



ORIGINAL ARTICLE

Effect of Intra Hippocampal CA1 Injection of Spexin on Pain Sensitivity in Female Rat

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ABSTRACT

Spexin is a neuropeptide which was recently identified through the bioinformatics approach. It seems that spexin is central modulators of nociception. The aim of present study was to investigate the effect of intra hippocampal CA1 injection of spexin on pain sensitivity during proestrus stage of estrous cycle in female rat. 18 adult female rat in standard conditions with temperature 21-24°C and 12 h light-dark cycle were used. Hippocampal CA1 was cannulated unilaterally by stereotaxic procedure. Pain test was performed by formalin test (formalin 2.5%) in proestrus stage of estrous cycle. Animals were divided into three groups: 1- sham (received 0.5 ul ACSF); 2- experimental1 (received 0.5ul spexin 10nmole/rat) and 3- experimental2 (received 0.5 ul spexin 30nmole/rat). Present data showed that injection of spexin in hippocampal CA1 at doses of 10 and 30 nmole/rat significantly ($P < 0.05$) decreases pain sensitivity in proestrus stage of estrous cycle. According to present results spexin in hippocampal CA1 has modulatory effect on pain sensitivity in formalin test procedure.

Keywords: Spexin, CA1, Brain

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INTRODUCTION

The pain is undesirable feeling that indicate tissue damage and needs immediate attention to the patient; Severity of pain can be related on the patient's social and cultural background, previous experience of pain can vary according to position so the amount of pain is directly related to the amount of tissue damage. Crippling nerve pain is a chronic condition caused by damage to the peripheral nerves is most commonly used pain medications such as analgesics and anti-inflammatory drugs do not work [1].

Neuropeptides are a chain of amino acids that are made in the central nervous system and are produced by proteolytic enzymes from a longer protein precursor in the vesicles transport, which can cause nerve response with a slow and long-term onset. Using the bioinformatics methods, a research team succeeded to discover one of these neuropeptides called Spexin in 2007. This completely new neuropeptide has been observed in many tissues of body and different parts of the nervous system, including several different areas in which there is a synthesis of neurosteroids, such as hippocampus [2].

The neuropeptide exists in places, in which there is the synthesis of steroids, including in the gonads and the adrenal cortex. Hippocampus, which is a part of the lateral system, is one of the most important and basic structures in different mechanisms of the body, including the feeling of pain [3,4].

On the one hand, it is one of the brain structures, in which the synthesis of neurosteroids occurs. Therefore, it seems that neuropeptide spexin to be involved in this part of because the presence of this peptide has been demonstrated by PCR method. Thus, this study tries to investigate and evaluate that possible role of Spexin in the process of tonic pain perception in female rats.

MATERIALS AND METHODS

Selection and Description of Samples

Eighteen female rats were used in standard conditions 21-24°C, 12 h light and 12 h dark cycle. Hippocampal CA1 was unilaterally cannulated by stereotaxic procedure. The animals were divided into six groups of control (received 0.5ul ACSF), experimental1 (received 0.5 ul spexin 10 nmole/rat), and

experimental 2 (received 0.5ul spexin 30nmole/rat). Pain testing was performed by formalin test (formalin 2.5%) in proestrus stage of estrous cycle.

Surgical procedure of brain

In order to be operated and be set the cannula, animals were anesthetized by Ketamine and xylazine. Then, they were placed in a stereotaxic apparatus and according to the mouse brain atlas (Paxinos Atlas), a cannula was produced in the hippocampal CA1 region by a needle (23) and fixed by the dental cannula cement. In order to recover the animals, they were kept in the animal house for one week and then tested according to the above groups. After pain testing, methylene blue was injected through the cannula in all of the samples, the animals immediately were deeply anesthetized with ether, the brain was removed, and validity of the cannula-setting was confirmed.

Formalin pain test: In this test, 50 µl formalin 2.5% was injected into the animal's foot and her behavior was evaluated in a system where the animal behaviors can be seen the mirror. If the animal had no reaction, the score would be (0), and if she did not rely on the injected paw, the score would be (1). If she held her paw, the score would be (2), and finally if she was beginning to lick and bit the injected paw, the score was score (3). The scoring was performed every 15 seconds for 60 minutes. The results were averaged every 5 minutes for statistical test.

Data analysis

SPSS 19 was used to analyze the data of the study. Data was investigated by statistical analysis of one-way variance and diagnostic testing.

