Evaluation of the Effect of Peripheral Injection of Leptin on Spatial Memory

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Citation

S Rasi, G Mohaddes, N Naghdi, M Ilkhani Pour, V Hajali. *Evaluation of the Effect of Peripheral Injection of Leptin on Spatial Memory*. The Internet Journal of Neurology. 2006 Volume 8 Number 2.

Abstract

Introduction: Leptin is a peptide hormone secreted by adipose tissue. Some studies have also suggested that leptin affect learning and memory. The hippocampus has been implicated in many learning and memory functions including spatial memory. The present study is scheduled to investigate the effect of intraperitoneal (IP) injection of different doses of leptin on spatial memory formation.

Material and methods: 60 male rats were divided into 6 groups in our experiments: (1) control, (2) sham, and (3), (4), (5), (6) intraperitoneal injection of 0.05, 0.1, 0.25 and 0.5 mg/kg doses of leptin respectively. All groups were trained in Morris water maze for two days. Learning parameters were compared among groups.

Results: Our results showed, there were significant differences of learning parameters between sham group and test groups in spatial learning.

Conclusion: In conclusion, our findings suggest that intraperitoneally injection of leptin improved spatial memory in rat. Leptin shows its highest effect with medium doses.

INTRODUCTION

Leptin is a hormone that regulates body weight and energy homeostasis via its actions on specific hypothalamic nuclei ([1]). Leptin is the product of the obese (ob) gene that is synthesized predominantly, although not exclusively, by white adipose tissue ([2]). Adipocytes secrete leptin into the blood. As it circulates through the cerebrovasculature, transporters for leptin carry it across the BBB to enter the interstitial fluid of the brain ([3], [4]). Leptin functions are thought to occur through the leptin receptors mainly in the hypothalamic nuclei. However, leptin receptors exist throughout the brain including the hippocampus ([5]). Immunoreactivity for leptin receptors has been found in the hippocampus especially in the dentate gyrus and CA1 ([6]). Moreover, it has been demonstrated that leptin receptor-deficient animals show impaired LTP in CA1 and poor spatial memory. In the Morris water-maze test, their poor performances in the invisible-platform situation may suggest a spatial memory deficit in both Zucker fatty rats and db/db mice ([7]).

The hippocampus has a well-documented role in spatial memory acquisition ([8]). It has been also determined that hippocampus has an essential role in rodent spatial memory and navigation ([9]). Hippocampal lesions produce memory deficits, but since hippocampal lesions do not eradicate previously established memory traces, the hippocampus could be a temporary store for information, particularly spatial information that is subsequently encoded in other cortical regions ([10]). Some studies have been conducted on leptin effect on different type of learning and memory.

Farr and colleagues reported the role of leptin in learning and memory using an animal model. They found that mice navigated a maze better after they received leptin. Their research indicated that administration of leptin to mice improved retention of T-maze footshock avoidance and step

down inhibitory avoidance ([5]). In addition, Oomura et al. showed a facilitation effect on learning and memory performance in passive avoidance and Morris water maze task after daily intravenous injection of leptin (50 μg/kg) in rats ([11]). The other study suggested that leptin applied directly into the dentate gyrus; enhanced normal LTP at 1.0 μM but inhibited LTP at lower and higher doses in the Morris water maze in urethane anesthetized rats ([6]). Just one experiment reported that leptin exhibit no effect on memory processes ([11]).

Since only a few studies investigated the involvement of systemic leptin in spatial memory formation and the subject is somehow controversial, we decided to assess the effect of different doses of intraperitoneal leptin on spatial memory in a Morris water maze task.

MATERIAL AND METHOD

Animals and substances. Adult male Wistar rats (220–250 g, aged 12 week) were obtained from colony of Tabriz university of Medical Sciences. They were housed in a temperature (22 ± 2° C) and humidity-controlled room. The animals were maintained under a 12:12-h light/ dark cycle, with lights off at 8:00 p.m. Food and water provided ad libitum except for the periods of behavioral testing in Morris Water Maze (MWM). The behavioral testing was done during the light phase. All experimental procedures were approved by the Regional Ethics Committee of Tabriz University of Medical Sciences. Leptin was purchased from Peprotech Pharmaceutical Company and was Solved in phosphate buffer ([12]) and then diluted in sterile 0.9% saline.

