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Exploring the effects of TRPV1 channel activation in the locus coeruleus on morphine-induced analgesia in rats with diabetic neuropathy using the hot-plate test

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ABSTRACT

Introduction: Diabetic neuropathies (DN), a group of neuropathic disorders linked to diabetes mellitus, often manifest as peripheral neuropathy characterized by hyperalgesia and can affect all forms of diabetes. Morphine is known to inhibit the activity of locus coeruleus (LC) neurons, which play a crucial role in pain modulation. The TRPV1 receptor, associated with capsaicin, is expressed in several brain nuclei involved in pain perception, including the LC nucleus. This study aims to investigate the influence of TRPV1 in the LC on thermal hyperalgesia in both morphine-dependent and non-dependent rats suffering from DN.

Methods: This study involved male Wistar rats, with diabetic neuropathy (DN) induced by administering streptozotocin (STZ) at a dose of 60 mg/kg. Morphine sulfate, at a dosage of 3 mg/kg, was administered intraperitoneally once daily for three consecutive days. Subsequently, we explored the activation of TRPV1 channels using capsaicin, at a concentration of 10 nmol, in mediating thermal hyperalgesia in both normal and neuropathic rats.

Results: Our findings revealed that activation of TRPV1 receptors in the LC significantly restored the decreased hot plate threshold in diabetic non-dependent rats. Conversely, this activation had no impact on diabetic-morphine-dependent rats.

Conclusion: The findings of this study imply that targeting the capsaicinoid system may serve as an effective pharmacological strategy for treating patients with peripheral neuropathy.

Keywords:

Diabetic neuropathy; Thermal hyperalgesia; Capsaicin, Morphine, Locus coeruleus; Hot plate test.

INTRODUCTION

Diabetic neuropathy is a severe and debilitating complication of diabetes mellitus that afflicts millions of people globally. This condition is marked by nerve damage resulting from prolonged exposure to elevated blood glucose levels. Diabetic neuropathy can manifest in a variety of symptoms, including pain, numbness, muscle weakness, autonomic dysfunction, and foot ulcers, all of which can significantly impair the quality of life for those affected (1).

The locus coeruleus (LC) is a bilateral nucleus consisting of approximately 20,000 neurons, strategically located adjacent to the fourth ventricle near the ponto-mesencephalic junction. This nucleus features a relatively small population of large neurons, organized into a central "core" where the majority of cell bodies are concentrated. Surrounding this core is a prominent "crust" known as the peri-coeruleus, characterized by the interspersion of glial cells and GABAergic neurons among the LC dendrites (2-5).

The LC serves as the sole source of norepinephrine (NE) for the cortex and also releases it down the spinal cord to engage in various modulatory roles. Research has demonstrated that pain conditions are linked to alterations in LC neuron activity and the release of NE (6).

Capsaicin, a potent and volatile compound derived from peppers, is renowned for its intense spiciness and irritating properties. Recent research has uncovered a wide array of health benefits associated with capsaicin, including its

antioxidant and anti-inflammatory effects. Additionally, capsaicin has been shown to effectively reduce and alleviate pain (7, 8). One of the primary targets for capsaicin interaction is the transient receptor potential vanilloid 1 (TRPV1) receptor, which is mainly found in sensory neurons (9). TRP channels are non-selective cation channels that encompass a diverse array of channels (10). These channels are highly expressed on C fibers and some Aδ nociceptor nerves and play a crucial role in thermosensory and thermal pain sensitization (11). Upon injury, TRP nociceptors in the periphery become activated, sending action potentials along afferent sensory fibers to synapses in the dorsal horn. The signal is then relayed through the spinothalamic tract to the thalamus and the sensory cortex of the parietal lobe, localizing the pain (12). Activation of TRPV1 in the periaqueductal gray region triggers the release of glutamate, which in turn activates antinociceptive neurons in the rostral ventromedial medulla, modulating pain signal transmission and antinociceptive responses within the central nervous system (CNS) (13).

