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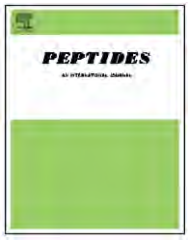

Effects of leptin on memory processing

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ABSTRACT

Leptin is a peptide hormone secreted by adipose tissue. Studies have shown that leptin crosses the blood–brain barrier (BBB) by a saturable transport system where it acts within the hypothalamus to regulate food intake and energy expenditure. Leptin also acts in the hippocampus where it facilitates the induction of long-term potentiation and enhances NMDA receptor-mediated transmission. This suggests that leptin plays a role in learning and memory. Obese mice and rats, which have leptin receptor deficiency, have impaired spatial learning. In disease states such as diabetes, humans and animals develop leptin resistance at the BBB. This suggests that low leptin levels in the brain may be involved in cognitive deficits associated with diabetes. In the current study, the effects of leptin on post-training memory processing in CD-1 mice were examined. Mice were trained in T-maze footshock avoidance and step down inhibitory avoidance. Immediately after training, mice received bilateral injections of leptin into the hippocampus. Retention was tested 1 week later in the T-maze and 1 day later in step down inhibitory avoidance. Leptin administration improved retention of T-maze footshock avoidance and step down inhibitory avoidance. Leptin administered 24 h after T-maze training did not improve retention when tested 1 week after training. SAMP8 mice at 12 months of age have elevated amyloid-beta protein and impaired learning and memory. We examined the effect of leptin on memory processing in the hippocampus of 4 and 12 months old SAMP8 mice. Leptin improved retention in both 4 and 12 months old SAMP8 mice; 12 month SAMP8 mice required a lower dose to improve memory compared to 4 months SAMP8 mice. The current results indicate that leptin in the hippocampus is involved in memory processing and suggests that low levels of leptin may be involved in cognitive deficits seen in disease states where leptin transport into the CNS is compromised.

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1. Introduction

Leptin is a peptide hormone involved in modulation of food intake and energy balance [2,3,5,6,20]. These actions are thought to occur through the leptin receptors mainly in the hypothalamic nuclei. However, leptin receptors exist throughout the brain including the hippocampus, an area of the brain involved in learning and memory [14]. Leptin has been found to facilitate long-term potentiation in the hippocampus, a process important for memory processing [31,35]. The facilitation of synaptic plasticity occurs via enhanced NMDA receptor-mediated Ca^{2+} influx. Leptin receptor deficient rodents have impaired spatial memory, a process that relies heavily on the hippocampus [21]. This

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has lead to the suggestion that low levels of leptin in the brain may be related to impaired memory found in disease states such as diabetes where leptin transport into the brain is impaired.

Leptin modulates hypothalamic feeding circuits [16]. In normal weight humans and animals leptin serves to signal the brain to stop eating [3,23]. The hypothalamus, the primary regulator of energy expenditure, is comprised of two populations of neurons: the orexigenic (appetite-stimulating) neurones containing neuropeptide Y (NPY) and agouti-related protein (AGRP), and the anorexigenic (appetite-suppressing) neurones, which produce the neuropeptides pro-opiomelanocortin (POMC) and cocaine-and-amphetamine-regulated transcript (CART). Both groups of neurones express leptin receptors, but are differentially effected by the hormone. Leptin increases the expression of POMC mRNA while decreasing the expression of mRNA encoding for NPY [30,32]. Similarly, leptin is thought to counteract the effects of the orexigenic peptide ghrelin and attenuate adiponectin and insulin levels [34]. Deficiencies in leptin result in obesity [4,36].

Leptin works outside the hypothalamus. Patients with both schizophrenia and depression with normal body mass indices have low leptin levels [18]. This occurs even if the patient is being treated with psychotropic medication suggesting a need to examine leptin in these psychiatric conditions. Evidence also exists for a role of leptin in brain development. Studies of ob/ob mice, which are leptin-deficient, find that their brains are smaller in both weight and cortical volume [33]. Administration of leptin for 2 weeks in 4-week-old mice resulted in a 10% increase in brain weight and a 19% increase in total brain DNA indicating that leptin increases cell numbers.