Apparatus. The water maze was a black circular pool with a diameter of 136 cm and a height of 100 cm, filled with 20± 1°C water to a depth of 60 cm. The maze was divided geographically into four equal quadrants and release points that were designed at each quadrant as N, E, S, and W. A hidden Square platform (10 cm each side), was located in the center of the southwest quadrant, submerged 1.5 cm beneath the surface of the water. Fixed, extra maze visual cues were present at various locations around the maze (i.e., computer and signs). A video camera was mounted above the center of the maze so the animal motion can be recorded and sent to the computer. A tracking system was used to measure the escape latency, traveled path and swimming speed.

Injection of intraperitoneal. The injections were made using a 1 ml insulin syringe. Saline or leptin (0.05, 0.1, 0.25, 0.5 mg/kg) were injected (0.5 ml) into peritoneal.

Behavioral procedure. The rats were trained in the water maze. The single training session consisted of eight trials with four different starting positions that were equally distributed around the perimeter of the maze. The task requires rats to swim to the hidden platform guided by distal spatial cues. After mounting the platform, the rats were allowed to remain there for 20 s, and were then placed in a holding cage for 30 s until the start of the next trial. Rats were given a maximum of 60 s to find the platform and if it failed to find the platform in 60 s, it was placed on the platform and allowed to rest for 20 s. Latency to platform and distance traveled were collected and analyzed later. After completion of the training, the animals were returned to their home cages until retention testing 24 h later. The probe trial consisted of 60 s free swim period without a platform and the time swum in the target quadrant was recorded ([13]).

In order to assess the possibility of drug interference with animal sensory and motor coordination or the animal motivation, the capability of rats to escape to a visible platform was tested in this study. The trained rats were given four trials for visuo-motor coordination on the visible platform.

Experimental groups. The aim of this experiment was to evaluate the effect of intraperitoneal leptin injection on memory. The intraperitoneally injected rats were randomly divided into six groups (ten rats in each): control, saline treated and leptin with doses 0.05, 0.1, 0.25, 0.5 mg/kg. Saline or leptin was injected intraperitoneally 30 min before training. The retention testing was done 24 h later as a 60 S probe trial (leptin or saline were injected 30 min before probe trial.)

Statistical analysis. Data are expressed, as means ± S.E.M. The statistical analysis of the data was carried out by one-way ANOVA. When appropriate, significance of specific comparisons between group means was determined by means of the Tukey method, $P < 0.05$.

RESULTS

During acquisition, the performance (traveled distance) of all groups improved with subsequent blocks of training. The difference between T1–T4 (block 1) and T5–T8 (block 2) was significant in each group (Fig. 1).

The one-way ANOVA of the escape latency of block 2 revealed significant differences between groups. Injection of 0.05 mg/kg ($p= 0.050$) and 0.1 mg/kg ($p= 0.003$) doses of

leptin demonstrated better spatial learning than that of the saline treated animals. Animals treated with higher doses of leptin (0.25, 0.5 mg/kg) did not show any significant difference on water maze acquisition (Fig. 2).

Probe test data were compared between groups. One-way ANOVA of the time spent and distance traveled in the target quadrant revealed significant differences between groups. Animals treated with 0.1 mg/kg (p= 0.001) and 0.25 mg/kg (p= 0.029) of leptin significantly spent more time in the target quadrant than did the saline-treated group, indicating memory improvement in these animals. But Animals treated with 0.05 and 0.5 mg/kg of leptin show no significant difference in time spent in the target quadrant comparing with saline treated group (Fig. 3A). The average distance traveled in the target quadrant on the probe test showed that animals treated with doses of leptin 0.1 (p= 0.000), 0.25 (p= 0.017), 0.5 (p= 0.011) mg/kg demonstrated better spatial learning than that of the saline treated animals, as distance traveled in the target quadrant was significantly longer in this groups (Fig. 3B). Our data showed that leptin had no significant effect on swimming speed (data not shown).