Morphine is universally acknowledged as one of the most powerful analgesics for managing post-operative and cancer-related pain. Despite its effectiveness, prolonged use of morphine is associated with a substantial risk of dependency and abuse (14).

This research aimed to explore the impact of activating both the opioid and capsaicinoid systems on thermal hyperalgesia in healthy and diabetic rats, both morphine-dependent and non-dependent.

MATERIALS AND METHODS

Experimental subjects and ethical standards

In this research, a total of forty-eight male Wistar rats, each weighing between 250 and 300 grams, were obtained from the Pasteur Institute of Iran, Tehran. These rats were housed in groups under meticulously controlled environmental settings. They were subjected to a consistent 12-hour light-dark cycle, with illumination from 7 AM to 7 PM, and an ambient temperature consistently maintained between 22 and 25°C. Throughout the experimental period, the rats had unrestricted access to standard rodent chow and sterile drinking water. All experimental procedures were conducted in strict accordance with the National Institutes of Health's Guide for the Care and Use of Laboratory Animals (NIH Publications No. 8023, revised 1978), ensuring the humane and ethical treatment of the animals involved.

Surgical technique and recovery period

The surgical procedure commenced by administering a deep anesthesia to the rats with an intraperitoneal injection of sodium pentobarbital at a dosage of 10 mg/kg. To minimize both pain and bleeding, a local anesthetic solution of 0.2 ml of lidocaine combined with epinephrine (Persocaine E) was applied at the surgical site. The animals were then securely positioned in a stereotaxic apparatus (Stoelting, USA) to ensure precise surgical placement.

A midline scalp incision was made, exposing the surface of the skull. The cranial landmarks, bregma and lambda, were identified and thoroughly cleaned. A stainless steel guide cannula was implanted to target the locus coeruleus (LC) region, using stereotaxic coordinates relative to bregma: antero-posterior = -10 mm, medio-lateral = +1.4 mm, and dorso-ventral = 7 mm, as per the rat brain atlas. The guide cannula was firmly secured with two stainless steel screws, and the incision was closed using dental cement.

Following the surgery, the animals were allowed a recovery period of one week before the commencement of the experiments.

Diabetes induction

Diabetes was induced in the experimental group through a single intraperitoneal injection of freshly prepared streptozotocin (STZ) at a dosage of 60 mg/kg. Three days following the STZ injection, blood samples were collected via tail prick to measure blood glucose levels using a digital glucometer (AVAN AGM01®, Iran). Rats with blood glucose levels exceeding 250 mg/dL were classified as diabetic, and the day hyperglycemia was confirmed marked the commencement of the study. The control group received an equivalent volume of citrate buffer as a vehicle. Throughout the study, the body weights and blood glucose levels of the rats were monitored at both the beginning and end of the experimental period.

Drugs and mode of application

In this study, three principal pharmacological

agents were employed:

Streptozotocin (STZ): This compound, sourced from Sigma–Aldrich Co. (USA), was utilized as a diabetogenic agent. It was meticulously prepared by dissolving it in a 0.1 M sodium citrate buffer with the pH adjusted to 4.5. The experimental group received a single intraperitoneal injection of this STZ solution at a dose of 60 mg/kg.

Capsaicin: Also obtained from Sigma–Aldrich Co. (USA), capsaicin was dissolved in 10% ethanol for administration purposes. For intracerebral microinjections, a uniform volume of 0.5 µl was used across all experimental groups, regardless of whether the injection contained capsaicin (10 nmol/0.5 µl) or the vehicle (10% ethanol). The injections were performed using a 1-µl Hamilton syringe connected to a stainless steel injector (30 gauge, 12 mm needle, 1 mm longer than the implanted guide cannulas) via a polyethylene tube (PE-20). Each injection was delivered over a span of 50 seconds, with the cannulas left in place for an additional 60 seconds to ensure effective delivery of the substances into the target brain region, the locus coeruleus (LC).