Diseases of aging are another state where changes in leptin are starting to be considered an important factor. Circulating leptin has been found to be lower in patients with Alzheimer's disease and vascular dementia with anorexia [29]. In addition, leptin has been found to decrease amyloid-beta load in transgenic mice with elevated Aβ [10]. Together these findings suggest that decreased leptin, perhaps due to neuroendocrine dysfunction, may be associated with elevated Aβ and dementia. The SAMP8 mice are a strain of mice with elevated amyloid-beta protein (Aβ) and learning and memory impairments by 12 months of age [13,19,24–26]. Aβ protein is considered to be a major contributor to the dementia of Alzheimer's disease (AD). In SAMP8 mice, administration of Aβ antibody or antisense directed at the Aβ region of the APP peptide reverses the memory impairment seen in 12 months SAMP8 mice. In addition, SAMP8 mice have a decreased sensitivity to memory-enhancing compounds such as arecoline, a cholinergic agonist, and glutamate when injected into the hippocampus. The decreased sensitivity can be reversed when Aβ antibody is given 24 h prior to training and drug administration [24]. Recent studies have suggested that administration of leptin significantly reduced Aβ levels in transgenic mice, which overexpress Aβ [10].

The purpose of the current studies is to examine the effect of leptin on memory processing in the hippocampus in both normal outbred CD-1 mice and in SAMP8 mice, which developed elevated amyloid-beta and memory deficits with advancing age.

2. Materials and methods

2.1. Mice

SAM-P8 male mice, 4 and 12 months of age, were obtained from our breeding colony. This colony has been maintained for 15 years as an inbred strain from siblings provided by Dr. Takeda of Kyoto University, Japan. Sentinels from the colony have remained free of pathogens, including salmonella, ectoparasites, pneumonia virus, mouse hepatitis, and ectromelia, for over 14 years. CD-1 male mice from our breeding colony, 4 months of age, are also tested regularly. This colony has been maintained for 3 year as an outbred strain obtained from Charles Rivers Breeding Laboratories of Wilmington, MA. Water and food were available ad libitum. All subjects were experimentally naïve. Mice were on a 12 h light:12 h dark cycle with lights on at 0600 h.

2.2. Drugs

Leptin (mouse recombinant) was purchased from Sigma (St. Louis, MO). The drug was dissolved in saline and then injected into the hippocampus in a volume of 0.5 (l per injection. Dose-response curves were first established in CD-1 mice. Drug concentrations were coded to prevent experimenter bias.

2.3. Surgery and drug administration

Forty-eight hours prior to training, mice were anesthetized with tribromoethylene, placed in a stereotaxic instrument, and the scalp was deflected and a hole drilled through the skull over each injection site. The injection coordinates for bilateral hippocampal injections were 1.6 mm posterior to the bregma and 1.6 mm to the right and left of the saggital suture. The injection depth was 1.6 mm. Mice were trained 48 h after surgery. Immediately after training, mice were again placed in the stereotaxic apparatus under light isoflurane anesthesia. Within 3 min after training, a 0.5 μl solution of saline or drug solution was injected into each injection site over 60 s through 30-gauge blunt stainless steel hypodermic tubing (Small Parts Inc., Miami, FL) attached to a 10 ml syringe with PE-10 tubing and driven by a Stoelting Syringe Pump (Wood Dale, IL).

2.4. Training and testing

2.4.1. Maze training and testing procedures

The T-maze consisted of a black plastic alley with a start box at one end and two goal boxes at the other. The start box was separated from the alley by a plastic guillotine door, which prevented movement down the alley until training began. An electrifiable stainless steel rod floor ran throughout the maze to deliver scrambled footshock [8].

Mice were not permitted to explore the maze prior to training. A block of training trials began when a mouse was placed into the start box. The guillotine door was raised and a buzzer sounded simultaneously; 5 s later footshock was applied. The goal box entered on the first trial was designated "incorrect" and the footshock was continued until the mouse entered the other goal box, which in all subsequent trials was designated as "correct" for the particular mouse. At the end of

each trial, the mouse was returned to its home cage until the next trial.