Intraperitoneal leptin injection 30 min before visual trial (visible platform) also showed no significant difference in time spent to find the visible platform, compared to the control (Fig. 4).

Figure 1

Figure 1: The effects of different doses of leptin on the distance traveled between block 1 and block 2. All leptin doses improved acquisition, * p

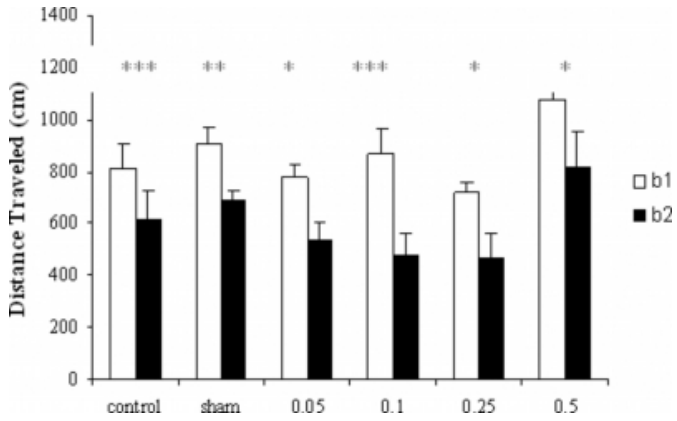


Figure 2

Figure 2: The effects of pre-training intraperitoneal administration of 0, 0.05, 0.1, 0.25 and 0.5 mg/kg leptin on acquisition in Morris Water Maze. Average escape latency of the block 2 between sham and test groups is shown. Leptin 0.05 and 0.1 mg/kg improved acquisition, * p<0.05, ** p<0.01.

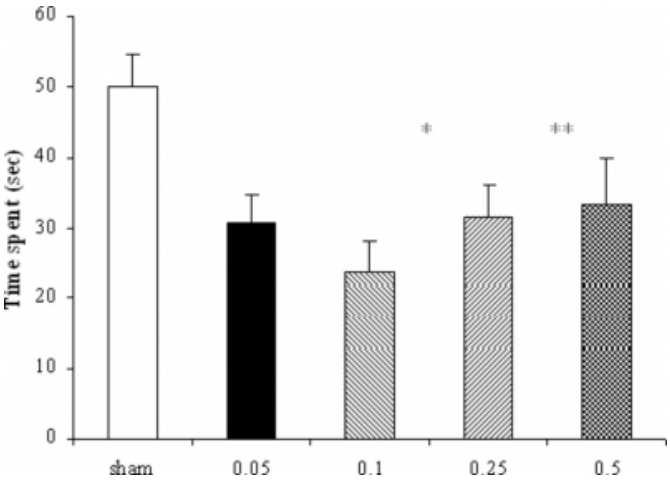


Figure 3

Figure 3: The effects of pre-training administration of different doses of leptin on probe test. (A) Time spent in target quadrant. Leptin 0.1 and 0.25 mg/kg improved retention significantly. (B) Distance traveled in target quadrant. Leptin 0.1, 0.25 and 0.5 mg/kg also significantly improved retention, * p<0.05, *** p<0.001.

