Effects of central and peripheral administration of leptin on pain threshold in rats and mice

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Abstract

OBJECTIVE: This study was planned to investigate the effects of exogenous leptin on the pain threshold.

METHODS: Adult male Wistar rats weighing 250–300 g and mice weighing 25–30 g were used in this study. Leptin was intracerebroventricularly (i. c. v.) injected in a dose of 3.5µg/rat. Mice were intraperitoneally (i. p.) injected with leptin in a dose of 25µg/mouse. Control animals were injected with the respective vehicle. The pain threshold test was performed using hot plate analgesia meter. The experiments were performed during the day and at night. The data were statistically analysed by Mann-Whitney U test. Level of significance was set at p<0.05.

RESULTS: During the day, there were no significant changes in hot plate latencies half an hour after i.c.v. injection of vehicle or leptin in the control and leptin-treated rats, respectively. At night, like during the day, i.c.v. injection of neither vehicle nor leptin caused any significant change in pain sensitivity. In mice, i.p. injection of leptin decreased latencies significantly (p<0.05) during the day and at night. Thus, leptin caused an increase in pain sensitivity during the day and at night.

CONCLUSION: These results clearly demonstrated that leptin has a decreasing-effect on pain threshold if it is peripherally administered in mice.
**Introduction**

It has been shown that obese people or animals may have different responses to pain stimuli. The sensory and pain threshold were found to be higher in the obese people than in the control subjects [1]. The patients with fatness had higher pain sensitivity threshold than people of other categories, so they felt less pain [2]. Dietary-induced obese rats were found to be similar to obese humans in being less sensitive to painful stimuli [4]. Morphine was found to be less potent in obese than in lean rats in this study.

In contrast to these studies, obese people or animals may show more sensitivity to painful stimuli. In the obese women, the threshold of the nociceptive flexion reflex was found to be significantly lower than in controls [4]. Obese female and male subjects were found to show similar reaction to painful stimuli, being more sensitive [5]. Obese Zucker rats were also observed to have lower pain threshold [6], which was suggested to result from a defect in the endogenous opioid systems.

Although obesity is generally accepted to change the pain threshold, the mechanism is not known very well. Endocrine changes caused by obesity may be responsible for the increase or decrease in the pain threshold. One of the main hormones the secretions of which are affected by obesity is leptin. Leptin has been shown to lead to changes in the secretions of neuroendocrine factors such as alpha-melanocyte stimulating hormone (alpha-MSH) and beta-endorphin, which are known to be related to nociception [7]. We hypothesized that the increased leptin release in obesity might be responsible for the changes observed in the pain threshold. So, we planned this study to investigate the effects of exogenous leptin on the pain threshold.

**Material and methods**

Adult male Wistar rats weighing 250–300 g (Firat University Biomedical Unit, Elazig) were employed in this study. They were housed under controlled light (12-h light and 12-h dark, lights on at 07.00 h) and temperature (21±1°C) conditions. Food and water were supplied ad libitum. All the protocols in the present study were approved by the local ethics committee in the Medical School. Rats were intracerebroventricularly (i.c.v.) implanted with a 22-gauge guide cannula for injection of leptin. Cannulation was performed under general anesthesia with ketamine 60 mg/kg plus xylazine (rompun) 5 mg/kg. Following anaesthesia, rats were placed in a stereotaxic apparatus. A cannula was inserted into the lateral ventricle. The coordinates which were used as follows: 0.92 mm posterior to bregma, 1.4 mm lateral to the midline and 3.2 mm below the surface of the brain. The cannula was placed with a stainless steel screw and dental resin. Rats were allowed to recover from operation completely before the experiments were started. Leptin (Mouse Recombinant) was injected in a dose of 3.5µg/rat. Leptin solution (10µl) was injected into the lateral ventricle within 1 min. Control animals were injected with the respective vehicle (0.5 ml 15 mM HCL and 0.3 ml 7.5 mM NaOH).

Male mice weighing 25–30 g (Firat University Biomedical Unit, Elazig) were also used in this study. They were kept in the same conditions above mentioned for the rats. Mice were intraperitoneally injected with leptin in a dose of 25µg/mouse in 0.1 ml of the solution.

The hot plate test was performed using the Harvard hot plate analgesia meter (Harvard Apparatus Ltd., England). Animals were allowed to acclimate to the hot plate for a period of 1 week prior to the experiment. The experiments were performed during the day and at night. Each group consisted of 6 animals. During the day, the animals were placed on the hot plate between 13.00 and 13.30 prior to treatment. Half an hour after leptin or vehicle injections, rats were placed on the hot plate. The response latency to either a hind paw lick or a jump was recorded. In the absence of a response, the animal was removed 60 s after the placement into the hot plate to prevent tissue damage. At night, hot plate test was performed between 01.00 and 02.00 in the same manner.

The same protocol mentioned above was performed for rats and mice separately except the temperature of the hot plate, which was 52°C and 50°C for rats and mice, respectively. The data were statistically analysed by Mann-Whitney U test. Level of significance was set at p<0.05.