RESULTS

The effect of intra-CA1 hippocampal Spexin on Pain Sensitivity

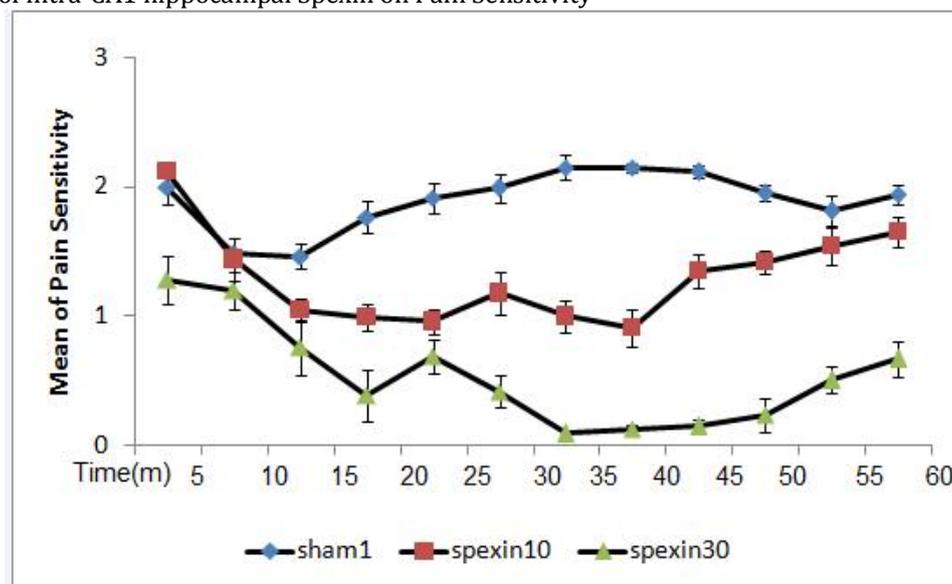


Diagram 1. The effect of intra-CA1 hippocampal Spexin on Pain Sensitivity in Healthy Female Rat

Except for the first and second five minutes, a significant difference existed in pain sensitivity between the healthy control group and the healthy groups of receiving Spexin in other times.

According ANOVA (diagram 4-1), there was a significant reduction ($P < 0.05$) in pain sensitivity of the groups receiving 10 and 30 nmole/rat doses of Spexin in comparison with the control group in the first phase of formalin test. Moreover, there was a significant reduction ($P < 0.05$) in pain sensitivity between the group receiving 30 nmole/rat doses of Spexin in comparison with the control group. According to the one-way variance analysis, there was a significant reduction ($P < 0.05$) in pain sensitivity at the first five minutes of 30 nmole/rat doses of Spexin in comparison with the groups of 10 nmol and the control group. There was not a significant reduction ($P < 0.05$) in pain sensitivity between the groups receiving receiving 10 and 30 nmole/rat doses of Spexin at the second five minutes.

At the third five minutes, there was not a significant reduction ($P < 0.05$) in pain sensitivity between the groups receiving 10 and of 30 nmole/rat doses of Spexin; however, there was a significant reduction ($P < 0.05$) in pain sensitivity at the third five minutes of 30 nmole/rat doses of Spexin in comparison with the control group. For the fourth five minutes, there was a significant reduction ($P < 0.05$) in pain sensitivity of 10 and 30 nmole/rat doses of Spexin in comparison with the control group. In addition, for the fifth five minutes, there was a significant reduction ($P < 0.05$) in pain sensitivity of 10 and 30 nmole/rat

doses of Spexin in comparison with the control group. For the sixth, seventh, eighth, ninth, and tenth five minutes, there was a significant reduction ($P < 0.05$) in pain sensitivity of 10 and 30 nmole/rat doses of Spexin in comparison with the control group.

For the eleventh and twelfth five minutes, there was not a significant reduction ($P < 0.05$) in pain sensitivity at 10 nmole/rat doses of Spexin in comparison with the control group; however, there was a significant reduction ($P < 0.05$) in pain sensitivity at 30 nmole/rat doses of Spexin in comparison with the control group the group receiving 10 doses.

DISCUSSION

The present data show that Spexin in two doses of 10 and 30 nmol per rat in the first phase of the formalin test causing significant decrease in pain sensitivity in the proestrus stage of the estrous cycle were compared with the control group. The second phase of the formalin test were significantly lower in proestrus stage of the estrous cycle pain sensitivity were observed between groups.

In this study, the formalin test was used to assess pain. Pain Test has two phases: The first phase of the formalin test pain from baseline to 10 minutes after the start of the test make the most direct effect on the materials and factors causing pain, skin pain receptors Analgesia for pain relief phase of the system and block the transmission of pain messages in the spinal dorsal horn region. The second phase of pain for 20 minutes to 60 minutes after starting the test, the test is, on the one hand due to the sensitive receptors in peripheral tissue inflammation and the flexibility of the central nervous system (e.g., changes and how synapses) in different parts of the CNS following pictures are the result of repeated pain signals. So when the pain increased in the first phase of the formalin test is usually the second phase of the test [5].