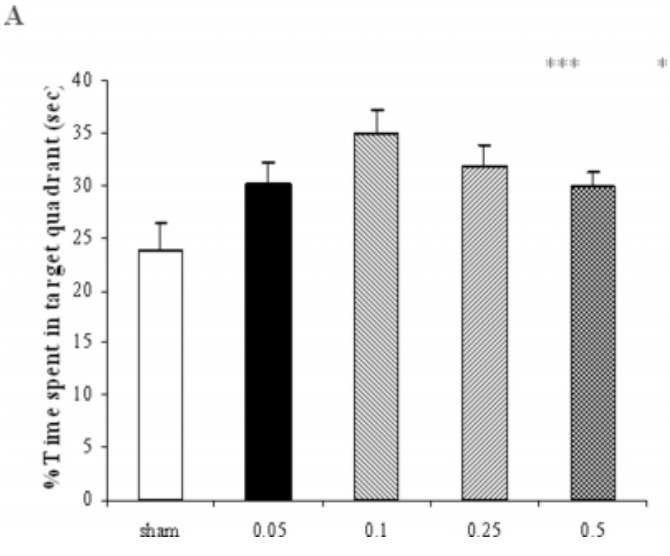


Figure 4

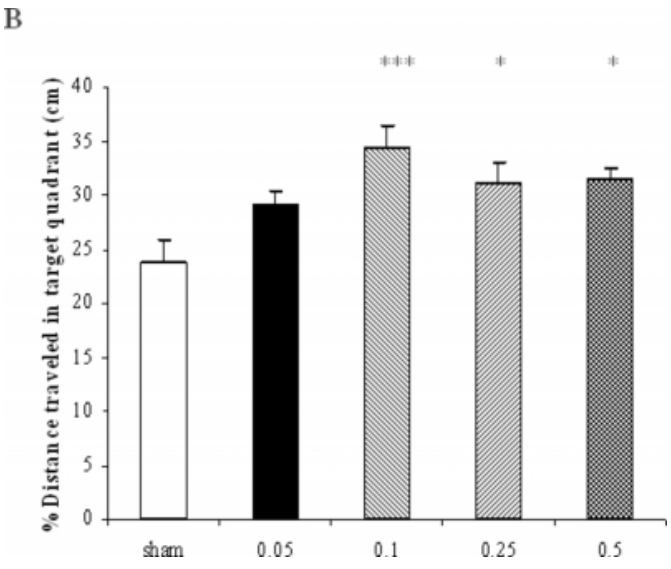
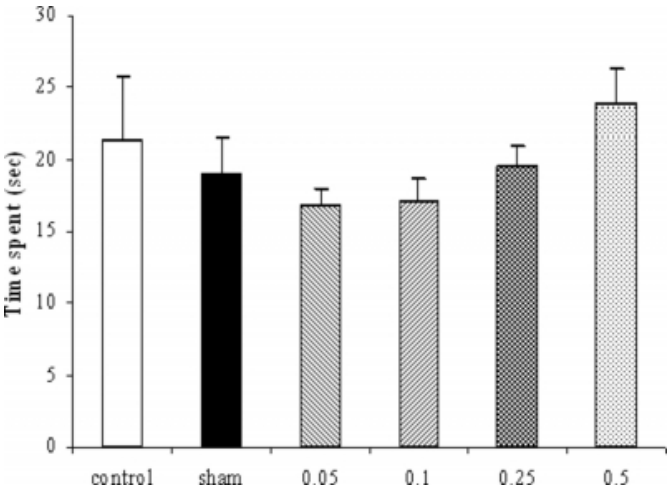


Figure 5

Figure 4: Visible test shows there is no significant difference to find platform between groups. Leptin did not affect visuo-motor and motivational factors in animals.



DISCUSSION

Our findings show that intraperitoneal injection of different doses of leptin improved spatial learning and memory. However, the improving effect of some doses was stronger than the improving effects of other doses.

Leptin enters areas throughout the brain by a system that is partially saturated at endogenous blood levels of leptin ([14]). Transporters for leptin carry it across the BBB to enter the interstitial fluid of the brain ([3], [4]) and Choroid plexus plays a key role in regulating leptin entry into the CSF under physiological conditions ([15]). Leptin outside the hypothalamus, where it plays a key role in energy expenditure and food intake, improves memory processing in the hippocampus ([5]). The hippocampus has been shown to be critically involved in learning and memory processes ([16], [17], [18]). Lesion of the CA1 subfield in rat spatial learning has been evaluated by the MWM ([19]).

