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The influence of capsaicin receptors in the locus coeruleus on morphine-induced analgesia in diabetic neuropathy: A comparison between morphine-dependent and non-dependent rats

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ABSTRACT

Introduction: Diabetic neuropathies (DN) are neuropathic disorders associated with diabetes mellitus. Peripheral neuropathy, characterized by hyperalgesia, can occur in all types of diabetes. Morphine inhibits the activity of locus coeruleus (LC) neurons, which are involved in pain modulation. The capsaicin receptor (TRPV1) is expressed in several brain nuclei involved in pain perception, including the LC nucleus. This study was conducted to examine the role of TRPV1 in the LC on morphine-induced analgesia in morphine-dependent and non-dependent rats affected by DN.

Methods: This study was conducted on male Wistar rats. Diabetic neuropathy (DN) was induced by a single dose of STZ. Morphine sulfate was injected intraperitoneally (3 mg/kg) once daily for 3 days. Finally, we investigated the role of TRPV1 receptors (10 nmol) in the locus coeruleus (LC) in morphine analgesia in both normal and neuropathic rats.

Results: Our results indicated that activating TRPV1 receptors in the locus coeruleus (LC) has no effect on morphine analgesia in normal, non-dependent rats. However, in morphine-dependent animals, it can potentiate morphine analgesia 45 minutes after the injection of a TRPV1 agonist. In diabetic neuropathy (DN) and non-dependent rats, TRPV1 activation increased morphine analgesia 30 minutes after injection but had no effect on dependent rats.

Conclusion: The results of this study suggest that activating the capsaicinoid system could be a useful approach in pharmacological therapy for patients with peripheral neuropathy.

Keywords: Diabetic neuropathy; Pain; Capsaicin, Morphine, Locus coeruleus; Tail-flick test.

INTRODUCTION

Diabetes mellitus, a persistent metabolic disorder, is rapidly becoming a worldwide issue with significant social, health, and economic impacts (1). Unmanaged diabetes can result in blindness, limb amputations, kidney failure, as well as vascular and heart diseases (2).

The locus coeruleus (LC) houses neurons that synthesize norepinephrine (NE), which project widely throughout the central nervous system (3). The noradrenergic system plays a crucial role in regulating the sensation of pain (4).

Capsaicin (trans-8-methyl-N-vanillyl-6-nonenamide, C18H27NO3) is a powerful and

volatile substance sourced from peppers, well-known for its intense spiciness and irritating characteristics. Recent research has highlighted a spectrum of capsaicin's health advantages, such as its antioxidant and anti-inflammatory properties. Moreover, capsaicin has been found to reduce blood pressure, support weight loss, alleviate pain, and even potentially prevent cancer (5, 6).

Morphine is widely regarded as the most potent pain reliever for managing post-operative and cancer-related pain. However, its prolonged use is linked with a significant risk of dependency and abuse (7).

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

MATERIALS AND METHODS

Experimental subjects and ethical standards

In this study, twenty-four male Wistar rats, each weighing between 250 and 300 g, were sourced from the Pasteur Institute of Iran, Tehran. These rats were housed in groups under carefully controlled environmental conditions. They experienced a consistent 12-hour light-dark cycle, with lights on from 7 AM to 7 PM, and an ambient temperature maintained between 22 and 25°C. Throughout the duration of the experiments, the rats had unrestricted access to standard rodent chow and sterile drinking water. All experimental procedures were carried out 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 animal subjects involved.

Surgical technique and recovery period

The surgical procedure commenced by deeply anesthetizing the rats with an intraperitoneal injection of sodium pentobarbital (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 administered at the surgical site. The animals were then secured in a stereotaxic apparatus (Stoelting, USA) for accurate surgical positioning.

A midline scalp incision was made, exposing the skull surface. The cranial landmarks, bregma and lambda, were identified and meticulously cleaned. A stainless steel guide cannula was implanted targeting 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, according to the rat brain atlas. The guide cannula was firmly anchored with two stainless steel screws, and the incision was closed with dental cement.

Post-surgery, the animals were given a one-week recovery period before starting the experiments.

