main effect of intra-amygdalar injection of the high and low doses of leptin hormone was not significant on the total distance travelled, F (2, 55) = 2.12, p = 0.13. The main effect of intra-amygdalar injection of the high and low doses of leptin hormone was significant on the measurement time, F (2, 55) = 6.77, p = 0.002 (Fig. 2a). The main effect of intra-amygdalar injection of the high doses of leptin hormone was significant related to the measurement time, F (2, 55) = 9.97, p =0.001 (Fig. 2b). The main effect of intra-amygdalar injection of the high and low doses of leptin hormone was not significant on the mobility, F (2, 55) = 1.08, p = 0.34. The main effect of intra-amygdalar injection of the high and low doses of leptin hormone was not significant related to the measurement time F (2, 55) = 1 .15, p = 0.33.

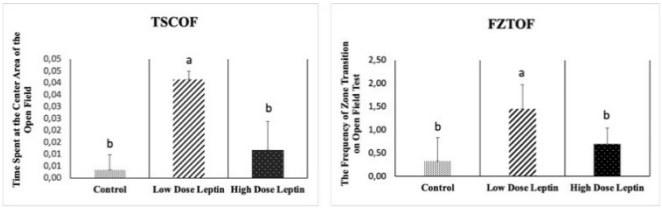

Fig. 1. a) Time spent at the center area of the open field test: control, low dose leptin and high dose leptin; b) Frequency of zone transition on open field test: control, low dose leptin and high dose leptin ($p < 0.05$). [a] $p < 0.05$ vs low dose leptin, [b] $p < 0.05$ vs control

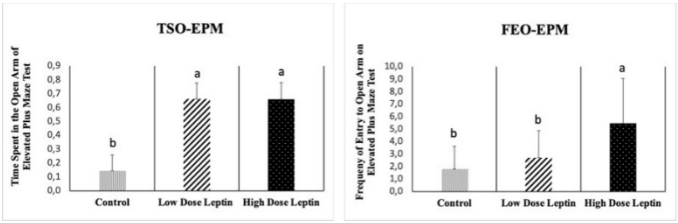

Fig. 2. a) Time spent in the open arm of elevated plus maze test: control, low dose leptin and high dose leptin (mean±SD, n=10) ($p < 0.05$); b) Frequency of entry to open arm on elevated plus maze test: control, low dose leptin and high dose leptin (mean±SD, n=10) ($p < 0.05$). [a] $p < 0.05$ vs low dose leptin, [b] $p < 0.05$ vs control

3.3. Porsolt measurements

The main effect of intraamygdalar injection of the high and low doses of leptin hormone was not significant on the total distance traveled in the Porsolt test, F (2, 55) = 0.25, p = 0.78. The main effect of intra-amygdalar injection of the low doses of leptin hormone was significant in the terms of immobility duration, F (2, 55) = 4.30, p = 0.02 (Fig. 3). The main effect of intra-amygdalar injection of the high and low doses of leptin hormone was not significant on the frequency of mobility, F (2, 160) = 2.91, p = 0.05.

3.4. Morris Water Maze

The effect of leptin hormone injection was not significant on total distance traveled. F (2, 45) = 1.08 p = 0.35. The effect of leptin hormone injection was not significant on the total time to find the platform. F (2, 45) = 0.82 p = 0.45. The effect of leptin hormone injection was significant, F (2, 45) = 3.50 p = 0.04. The subjects exposed to a high dose of leptin (M = 0.05) had less time spent around the platform than controls (M = 0.08). The effect of leptin hormone injection was not significant, F (2, 45) = 0.35 p = 0.70.

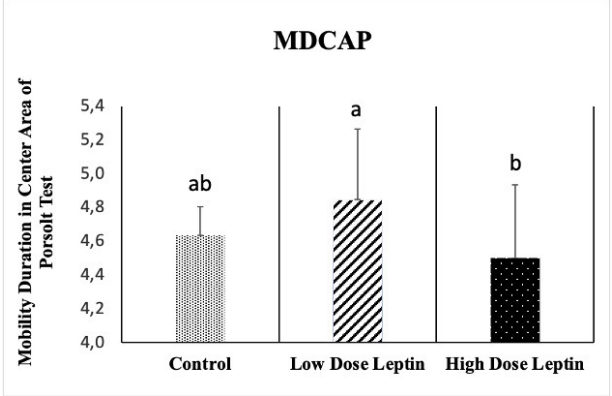

Fig. 3. Mobility duration in center area of Porsolt test. [a] $p < 0.05$ vs low dose leptin, [b] $p < 0.05$ vs control

3.5. Results of HPLC

Serotonin and glutamate levels were observed to have increased after the low dose leptin administration ($p<0.05$). This supports the conclusion that low dose leptin reduces anxiety. GABA, Noradrenaline, and Melatonin concentrations were not determined as their concentrations were below the limits. (Table 1 and 2).