Morphine sulfate: Procured from Temad, Iran, morphine sulfate was dissolved in normal saline (0.9% NaCl). In the morphine-induced analgesia groups, the animals received a subcutaneous injection of morphine at a dosage of 9 mg/kg, administered 20 minutes prior to the hot plate test.

Thermal hyperalgesia (hot plate test)

Thermal hyperalgesia was evaluated by measuring the paw withdrawal latency (PWL) in response to noxious radiant heat applied to the hind paw, using a hot plate apparatus (Tahgos teb, Iran). The rats were individually placed in a transparent plexiglass cage, and a 30-minute acclimation period was allowed prior to testing to minimize stress and ensure consistent results.

To assess the PWL, the plantar surface of the hind paw was exposed to a focused radiant heat source, and the time taken for the animal to lift or lick its hind paw was recorded as the PWL. This measurement reflects the animal's threshold for detecting and responding to painful thermal stimuli. An automatic cut-off time of 30 seconds was implemented to prevent potential tissue damage.

The thermal stimulus was applied three times to each rat, with a 5-minute interval between each application to allow for recovery and to avoid sensitization or habituation. The average PWL values were calculated and reported, providing a robust measure of thermal hyperalgesia.

Experimental design

The animals were randomly allocated to either the normal or diabetic groups. Within each experimental group, they were further subdivided into two subgroups: one that received vehicle treatment and the other that received morphine to induce analgesia. This study aimed to explore the effect of intra-locus coeruleus (LC) capsaicin on thermal hyperalgesia. Five minutes prior to the hot plate test, the animals were microinjected with either capsaicin or ethanol. For the groups undergoing morphine-induced analgesia, a subcutaneous injection of morphine (9 mg/kg) was administered 20 minutes before the test.

Histology

Upon completing the behavioral test, the animals were humanely euthanized by administering deep anesthesia intraperitoneally with a mixture of Ketamine and Xylazine (150-10 mg/kg). Following euthanasia, each animal's brain was meticulously extracted and immersed in a 10% formalin solution for a minimum of four days to ensure proper fixation. The fixed brain tissues were then coronally sectioned into thin slices, each 50 micrometers thick. These sections were analyzed using the Paxinos and Watson rat brain atlas as a reference. The final statistical analysis included only data from animals with correctly positioned microinjection cannulae in the LC region.

Statistical analysis

All data were presented as the mean ± standard error of the mean (mean ± S.E.M.). To evaluate the differences between experimental groups, a one-way analysis of variance (ANOVA) was employed, followed by Tukey's post hoc test for pairwise comparisons. A p-value of less than 0.05 was considered statistically significant.

RESULTS

Capsaicin microinjection into the locus coeruleus modulates thermal hyperalgesia in diabetic non-morphine-dependent rats

Our findings revealed a decrease in the hot plate threshold in diabetic non-morphine-dependent animals. However, capsaicin microinjection into the locus coeruleus

effectively modulated this response in these animals (Fig. 1).

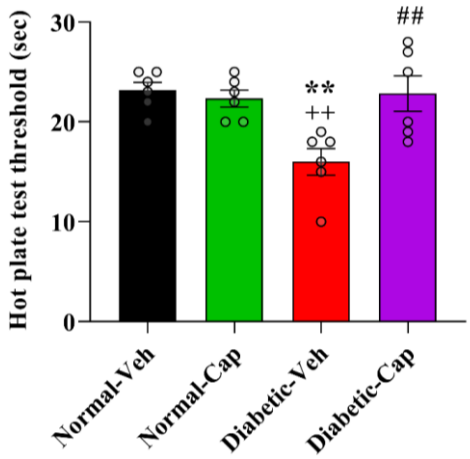

Figure 1. The bar graph illustrates the impact of the microinjection of capsaicin into the locus coeruleus (LC) nucleus on the hot plate threshold in healthy and diabetic non-morphine-dependent animals (n=6). Data represent mean ± SEM. **p < 0.01 compared with the normal-veh group, ++p < 0.01 compared with the normal-cap group, and ##p < 0.01 compared with the diabetic-veh group.