Diabetes induction

Diabetes was induced in the experimental group by administering a single intraperitoneal injection of freshly prepared streptozotocin (STZ) at a dose of 60 mg/kg. Three days after 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 start of the study. The control group received a comparable 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 key pharmacological agents were utilized:

Streptozotocin (STZ): Sourced from Sigma–Aldrich Co. (USA), STZ was prepared as a diabetogenic agent by dissolving it in a 0.1 M sodium citrate buffer, with the pH precisely 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. For intracerebral microinjections, a uniform volume of 0.5 µl was used for all experimental groups, 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 50 seconds, and the cannulas were left in place for an additional 60 seconds to ensure effective delivery of the substances into the target brain region, the LC.

Morphine sulfate: Sourced from Temad, Iran, morphine sulfate was dissolved in normal saline (0.9% NaCl). In the morphine-induced analgesia groups, animals received a subcutaneous injection of morphine (9 mg/kg; s.c.) 20 minutes before the tail-flick test.

Pain assay (tail-flick test)

The nociceptive responses in animals were assessed utilizing the tail-flick test with a specialized apparatus (Poya Armaghan Apparatus, Iran). During the test, the animal's tail was positioned in the groove of the apparatus, and a thermal stimulus beam was applied at two specific points: 3 cm and 5 cm from the tail tip. The time interval between the application of the thermal stimulus and the withdrawal of the tail, known as the tail-flick latency (TFL), was meticulously recorded. TFL measurements were taken at several intervals over the 60-minute experimental period: 0, 15, 30, 45, and 60 minutes from the start of the experiment. To prevent potential tissue damage, a cut-off time of 10 seconds was imposed for the tail-flick response.

Experimental design

The animals were randomly assigned to either the control or diabetic groups. Within each experimental group, they were further divided into two subgroups: one receiving vehicle treatment and the other receiving morphine to induce analgesia. This study explored the impact of intra-LC capsaicin on pain tolerance duration. The tail-flick test was conducted once the animals were securely fixed in the restraining device. Five minutes before this 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 tail-flick test.

Histology

After completing the tail-flick test, the animals were humanely euthanized using deep anesthesia administered intraperitoneally with a mixture of Ketamine and Xylazine. Post-euthanasia, each animal's brain was carefully extracted and placed in a 10% formalin solution for at least 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. Only data from animals with correctly positioned microinjection cannulae in the LC region were included in the final statistical analysis.

Statistical analysis

All data are expressed as the mean ± standard error of the mean (mean ± S.E.M.). Differences between experimental groups were assessed using a one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for pairwise comparisons. A p-value of less than 0.05 was deemed statistically significant.

RESULTS

Capsaicin microinjection into the LC does not change pain tolerance in healthy non-morphine-dependent rats

The statistical analysis indicated that there was no significant variation in tail-flick latency between the healthy non-morphine-dependent group administered capsaicin and the healthy non-morphine-dependent group given vehicle treatment (Fig. 1).

Capsaicin microinjection into the LC increases pain tolerance in healthy morphine-dependent rats

The statistical analysis demonstrated a notable difference in tail-flick latency among the experimental groups of healthy morphine-dependent rats. Specifically, 45 minutes after the initiation of the test, the healthy morphine-dependent group treated with capsaicin showed a significantly prolonged tail-flick latency compared to the healthy morphine-dependent group treated with the vehicle ($p < 0.01$) (Fig. 2).

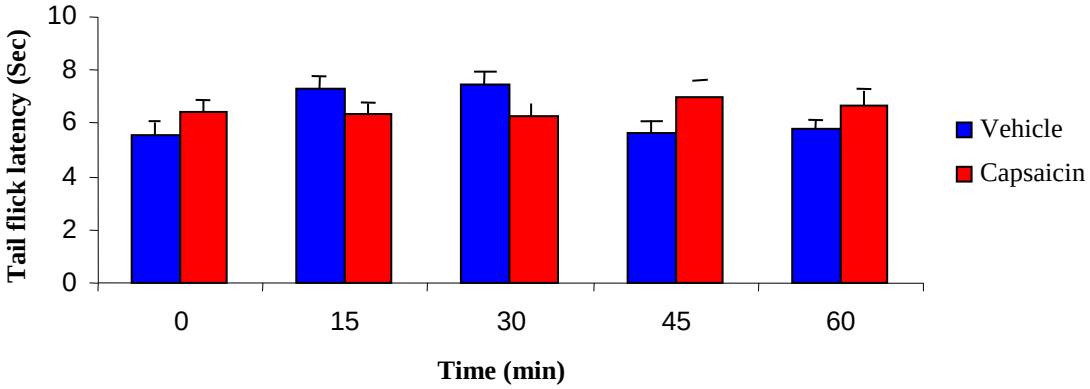

Figure 1. The bar graph illustrates the impact of microinjection of capsaicin into the locus coeruleus (LC) nucleus on tail flick latency in healthy non-morphine-dependent animals (n=6). Data represent mean ± SEM.

