Fırat Tıp Dergisi 2005;10(3): 89-91

Experimental Research

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Effect of Ghrelin on Pain Threshold in Mice

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ABSTRACT

Objectives: Ghrelin, a novel growth hormone-releasing peptide, was isolated from the rat stomach as an endogenous ligand for the growth hormone secretagogues receptor. The present study was planned to determine whether ghrelin affects pain threshold in mice.

Materials and Methods: Adult male BALB/C mice weighing 25–30g were used in this study. The hot plate test was conducted by placing the mouse on a metal surface maintained at 50±0.1°C by using hot plate analgesia meter. The latency to jumping or licking a hind paw was recorded as nociceptive threshold. Animals were allowed to acclimate to the hot plate for a period of 1 week prior to the experiment. Different doses of ghrelin were intraperitoneally administered to the animals after control latencies. Control group received saline alone. Hot plate test were performed in all animals individually in 30 th, 60 th, 90 th and 120 th minutes after injection. Pain threshold values were determined and analyzed by Mann-Whitney U Test and Wilkoxon Sign Ranks Test.

Results: Ghrelin didn't affect pain threshold throughout the experiment in 0.3pmol and 1pmol doses compared to control values. There were significant decreases in pain threshold when it is given in a dose of 3pmol in 30 th and 60 th minutes ($p<0.05$ and $p<0.01$, respectively).

Conclusion: The results of this study have presented that ghrelin may have a decreasing effect on pain threshold in mice. Further studies are needed to determine the mechanism by which ghrelin exerts its nociceptive effect. *©2005, Fırat University, Medical School*

Key words: *Ghrelin, pain threshold, hot plate and mice*

ÖZET

Farelerde Ağri Eşiği Üzerine Grelinin Etkisi

Amaç: Yeni bir büyüme hormonu salgılatıcı peptit olan grelin, büyüme hormonu salgılatıcı reseptörlerin endojen bir ligandı olarak sıçan midesinden izole edilmiştir. Bu çalışma, grelinin farelerde ağrı eşiği üzerindeki olası etkilerini belirlemek amacıyla planlanmıştır.

Gereç ve Yöntem: Çalışmada 25-30 gram ağırlığında yetişkin erkek BALB/C fareler kullanıldı. *Hot plate* testi, farelerin analjezimetrenin 50±0.1 °C'deki metal yüzeyine bırakıldıkları andan itibaren, ayaklarını hızla çırptıkları veya yaladıkları süre saniye olarak ağrı eşiği değeri için kaydededilerek uygulandı.. Deneylerden önce hayvanlar 1 hafta süreyle *hot plate*'e alıştırıldı. Deney günü, analjezimetrede kontrol kayıtları alındıktan hemen sonra, intraperitoneal yolla farlı dozlardaki grelin uygulamaları yapıldı. Kontrol grubuna serum fizyolojik verildi. Bütün hayvanlara enjeksiyonlardan 30, 60, 90 ve 120 dakika sonra test uygulandı. Ağrı eşiği değerleri belirlendi ve *Mann-Whitney U Test* ve *Wilkoxon Sign Ranks Test* kullanılarak analiz edildi.

Bulgular: Grelin kontrol grubuyla karşılaştırıldığında 0.3 pmol ve 1 pmol dozlarında ağrı eşiğini etkilemedi. 3 pmol dozda ise, 30. ve 60. dakikalarda ağrı eşiğinde belirgin azalmalar ortaya çıktı ($p<0.05$ ve $p<0.01$).