4. Discussion

Leptin is involved in regulating brain development, improves angiogenesis, promotes nerve regeneration, energy homeostasis, reproduction, and cognition (Pasco et al., 2008). Leptin has an antidepressant effect in rodents (Zeman et al., 2009). Moreover, systemic administration of leptin lowers levels of corticosterone (Farr et al., 2006). Decreased serum leptin levels were found in individuals with major depressive disorder compared to healthy controls (Kraus et al., 2002; Farr et al., 2006). Similarly, some studies suggest that leptin levels increase (Kraus et al., 2002; Esel et al., 2005), or do not change with antidepressant treatment in a variety of ways (Kraus et al., 2002; Farr et al., 2006).

Leptin receptor localization has been identified mostly in the arcuate nucleus, dorsomedial hypothalamus, and lateral hypothalamus in the mouse brain. On the other hand, there is a moderate amount of leptin receptors in the amygdala region (Patterson et al., 2011). In this study, there may be a plausible explanation for why low-dose leptin is more effective.

Antidepressant and anxiolytic activity of 5-HT3 receptor antagonists has been proposed in animal models (Schilling et al., 2013, Romanova et al., 2018). The administration of intra-amygdalar leptin leads to modulation of the serotonergic system for antidepressant and anxiolytic effect and the increase in serotonergic neurotransmission. The results of this study showed that low-dose intra-amygdalar leptin administration in anxiety tests reduced anxiety, while in HPLC analysis, serotonin levels were higher in the low-dose leptin group. Considered together, these results support each other.

Intrahippocampal leptin injections improved dose-dependent performance in the T-maze and inhibitory avoidance test (Pasco et al., 2008). High-dose leptin administration did not affect behavior in the avoidance test, possibly indicating the absence of the effect of intrahippocampal leptin reported in Wistar rats (Kurhe et al., 2015). Administration of leptin to the CA1 region of the hippocampus was found to not alter learning and memory in the radial maze test in Wistar rats (Kurhe et al., 2015). Similarly, administration of leptin to the amygdala in this study was the dose dependent.

While leptin administered to the hypothalamus improved appetite, nutrition and spatial memory (Zarrindast et al., 2015), it showed no effect on high and open field tests (Kanoski and Davidson, 2011). No effect of intra-amygdalar leptin was observed on learning and memory in this study; on the other hand, this effect is evident in open field, high plus maze and porsolt tests. According to these results, the effect of leptin depends on both the dose and the area of administration.

Depression is associated to abnormalities in the frontal and limbic neural circuits, including the amygdala (Sharma et al., 2010). In the hippocampus, amygdala, and postrema region, the 5-HT3 receptor is highly expressed in the unique ion channel type in the family of serotonergic receptors (Canli et al., 2005). Leptin increases serotonin in the forebrain region and reverses the increased corticosterone, so that HPA axis hyperactivity is regulated by leptin, supporting the antidepressant effect of leptin (Tecott et al., 1993).

Low-dose leptin (0.1 ul / kg) was found to reduce anxiety and depression in intra-amygdalar leptin administration. In this study, high levels of glutamate and serotonin were found in extracellular fluid collected from the amygdala region in the low-dose leptin group. Moreover, increased glutamate and serotonin expression was found to reduce anxiety and depression. Leptin mediated an increase in glutamate and serotonin. In this study, CSF was collected between 14:30 and 18:30, and these samples were collected in separate tubes at 30-minute intervals. Accordingly, glutamate increased 30 minutes after the administration of leptin, and serotonin increased two hours later. Taken together, intra-amygdalar injection of low-dose leptin can reduce the anxiety and depression-like behavior in male rats by increasing serotonin and glutamate levels in the amygdala. Further studies should investigate why leptin mediates the effects of glutamate and serotonin in order to establish a new strategy for treating anxiety and depression

Conflict of interest

The authors declare that they have no conflict of interest.

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