Our data support previous results on leptin effects on different type of memory, such as in a water maze performance ([6], [7], [20]), T-maze footshock avoidance ([5]) and passive avoidance tasks ([20]). Oomura's rat study shows that leptin facilitated learning and memory in the Morris water-maze test, enhanced CA1 LTP maintenance, attenuated LTD, and led to increased CaMK II activity in the CA1 area ([21]). In addition, a close association between enhanced hippocampal LTP and facilitated learning and memory has been demonstrated ([22], [23], [24], [25], [26] , [27]). Farr and colleagues assessed the role of leptin in memory processing using two different avoidance paradigms. Their results indicate the leptin improves memory processing for T-maze footshock avoidance in SAM-P8 male mice ([5]).

Recent studies investigated hippocampal long-term potentiation (LTP) and long-term depression (LTD), and the spatial-memory function in two leptin receptor-deficient rodents (Zucker rats and db/db mice). In brain slices, the CA1 hippocampal region of both strains showed impairments of LTP and LTD; leptin did not improve these impairments in either strain ([7]). Another experiment determined the effects of leptin, 0.0, 1.0, 100 nM, 1, and 10 M, applied directly into the dentate gyrus, on LTP in medial perforant path dentate granule cell synapses in male Harlan Sprague-Dawley rats. Its findings suggest that leptin enhances normal LTP at 1.0 M but inhibits LTP at lower and higher doses ([6]).

In the present behavioral study intraperitoneal injection of leptin improved spatial learning and memory. However, the improving effect of dose (0.1mg/kg) was stronger than the improving effects of other doses. Leptin with lower (0.05 mg/kg) and also higher doses (0.25, 0.5 mg/kg) had weaker effects on water maze task, which indicates there is an optimal dose for memory.

Recent behavioral and LTP experiments has also demonstrate that leptin shows an inverted-U dose related function in terms of its effects on learning and memory and LTP. Similar inverted-U-shaped nature on the dose–response curve for leptin has been reported in memory processing in mice performing step down passive avoidance test ([5]), and in LTP in the dentate gyrus of anesthetized rats ([6]), when leptin is administered directly into the CA1 region and the dentate

gyrus of the hippocampus, respectively.

Collectively, it is possible that the 0.05 mg/kg dose of leptin is not enough to trigger cellular effects. Also, the weak effect of higher doses of leptin on spatial learning and memory probably could be attributed to the limiting effect of choroids epithelium in transferring of hormone to cerebrospinal fluid. Because leptin transport across the blood-CSF barrier is fully saturated at higher leptin physiological plasma concentrations, it is possible that the choroid epithelium acts as a rate-limiting step to prevent increases in CSF leptin concentrations ([15]).

On the other hand, it is possible that higher doses of leptin trigger other types of receptors or other intracellular signaling pathways. For example, it may be related to the effect of leptin on internalization of AMPA receptors in hippocampal CA1 neurons ([28]). AMPA receptor-mediated synaptic transmission in the hippocampus is critical for encoding and consolidation of spatial ([29]), aversive ([30]) and recognition memory ([31], [32]). Maybe, leptin inhibits hippocampal cells through AMPA receptor down-regulation. It had also shown that leptin inhibits rat hippocampal neurons by increasing a K+ conductance ([33]).

Other findings show that leptin; at concentrations comparable with those circulating in the plasma ([34]) can modulate hippocampal synaptic plasticity, by conversion of STP into LTP. A key process underlying this effect is the enhancement of NMDA responses; a process not only requiring activation of PI 3-kinase, but also MAPK and Src tyrosine kinases. A crucial intracellular process regulating NMDA receptor function is phosphorylation ([35]), and both serine– threonine and tyrosine phosphorylation regulate NMDA receptor function. In particular, Src tyrosine kinases can directly phosphorylate NMDA receptor NR2A ([36]) and NR2B ([37]) subunits. Functionally this may be important in hippocampal synaptic plasticity because it has been hypothesized that during LTP induction, Src is rapidly activated leading to enhanced NMDA receptor function ([38]).

Taken together, our results and previous studies indicate that the same peptide could possibly have different modulator post synaptic effects in different hippocampal synapses dependent upon different types of post synaptic receptors ([5], [6]).

In summary, we found that leptin improves memory and it shows its highest effect with medium doses.

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