**Results**

The latency results of the hot plate test performed in the rats are seen in Fig 1 and 2. During the day, there were no significant changes in hot plate latencies half an hour after i.c.v. injection of vehicle or leptin in the control and leptin-treated groups, though the latency was slightly lower after the injection of leptin. The response times to hot plate changed from 20.8 ± 5.3 and 22.5 ± 7.2 to 19.3 ± 6.3 and 19.1 ± 3.1 sec in the control and in the leptin-treated groups before and after vehicle or leptin administration, respectively. Besides the fact that the pre- and post-injection latencies did not show significant changes in vehicle and leptin injected rats, there were no significant changes between the control and leptin-injected rats compared to each other. At night, like during the day, i.c.v. injection of neither vehicle nor leptin caused any significant change in pain sensitivity. The mean latencies were 20.2 ± 8.1 and 22.3 ± 4.2 sec in the control and 18.7 ± 3.9 and 17.5 ± 4.2 sec in the leptin-treated groups before and after vehicle or leptin administration, respectively. Compared to day and night values, there were no significant changes between the respective values in the control and leptin-injected groups.

As seen in Fig. 3 and 4, i.p. injection of leptin to mice decreased significantly (p<0.05) latencies during the day and at night. Thus, leptin caused an increase in pain sensitivity during the day and at night. During the day, the control rats had pre- and post-injection latencies of 17.8 ± 4.5 and 19.4 ± 3.7 sec, respectively.
Leptin injection decreased latency from 18.2 ± 1.7 and 14.6 ± 3.7 sec to 14.5 ± 3.2 and 9.6 ± 3.3 sec during the day and at night, respectively. In addition to a decreasing effect of leptin on pain threshold, vehicle or leptin injected mice had higher latencies (p<0.05) in both pre- and post-injection periods during the day than at night.

Discussion

Our data show that i.p. administration of leptin affects pain threshold in mice. Mice had lower latency to hot plate test after receiving i.p. leptin. Leptin is known to be released in a circadian pattern, being higher at night than during the day [8,9]. Pain threshold is also known has a circadian rhythm in mice [10]. So, the hot plate test was performed during the day and at night. Leptin caused a decrease in pain threshold during the day and at night. In the present experiment, pain threshold was measured between 13.30 and 14.00 p.m. during the day and respective a.m. hours. Both vehicle and leptin injected mice had lower latencies in pre- and post-injection periods at night. Thus, circadian rhythm of pain threshold was not changed by leptin administration.

In contrast to the nociceptive effects of i.p. leptin administration in mice, i.c.v. leptin administration to rats did not cause any significant change in pain threshold during the day or at night times. Slight differences in the primary structures of mouse vs. rat leptin may cause the lack of effect of mouse leptin in the rat. Although it has been reported that pain threshold may have a circadian rhythm in rats [11], in our study the latency did not show significant change which indicates a presence of circadian rhythm of pain threshold during the day and at night. In this study, the presence of a circadian rhythm of pain threshold in the rat may not be observed because pain threshold needs measuring at more frequent intervals to determine whether it has a circadian rhythm or not.

There is no previous knowledge about the effects of leptin on pain threshold. But, many strong reasons are present to hypothesise that leptin may have effects on pain threshold. Obesity and fasting are the two main situations in which leptin secretion is mostly modified. In obese people or animals, leptin is known to be secreted in high levels due to an increase in the mass of...
adipose tissue. The studies on the effects of obesity on pain threshold have given controversial results. In the obese people who were exposed to mechanical pressure test [2] or electrical stimulation [12] to determine pain threshold, an increased pain threshold was observed. Contrarily, in the studies [4] using the electrophysiological tests such as the measurement of withdrawal reflex time, obese people had lower pain threshold, which indicates a reverse correlation between degree of overweight and the threshold of the nociceptive reflex. Thus, according to the methods that are used to determine pain threshold, different results may be obtained. The rat foot withdrawal response to noxious radiant heat has been used as a model of nociception, and latency can be used as an accurate measure of changes in nociceptive threshold produced by drug treatments [13]. Peripheral factors such as skin thickness and innervation density were suggested to be able to affect pain sensitivity [14,15]. Therefore, due to these factors, the pain threshold tests may not always be able to reflect the real pain threshold because of the changes in the stimulation of nociceptors in the skin. The more adipose tissue people gain, the more difficult it is to stimulate nociceptors. Moreover, the density of nociceptors may be reduced by the increased mass of the body because of fatness, which makes the stimulation of nociceptors more difficult. The measurement of the latency to heating stimuli is accepted as an accurate test to determine nociceptive threshold.

The results of the studies on pain sensitivity in laboratory animals are also controversial like in humans. Pain threshold was found to be higher in the dietary-induced obese rats [3] whereas Zucker rats, which have been reported to have hyperleptinemia [16] exhibited shorter latencies to tail flick tests [6].

Leptin administration forms a kind of obesity at least in terms of endocrinological point. So, behavioural changes such as increased or decreased pain sensitivity observed in obesity may at least partly result from the changes in leptin secretion or leptin-induced changes. It has not been shown that spinal cord includes leptin receptors. So, it is difficult to talk about a spinal effect of leptin itself at least now. Leptin has been recently reported to enhance NMDA receptor function in the hippocampus [17]. Leptin may sensitize nociceptors by activating NMDA receptors in spinal cord. Leptin may also have an indirect effect on pain sensitivity due to its interaction with the some neuroendocrinological factors [18]. Leptin has been reported to increase alpha-melanocyte stimulating hormone synthesis [7], which was shown to increase pain sensitivity [19]. To determine the mechanism by leptin exert its nociceptive effect, antagonism of melanocortin system, which is the one of the main ways leptin uses to show its central effect [20], may be useful.

In conclusion, leptin may have an effect on the regulation of pain sensitivity, but further studies are needed to determine the mechanism by which leptin exerts its nociceptive effect in mice.

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REFERENCES