The parameters for this training condition were set so that the control groups would have poor retention (mean trials to criterion between 9 and 10) so that drug-induced improvement of retention could be detected. Training used an intertrial interval of 35 s, the buzzer was of the door-bell type, sounded at 55 dB and shock was set at 0.35 mA (Coulbourn Instruments scrambled grid floor shocker model E13-08). Retention was tested 1 week later by continuing training until mice reach a criterion (five avoidances in six consecutive trials). The results were reported as the number of trails to criterion for the retention test.

2.4.2. *Step down inhibitory avoidance paradigm*

Two days after surgery, mice were submitted to a step down inhibitory avoidance training paradigm. The box was 55 cm × 55 cm × 20 cm high with a stainless steel rod floor. A 7.5 cm × 7.5 cm × 2.5 cm high platform was placed in the center of the apparatus. Mice were placed on the platform upon stepping four paws onto the grid floor they received a 0.28 mA, 2 s footshock. The latency to step down was recorded. Training consisted of one trial. During the retention test session mice were placed on the platform, but no footshock was given when they stepped down. The latency to step down onto the grid was recorded. Retention test scores were expressed as test minus training step down latency (ceiling, 180 s).

2.5. *Statistics*

Results were expressed as means with their standard errors. The retention test scores were analyzed by one-way analysis of variance (ANOVA) for each group followed by Dunnett's *t*-test post hoc analysis.

3. Results

3.1. *Dose-response curve for leptin in T-maze footshock avoidance retention*

The one-way ANOVA for mean trials to criterion (five avoidance in six consecutive trials) measure on the retention test showed a significant effect for group $F(4, 45) = 14.88$, $P < 0.0001$. Tukey's *t*-test post hoc analysis indicated that the mice that received 0.25 μg or 0.5 μg of leptin took significantly fewer trials to reach criterion on the retention test than the other groups (Fig. 1). Leptin (0.5 μg) given 24 h post-training had no effect on retention.

3.2. *The effect of leptin on step down inhibitory avoidance*

The one-way ANOVA for retention (latency to step down during retention test minus the latency to step down during acquisition test) measure on the retention test showed a significant effect $F(2, 45) = 5.14$, $P < 0.009$. Tukey's *t*-test post hoc analysis indicated that the mice that received 0.5 μg, but not 0.75 μg had significantly longer latencies on the retention test than the other groups (Fig. 2).

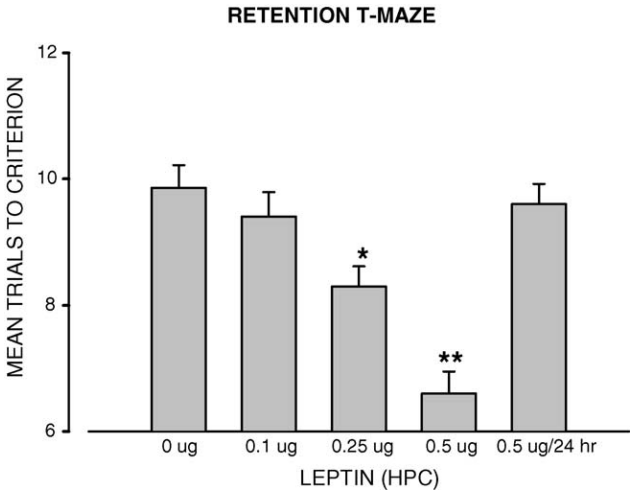

Fig. 1 – The effects of post-training intrahippocampal administration of leptin on retention in T-maze footshock avoidance. Leptin 0.25 and 0.5 μg improved retention. Leptin 0.5 μg 24 h post-training had no effect: $^{*}P < 0.05$, and $^{**}P < 0.01$.

3.3. *T-maze active avoidance in 4- and 12-month-old SAMP8 mice*

A one-way ANOVA for 4-month-old SAMP8 mice on trials to criterion on the retention test showed a significant effect $F(3, 33) = 13.85$, $P < 0.001$. Tukey's *t*-test post hoc analysis indicated that the mice that received 0.5 μg of leptin took significantly fewer trials to reach criterion than the other groups (Fig. 3, left panel).