Sonuç: Bu çalışmanın sonuçları grelinin farelerde ağrı eşiğini düşürdüğünü göstermektedir. Grelinin bu hiperaljezik etkisinin mekanizmasını belirlemek için ek çalışmalara ihtiyaç vardır. *©2005, Fırat Üniversitesi, Tıp Fakültesi*

Anahtar kelimeler: *Grelin, ağrı eşiği, hot plate ve fare*

Ghrelin, an acylated 28-amino acid peptide recently isolated from mammalian stomach, has been identified as an endogenous ligand for growth hormone secretagogue receptors (1). This hormone exerts a strong stimulatory effect on GH secretion in humans (2) and rats (3). Ghrelin stimulates food intake after central and peripheral administration (4). Food intake causes a decrease in ghrelin level in blood (5). In addition to these effects, this hormone may be involved in some physiological processes in the central nervous system. In the brain, receptors for ghrelin have been detected in multiple hypothalamic nuclei as well as in the hippocampus, substantia nigra, ventral tegmental area, and dorsal and median raphe nuclei (6; 7). Ghrelin acts at the nucleus of the solitary tract to suppress sympathetic activity and to decrease arterial pressure in rats (8). Ghrelin increase anxiety-like behavior and memory retention (9). It is suggested that ghrelin is an endogenous sleep-promoting factor in humans (10).

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. The patients with fatness had higher pain sensitivity threshold than people of other categories, so they felt less pain. Dietary-induced obese rats were found to be similar to obese humans in being less sensitive to painful stimuli. Ghrelin level is reduced in obese human (11) and rodents (12). Therefore, it may be thought that there is a relationship between ghrelin and nociception. In this study we investigated the possible effect of ghrelin on pain threshold in mice.

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MATERIALS AND METHODS

Adult male Balb/C mice weighing 25-30g obtained from Firat University Biomedical Unit (Elazig) were used in this study (n=36). They were housed under controlled light (12-h light and 12-h dark, lights on at 07.00h) and temperature (21±1 °C) conditions. Food and water were supplied *ad libitum*.

The hot plate test was conducted by placing the mouse on a metal surface maintained at 50±0.1 °C using the hot plate analgesia meter (Harward Apparatus Ltd., England). Hot plate was surrounded with a transparent plastic barrier. The latency to jumping or licking a hind paw was recorded. In the absence of a response, the animal was removed 60s after the placement into the hot plate to prevent tissue damage. Animals were allowed to acclimate to the hot plate for a period of 1 week prior to the experiment.

Different doses of ghrelin (0.3pmol (n=10), 1pmol (n=8) and 3pmol (n=8)) were intraperitoneally administered to the animals after obtaining control latencies (minute 0). Control group received saline alone (n=10). Hot plate test was performed on all animals individually in 30th, 60 th, 90 th and 120th minutes after injection. Pain threshold values were determined and analyzed by Mann-Whitney U Test and Wilkoxon Sign Ranks Test. P<0.05 was considered statistically significant.

RESULTS

The response latencies had similar values throughout the experiment in the control group. 0,3 pmol ghrelin did not have any significant effect compared to control group (Figure 1). The second dose of ghrelin (1pmol) slightly decreased the pain threshold in 30st and 60st minutes after treatment, but it was not statistically significant (Figure 1).

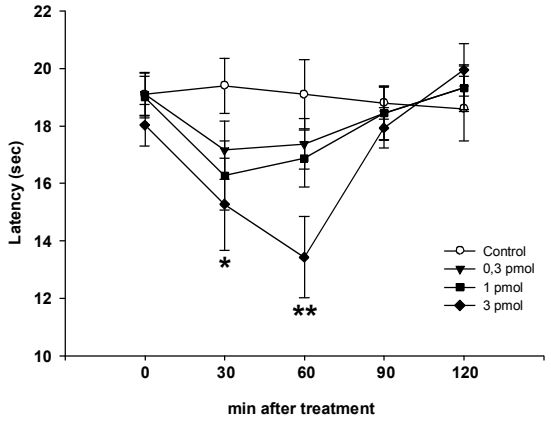

Figure 1. Hot plate latencies (Mean±SEM) of control (n=10,), 0,3 pmol (n=10), 1 pmol (n=8) and 3 pmol (n=8) ghrelin administered groups. *p<0.05 and **p<0.01 compared to control by using Mann-Whitney U Test

There were significant decreases in pain threshold when ghrelin was administered at a dose of 3pmol in 30 th and 60 th minutes compared to control group (p<0.05 and p<0.01, respectively, Figure 1). Additionally, significant decreases were occurred in 30 th and 60 th minutes compared to beginning value in 0th minute in 3pmol ghrelin administered group (p<0.05 and p<0.01, respectively, Figure 2).