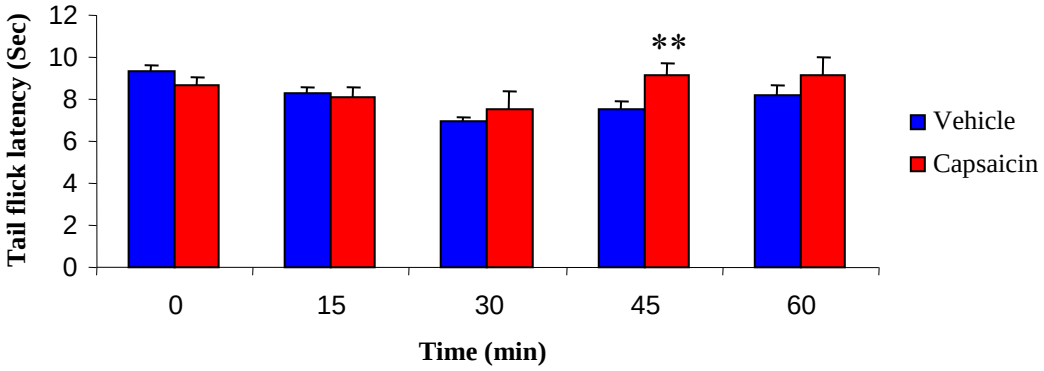

Figure 2. The bar graph illustrates the effect of directly microinjecting capsaicin into the locus coeruleus (LC) on tail flick latency in healthy morphine-dependent rats (n=6). Data are presented as mean ± SEM. **p < 0.01 compared with the healthy morphine-dependent + vehicle group.

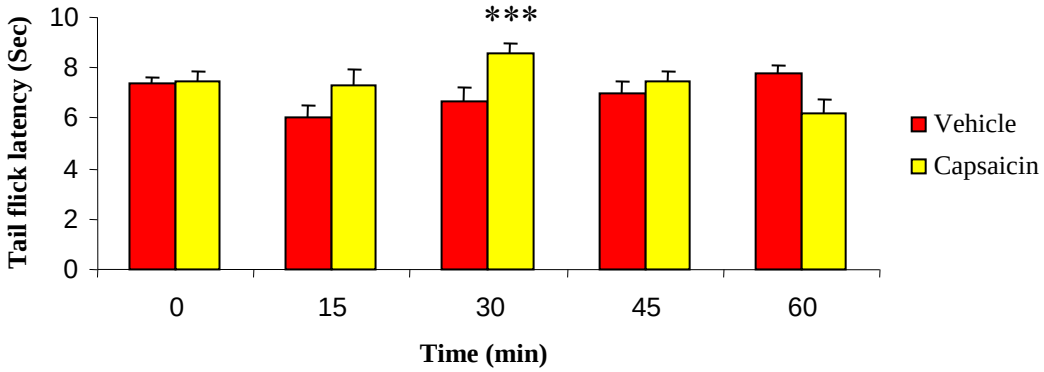

Figure 3. The bar graph illustrates the effect of directly microinjecting capsaicin into the locus coeruleus (LC) on tail flick latency in diabetic rats without morphine dependency (n=6). Data are presented as mean ± SEM. ***p < 0.001 compared with the diabetic non-morphine-dependent + vehicle group.