A one-way ANOVA for 12-month-old SAMP8 mice on trials to criterion on the retention test showed a significant effect

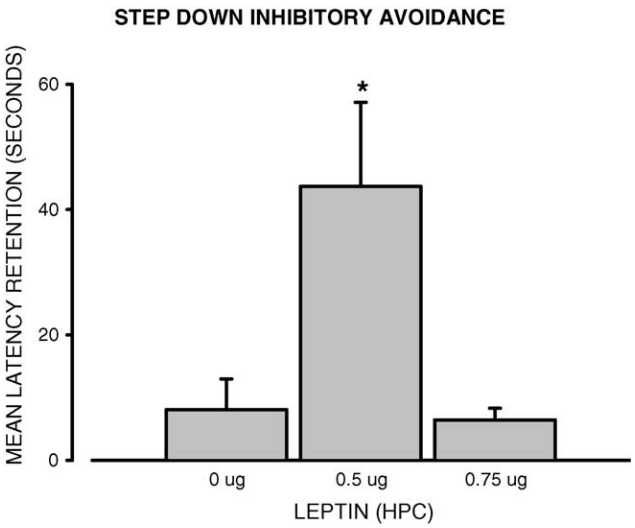

Fig. 2 – The effects of post-training intrahippocampal administration of leptin on retention in step down inhibitory avoidance. The retention score for each mouse was calculated by subtracting the training latency from the test latency score. Leptin 0.5 μ improved retention: $^{*}P < 0.05$.

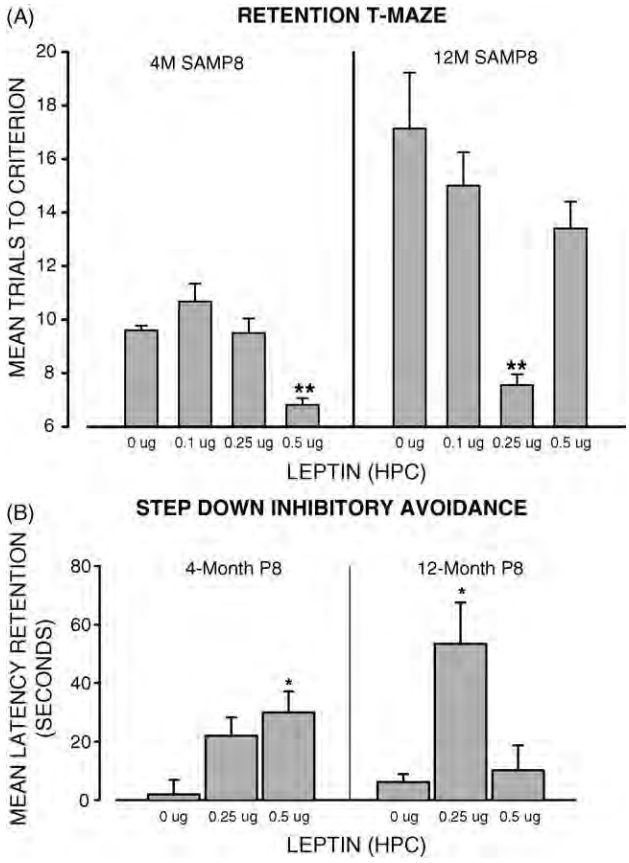

Fig. 3 – The effects of post-training intrahippocampal administration of leptin in 4- and 12-month SAMP8 mice in T-maze footshock avoidance (A) and step down inhibitory avoidance. Leptin 0.05 μg improved retention of both tasks in 4-month SAMP8 mice; however, 12-month SAMP8 mice required only 0.25 μg intrahippocampally to improve retention: $^{*}P < 0.05$ and $^{**}P < 0.01$.

$F(3, 33) = 13.00$, $P < 0.001$. Tukey's t-test post hoc analysis indicated that the mice that received 0.25 μg of leptin took significantly fewer trials to reach criterion than the other groups (Fig. 3A, right panel).