Capsaicin microinjection into the locus coeruleus does not effectively alleviate thermal hyperalgesia in diabetic-dependent-morphine rats

The statistical analysis revealed no notable difference in hot plate tolerance between the diabetic morphine-dependent rats treated with capsaicin and those administered the vehicle (Fig. 2).

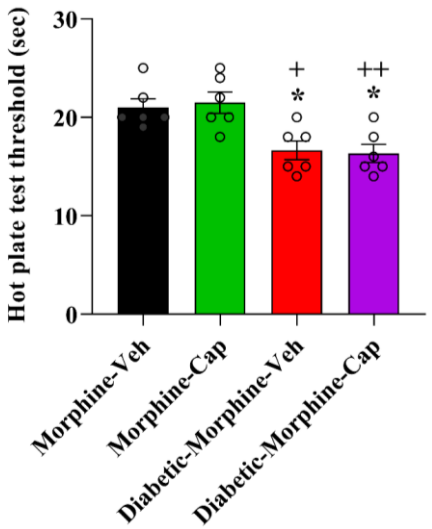

Figure 2. The bar graph illustrates the impact of the microinjection of capsaicin into the locus coeruleus (LC) nucleus on the hot plate threshold in healthy and diabetic morphine-dependent animals (n=6). Data represent mean ± SEM. *p < 0.05 compared with the morphine-veh group, +p < 0.05 compared with the morphine-cap group, and ++p < 0.01 compared with the morphine-cap group.

DISCUSSION

The data analysis conducted in this study has revealed several key findings:

1) Diabetic rats exhibited a reduction in thermal hyperalgesia when subjected to hot plate tests. 2) The administration of capsaicin into the LC did not affect thermal hyperalgesia in both healthy morphine-dependent and non-dependent groups. 3) Administration of capsaicin to non-morphine-treated diabetic rats restored thermal hyperalgesia when compared to the "Diabetic-Veh" group. 4) Conversely, capsaicin injection into the LC had no substantial effect on thermal hyperalgesia in diabetic rats that were morphine-dependent.

Among the myriad complications that stem from diabetes, the most prevalent is a collection of clinical syndromes resulting from the impairment of the peripheral and autonomic nervous systems. Collectively referred to as neuropathy, these syndromes are precipitated by both extensive and localized damage to the nervous system, and they impact nearly half of all individuals living with diabetes (15). Our findings demonstrated that diabetic rats exhibit a reduction in thermal hyperalgesia, which is consistent with the outcomes reported in previous studies (16).

Capsaicin, the pungent compound in hot chili peppers, engages with the transient receptor potential cation channel vanilloid subfamily member 1 (TRPV1). Through immunohistochemical and autoradiographic studies, TRPV1 channel expression has been detected in several brain regions, including the substantia nigra, ventral medulla, locus coeruleus, hypothalamus, ventral tegmental area, and periaqueductal gray (PAG). The extensive distribution of TRPV1 channels implies their involvement in the modulation of thermal responses, motor functions, anxiety, cardiovascular activity, and pain perception (17). Our results demonstrated that capsaicin microinjection into the LC increases the reaction time in diabetic non-morphine-dependent animals. It is hypothesized that capsaicin, through its interaction with peripheral TRPV1 channels, exhibits anti-nociceptive effects (18). However, there was no significant difference in reaction time between the diabetic, morphine-dependent rats treated with capsaicin and those administered the vehicle. This lack of effect may be attributed

to the activation of opioid receptors, which inhibits the sensitization of TRPV1 receptors mediated by cAMP-dependent PKA (19).

CONCLUSION

In conclusion, the findings of this study suggest that targeting the capsaicinoid system holds significant promise as a pharmacological strategy for treating patients afflicted with peripheral neuropathy.

DECLARATIONS

Authors have no conflict of interest to declare.

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