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Behavioral, hormonal and central serotonin modulating effects of injected leptin

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ABSTRACT

Leptin is viewed as an important target for developing novel therapeutics for obesity, depression/anxiety and cognitive dysfunctions. The present study therefore concerns behavioral, hormonal and central serotonin modulating effects of systemically injected leptin. Pharmacological doses (100 and 500 μg/kg) of leptin injected systemically decreased 24 h cumulative food intake and body weight in freely feeding rats and improved acquisition and retention of memory in Morris water maze test. Potential anxiety reducing, hormonal and serotonin modulating effects of the peptide hormone were determined in a separate experiment. Animals injected with 100 or 500 μg/kg leptin were tested for anxiety in an elevated plus maze test 1 h later. A significant increase in the number of entries and time passed in open arm of the elevated plus maze in leptin injected animals suggested pronounced anxiety reducing effect. Moreover, circulating levels of leptin correlated significantly with anxiety reducing effects of the peptide hormone. Serum serotonin increased and ghrelin decreased in leptin injected animals and correlated, positively and negatively respectively, with circulating leptin. Corticosterone increased at low dose and levels were normal at higher dose. Serotonin metabolism in the hypothalamus and hippocampus decreased only at higher dose of leptin. The results support a role of leptin in the treatment of obesity, anxiety and cognitive dysfunctions. It is suggested that hormonal and serotonin modulating effects of leptin can alter treatment efficacy in particularly comorbid conditions.

1. Introduction

The increasing prevalence of obesity, depression/anxiety is becoming a significant health concern throughout the world. Current medications for these disorders do not provide satisfactory remission [31,50] and are often associated with a number of adverse effects [6]. Investigations for novel therapeutic agents for the treatment of these disorders are therefore highly needed. In this context, role of peptide hormone leptin is becoming increasingly important. Obesity and depression are also often linked with dementia and cognitive deficits [12,14]. Animal research shows that high fat diet-induced deficits of leptin signaling in the hippocampus can impair spatial memory tasks in animal models suggesting that leptin is also involved in cognition.

Leptin, a 16 kDa protein, encoded by the obese (ob) gene was identified as a hormonal signal for satiety produced largely, but not exclusively, by adipocytes [8,54]. The peptide hormone can cross blood–brain barrier and acts via its receptors in the hypothalamus to elicit a negative feedback adiposity signal for the control of energy homeostasis [42]. Thus, high leptin levels signal the presence of sufficient energy stores, resulting in a decrease in appetite and increase in energy expenditure, whereas a decrease in leptin levels with the onset of starvation produces signal for the initiation of meal. Leptin receptors are also expressed in many

regions other than the hypothalamus [8] and accumulating evidence suggests that emotional, cognitive and stress reducing effects of leptin are mediated via its receptors located in different brain regions [1,5,22].

Rodent models such as leptin deficient ob/ob mice or leptin receptor deficient db/db mice are often used to evaluate a role of leptin in various physiological process and behaviors

Abbreviations: 5-HT, 5-hydroxytryptamine (serotonin); 5-HIAA, 5-hydroxyindole acetic acid.

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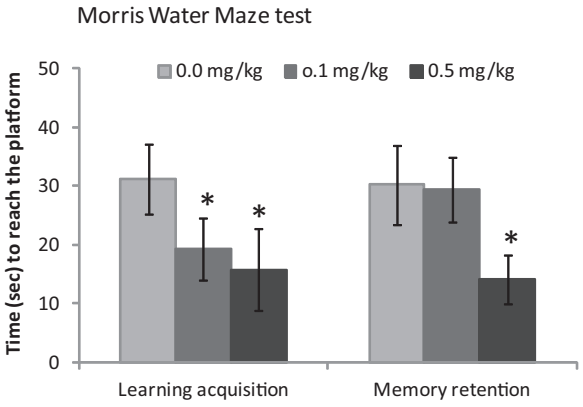

Fig. 1. Effects of injected leptin on learning acquisition and memory retention in Morris water maze test. Values are means ± SD ($n = 8$). Significant differences by Tukey's post hoc test: $^{*}p < 0.01$ from respective saline injected animals following two way ANOVA (repeated measure design).