3.4. *The effect of leptin on step down inhibitory avoidance in 4- and 12-month SAMP8 mice*

The one-way ANOVA in 4-month-old SAMP8 mice for retention (latency to step down during retention test minus the latency to step down during acquisition test) measure on the retention test showed a significant effect $F(2, 44) = 4.27$, $P < 0.02$. Tukey's t-test post hoc analysis indicated that the mice that received 0.5 μg, but not 0.25 μg had significantly longer latencies on the retention test than the other groups (Fig. 3B).

The one-way ANOVA in 2-month-old SAMP8 mice for retention (latency to step down during retention test minus the latency to step down during acquisition test) measure on the retention test showed a significant effect $F(2, 52) = 6.74$, $P < 0.002$. Tukey's t-test post hoc analysis indicated that the mice that received 0.25 μg, but not 0.50 μg had significantly longer latencies on the retention test than the other groups (Fig. 3B).

4. Discussion

In the current studies, we assessed the role of leptin in memory processing using two different avoidance paradigms. Our results indicate the leptin improves memory processing for T-maze footshock avoidance and one trial step down inhibitory avoidance. Leptin administered 24 h post-training did not improve retention. An effect of a substance when given immediately, but not later, after training shows that the effect occurs during consolidation of the information immediately after training and not due to lingering presence of the substance affecting retention test performance [22]. In 12-month SAMP8 mice leptin improved retention for T-maze footshock avoidance and step down inhibitory avoidance at a lower dose than in 4-month SAMP8 mice with much lower Aβ levels. We have previously shown that 12-month SAMP8 mice have age-related deficits in learning and memory which correlates with increased levels of Aβ in the brain and are less sensitive to several memory-enhancing drugs [13,24,25]. Here, leptin was able to improve memory at a lower dose despite elevated levels of Aβ.

Obesity and obesity-related diabetes mellitus are both associated with leptin resistance across the BBB [1]. Memory impairment has been associated with both obesity and diabetes [7,9,12]. Here, we found that leptin improves memory. These findings suggest that the resistance to leptin in the brain may play a part in the memory impairment seen with obesity and obesity-related diabetes.

The u-shaped nature of the dose-response curve for leptin, which indicates there is an optimal dose for memory. For example, as we have discussed above that resistance to leptin in the brain as in the case of obesity appears to be detrimental. However, there is a large body of literature on the detrimental effects of too much leptin, as well. For example, high levels of leptin have been associated with many inflammatory conditions. Elevated leptin is likely to play a role in autoimmune states such as those observed in inflammatory bowel disease, multiple sclerosis, diabetes type I and rheumatoid arthritis [27]. Currently, it is unknown if high leptin levels in these conditions are detrimental to memory.

Recent studies have found that leptin reduces Aβ load in transgenic mice [10]. We have previously shown that reducing Aβ with its antibody in SAMP8 mice improved learning and memory and restored sensitivity to memory-enhancing compounds [24]. Surprisingly, the 12-month SAMP8 mice required less leptin than the 4-month SAMP8 mice to improve retention.

Changes in leptin with aging are unclear. Leptin has been reported to both increase and decrease with age [17,28]. Both testosterone and dehydroepiandrosterone (DHEA) in males decrease with aging [17]. Testosterone and DHEA have been found to improve memory processing in 12-month SAMP8 male mice [9,11]. In addition, testosterone reduces Aβ release when applied to neuronal tissue [15]. Recent studies found that leptin reduced Aβ load [10]. Here, we see that leptin improves memory in 12-month SAMP8 male mice. These

findings suggest that dysregulation of hormonal pathways with aging and disease play a factor in memory decline seen with aging and disease.

The current studies show yet another role for leptin in the brain. Outside the hypothalamus, where leptin plays a key role in energy expenditure and food intake, leptin improves memory processing in the hippocampus. When given immediately, but not 24 h after training, leptin was able to improve retention in both T-maze footshock avoidance and step down inhibitory avoidance. Leptin was also able to improve memory processing in SAMP8 mice, a model of Aβ toxicity, as well. Interestingly, leptin was able to improve memory in aged SAMP8 mice at half the dose needed for young SAMP8 mice that have no memory deficits. Our findings indicate that leptin plays a role in memory and suggests leptin may contribute to memory impairment in diseases where leptin deficiencies or resistances occur.

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