Toll et al [2] studied the first time in vivo biological activity of two putative peptide of the precursor peptide that one of them Spexin / NPQ (NWTPQAMLYLKGAQ-NH₂) and anti NPQ53-70 (FISDQSRRKDLSDRPLE) is revealing that caused IV and ICV injections of each of the different renal and cardiovascular effects have been observed in rats. Both of them after ICV injection of 10 nmol warm water tail withdrawal test the analgesic activity had a naloxone did not inhibit this activity, this response is not dependent on the Opioid system. Furthermore, ICV injection of either no change in the behavior of rats is not clear, and therefore they did not have any anxiety or locomotor activity, also, no significant difference between the experimental groups with other groups in cleaning, Rearing and Sniffing is not considered.

With regard to the effect on pain spexin there is only one published report, we present our results and compare with other neuropeptide. Do peptides based on their distribution in the brain as much as regulatory actions, such as learning and memory are feeling the pain. These peptides include releasing factor Kortikotrof, Orokortin, Norotnsyn, galanin, opioid peptides, and oxytocin and are Nzivtansin. The study Ksmtly *et al* [6] Analgesic effects of oxytocin in the raphe nuclei in the presence of specific opioid receptor antagonist, was studied and it was found that beta-funaltrexamine receptor antagonist(μ) can inhibit this effect, with the opiate receptors hair covering - delta and kappa are classified. Basically "Mu receptor or receptors that preferentially" Morphine connected. And spinal cords are responsible for the analgesic delta antagonist naltrindole and the kappa antagonist nor-binaltorphimine, but the response time of pain is not effective. The raphe nucleus oxytocin receptor-mediated analgesic affects your hair effects of the opioid system in the analgesic effect of oxytocin confirmed several reports have been presented. For example, some studies have shown that a naloxone-nociceptive effect of oxytocin, which is injected into the abdominal cavity, is inhibited. It was also observed naltrexone (another opioid receptor antagonist), the effects of oxytocin on tolerance to thermal stimuli is diminished.

However, because of the different stages of estrous cycle in female rats have different pain sensitivity, pain sensitivity during proestrus were investigated in this study. This stage is the last stage of the estrous cycle. It takes about 12 hours. Vaginal smear contains a large number of squamous cells are nucleated. On the morning of proestrus estradiol secretion from the dominant follicle reaches its maximum, which causes LH Surge in the early hours of the afternoon of proestrus and ovulation occurs. In the first phase of a wave and a wave of estradiol and progesterone many studies have been conducted on the role of steroid hormones on pain. Although certain hormonal changes that cause the pain are not completely understood but there is evidence that 3 α , 5 α -THP hormone increases the gestation period. Progesterone and testosterone cause increased sensitivity to pain. In the central nervous system, internal medicine and sex hormones on opioid system regulates the activity of nerve pain such as GABA, glutamate and other chemical mediators such as epinephrine are effective.

In a study Taherianfard *et al* [7] The central effects of α 1-adrenergic receptors on tonic pain during the estrous cycle in female rats have been determined that phenylephrine induced a significant decrease in sensitivity to pain. This decrease was particularly evident in Phase Pro Cycling findings correspond with

the results of existing research. Prazosin significantly increased sensitivity to pain and this increase is particularly pronounced in the estrus phase.

It is likely that fluctuations in pain sensitivity during estrous cycle and changes in sex hormone levels during the cycle are relevant [7]. Frye and colleagues reported that metabolites of progesterone have different effects on pain 3α , 5α -THP are so many metabolites, including increased sensitivity to pain, and some like 7α Progesterone Azmtabolit no effect on pain. The relationship between pain sensitivity is modified by various chemical mediators. The most important chemical mediators in the descending pathways of pain are opioid peptides, serotonin, noradrenaline, GABA and acetylcholine. The analgesic effect of the interaction between GABA and sex steroid hormones, there is little information. The study Taherianfard et al [7] demonstrated that muscimol at all stages of the estrous cycle was significantly ($P < 0.05$) reduction in pain intensity but analgesia during estrus and estrus than in Peru January of estrus and estrus was higher than the results obtained are consistent with the existing research results. Pikrotosin also have been observed in all stages of estrous cycle was significantly ($P < 0.05$) increase in pain, but this Hyperalgesia during estrus and proestrus and estrus stages were higher than in December Strauss. According to the evidence GABAergic system regulates the intensity of pain during estrous cycle. [7]. The results of this study stated that Aspksyn in two doses of 10 and 30 Nanomol per rat. In healthy rats caused a significant decrease in pain sensitivity compared with the control group 1; these results indicate that Aspksyn a significant impact in reducing sensitivity to pain.

CONCLUSION

According to the results of the study, spexin in hippocampal CA1 has strong modulatory effects on pain sensitivity to formalin test procedure.

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Citation of This Article

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