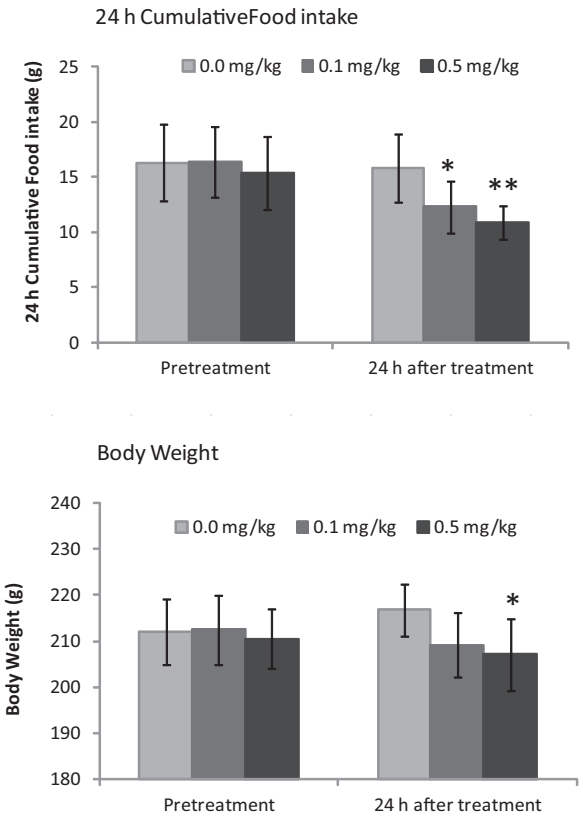

Fig. 2. Pretreatment and post treatment values of 24 h food intakes and body weights in leptin and saline treated animals. Values are means ± SD ($n = 8$). Significant differences by Tukey's post hoc test: $^{*}p < 0.05$, $^{**}p < 0.01$ from respective saline injected animals following two way ANOVA (repeated measure design).

[36,46,53]. Considering a potential role of leptin in the treatment of obesity, anxiety/depression and cognitive deficits; behavioral effects of exogenous leptin in normal (wild-type) animals are important. In these studies we have shown that systemically injected leptin inhibits stress-induced behavioral deficits [26] suggesting an important role of leptin in reducing emotional distress [22]. That exogenous leptin produces anxiolytic and antidepressant like effects in rat models [26,37,38] has been also shown. Effects of leptin on spatial memory are, however, not conclusive. Systemically injected leptin at doses of 5 or 50 μg/kg improves cognitive performance in passive avoidance and Morris water maze tests but higher doses (500 μg/kg) produce no effect [41]. Bilateral injections of leptin into the hippocampus improve performance in remembering the location of an aversive event (foot shock) in mice [11] but following bilateral injection of leptin in the ventral hippocampus, memory consolidation for spatial location of food is suppressed in rats [32].

Regarding hormonal effects of exogenous leptin, we have shown that low doses of leptin increase circulating levels of corticosterone while higher doses had no effect [26]. Functional systems for serotonin (5-hydroxytryptamine; 5-HT) synthesis, reuptake and receptor activation occur in adipocytes [49] and a decrease in circulating levels of leptin is reported to occur following short (5 days) and long (4 months) term systemic administration of serotonin. It is therefore possible that injected leptin can change circulating levels of serotonin which though cannot cross blood brain barrier, mediates its effects via brain–gut connection. In addition, ghrelin an orexigenic hormone [30] mainly secreted by cells in the stomach also plays an important role in controlling the motility of stomach and duodenum [13,34]. It is therefore possible that systemically injected leptin modulates circulating levels of serotonin and ghrelin.

The present study is, therefore, designed to investigate effects of systemically injected leptin on learning acquisition and memory retention in Morris water maze test. Effects of the administration on 24 h food intake, body weight change, behavior in an elevated plus maze and circulating levels of corticosterone, serotonin and ghrelin are also determined. In view of a role of central serotonin in the regulation of eating and emotional behavior as well cognition, associated changes of 5-HT and its metabolite 5-hydroxyindole acetic acid (5-HIAA) in the hypothalamus and hippocampus are also determined. The findings may help to evaluate importance of leptin as a therapeutic target for cognitive dysfunctions, anxiety, depression, obesity and gastrointestinal disturbances.

2. Methods and materials

2.1. Animals, treatment and recombinant rat leptin

Locally bred male albino Wistar rats, weighing 200–220 g were housed individually on a 12 h light dark cycle (lights on at 6:00 h) in a temperature controlled (24 ± 2 °C) room with free access to tap water and standard rodent diet. To establish familiarity with the environment, animals were housed 7 days before the start of the experiment. All treatments and behavioral monitoring were done in light phase. The experiments were conducted in a balanced design to avoid order and time effect and these were in accordance with the guidelines approved by the institutional Ethics and Animal Care Committee. Recombinant rat leptin (ALX-201-231) purchased from Enzo Life Sciences Inc., Farmingdale, NY, USA was dissolved in saline before use and injected intraperitoneally (i.p.) to rats at doses of 0.0, 100 and 500 μg/kg.

2.2. Effects of leptin on behavioral performance in Morris water maze test, 24 h food intake and body weight change

Twenty four animals were used in the experiment. The animals were weighed and randomly divided into three equal groups of eight each. Leptin was injected intraperitoneally at doses of 0.0, 100 and 500 μg/kg from 9:00 to 10:00 h.

2.3. Water maze test

The water maze used in the present study was a circular pool made up of white plastic. The maze, 90 cm in diameter and 60 cm high was filled with opaque water to a depth of 30 cm. The temperature of water was 22 ± 2 °C. The maze divided virtually into four