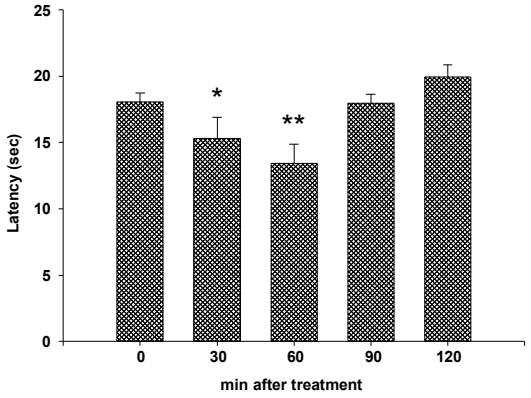

Figure 2. Hot plate latencies (Mean±SEM) of 3 pmol ghrelin. *p<0.05 and **p<0.01 compared to control (0th minute) by using Wilkoxon Sign Ranks Test

We also observed that mice started food intake at 15 minutes after injection of 3pmol ghrelin. But the aim of this study was not to determine the effect of ghrelin on food intake, so we ignored the feeding behavior of animals.

DISCUSSION

The results of the present study demonstrate that ghrelin has hiperalgesic effect in mice. This is only a preliminary study and no attempt was made to clarify the possible mechanism of action of ghrelin on pain threshold. This is the one possibility that ghrelin may induce the Ca^{2+} entry into the neuronal tract involved in transporting noxious stimuli. There is not any direct evidence about this insight. However, ghrelin induces an increase in the intracellular calcium concentration in porcine somatotropes via L-type calcium channel in a dose dependent manner (13). In a zero Na^{+} solution, the stimulatory effect of ghrelin on somatotropes was decreased, suggesting that besides calcium channel, sodium channels are also involved in ghrelin-induced calcium transients (13). Additionally, ghrelin directly interacts with NPY neurons in the arcuate nucleus to induce Ca^{2+} signalling via protein kinase-A and N-type calcium channel-dependent mechanisms in rats (14).

Histaminergic neurons in the tuberomamillary nucleus are implicated in nociception (15) and presence of GHS-R in the tuberomammillary nucleus was suggested by the existence of GHSR mRNA in this area (7). Therefore ghrelin may show its nociceptive effect indirectly by affecting histaminergic transmission. In an electrophysiologic study, it is demonstrated that ghrelin activates histaminergic neurons in the tuberomammillary nucleus by inhibiting G protein-coupled inwardly rectifier K^{+} channels (16). Injection of histamine into the rat dorsal raphe nucleus and periaqueductal grey region produces an antinociception, while its injection into the median raphe nucleus causes hyperalgesia (17,18). Intracerebroventricular administrations of low doses of histamine elicit hyperalgesia, while high doses of histamine produce antinociception (19, 20). The results of above studies suggest that the opposite effects of histamine on pain threshold may be

mediated through different subtypes of histamine receptors (20,21).

It is presented that serotonergic pathways originating from dorsal raphe nucleus is involved in pain modulation (22). Raphe nucleus is another target for the effect of ghrelin (9). Ghrelin has been found to decrease serotonin release in hypothalamus in vitro (23) and suggested to decrease serotonin release in dorsal raphe nucleus (24), which may have an additional role in food intake-increasing effect of ghrelin. Therefore, ghrelin may also affect analgesic system due to its effects on serotonergic transmission in brain stem, which also modulates pain transmission in medulla spinalis.

In conclusion, ghrelin may be a candidate for hormonal regulation of pain sensitivity. However, further studies are needed to establish its effect on nociception and the mechanism by which it exerts its effect.

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Kabul Tarihi:26.05.2005