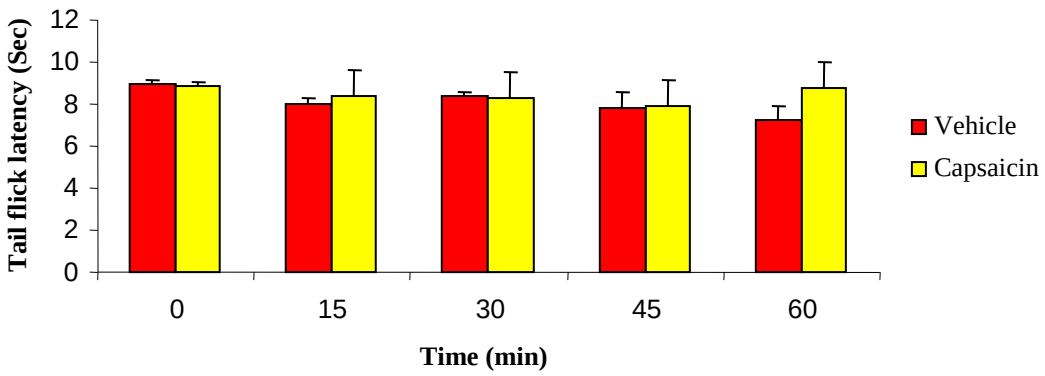

Figure 4. The bar graph illustrates the effect of directly microinjecting capsaicin into the locus coeruleus (LC) on tail flick latency in diabetic morphine-dependent rats (n=6). Data are presented as mean ± SEM.

Capsaicin microinjection into the LC enhances pain tolerance in diabetic rats without morphine dependency

Statistical analysis revealed a significant difference in tail-flick latency among the experimental groups of diabetic rats without morphine dependency. At the 30-minute mark, after the test began, the diabetic rats treated with capsaicin showed a significantly longer tail-flick latency compared to the diabetic group given the vehicle ($p < 0.001$) (Fig. 3).

Capsaicin microinjection into the LC fails to alter pain tolerance in diabetic morphine-dependent rats

The statistical analysis revealed no significant difference in tail-flick latency between the diabetic morphine-dependent rats treated with capsaicin and those given the vehicle (Fig. 4).

DISCUSSION

The data analysis in this study yielded the following results:

1) Capsaicin microinjection into the LC does not impact pain tolerance in healthy rats without morphine dependence. 2) Capsaicin microinjection into the LC significantly increases pain tolerance in healthy morphine-dependent rats, with a marked effect 45 minutes after the commencement of the tail-flick test. 3) Capsaicin microinjection into the LC markedly enhances pain tolerance in diabetic rats without morphine dependence, noticeable 30 minutes after the test begins. 4) Capsaicin microinjection into the LC does not significantly alter pain tolerance in diabetic rats that are morphine-dependent.

Of all the complications arising from diabetes, the most common are a set of clinical syndromes resulting from damage to the peripheral and autonomic nervous systems. Commonly known as neuropathy, these syndromes are triggered by widespread and localized nervous system damage and affect up to half of all people with diabetes (8). While not every neuropathy patient experiences motor or sensory symptoms, the neuropathic pain associated with symptomatic cases often proves to be troublesome, significantly restricting physical activity, quality of life, and work productivity (9). Capsaicin, the spicy component of hot chili peppers, interacts with the transient receptor potential cation channel vanilloid subfamily member 1 (TRPV1). Immunohistochemical and autoradiographic studies have identified TRPV1 channel expression in various brain regions, including the substantia nigra, ventral medulla, locus coeruleus, hypothalamus, ventral tegmental area, and periaqueductal gray (PAG). This widespread presence suggests that TRPV1 channels play roles in regulating thermal responses, motor functions, anxiety, cardiovascular activity, and pain (10). While capsaicin acts as a pro-nociceptive agent via peripheral TRPV1 channels, it exhibits anti-nociceptive properties when administered through microinjection into midbrain regions involved in supraspinal pain

modulation (11). Marinelli et al. demonstrated that capsaicin, through VR1 receptors, stimulates the spontaneous release of glutamate and adrenaline/noradrenaline in the LC in vitro (12). Large DRG neurons from diabetic rats (6–8 weeks) exhibit heightened oxidative stress and activation of cell injury markers compared to healthy controls. Capsaicin treatment results in reduced MitoTracker Red labeling, increased cytosolic cytochrome c, and activation of caspase 3 in large neurons isolated from diabetic rats (13). The activation of opioid receptors inhibits the sensitization of TRPV1 receptors mediated by cAMP-dependent PKA (14).

CONCLUSION

In conclusion, the findings of this study indicate that targeting the capsaicinoid system may offer a promising strategy for the pharmacological treatment of patients suffering from peripheral neuropathy.

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DECLARATIONS

Authors have no conflict of interest to declare.

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