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Activation of capsaicin receptors in the periaqueductal gray does not alter pain tolerance in a diabetic neuropathy model

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ABSTRACT

Introduction: To date, a multitude of neural circuits within the central nervous system have been recognized for their roles in pain modulation. Notably, the ventrolateral periaqueductal gray (vlPAG) region of the midbrain emerges as a pivotal element within the supraspinal pain modulation network. This region's significance has been thoroughly documented across diverse animal pain models. In our study, we concentrated on exploring the functions of capsaicin receptors in this specific area, particularly their involvement in mediating antinociceptive effects.

Methods: In this study, male Wistar rats were utilized to examine the antinociceptive effects of capsaicin when directly administered into the ventrolateral periaqueductal gray (vlPAG) region of the midbrain. The efficacy of this intervention was evaluated using the tail-flick test, conducted five minutes after injection. The research compared the outcomes of intra-vlPAG capsaicin administration between healthy control rats and those with diabetes.

Results: In the control groups, capsaicin induced a swift and temporary analgesic effect, but it did not produce antinociceptive effects in diabetic rats.

Conclusion: Acute microinjections of capsaicin failed to elicit significant antinociceptive effects in the diabetic animal group. To derive more precise conclusions, it is advisable to also examine the long-term impacts of these compounds.

Keywords: Pain, Tail-flick test, Capsaicin receptor, Ventrolateral periaqueductal gray, Diabetic neuropathy.

INTRODUCTION

The International Diabetes Federation reports that diabetes affects 425 million people globally (1), making it the most widespread epidemic of the 21st century (2). Among the numerous complications associated with diabetes, neuropathies—clinical syndromes resulting from damage to the peripheral and autonomic nervous systems—are the most common (3). These neuropathies, which can affect up to half of all individuals with diabetes, arise from both diffuse and focal nerve damage. This condition manifests as chronic or persistent neuropathic pain, characterized by altered pain perception, heightened responses to painful stimuli (hyperalgesia), and abnormal sensitivity to normally non-painful stimuli (allodynia) (4).

Capsaicin (trans-8-methyl-N-vanillyl-6-nonenamide, C18H27NO3) is a potent and volatile compound derived from peppers, renowned for its intense heat and irritant

properties. Recent studies have illuminated a range of capsaicin's health benefits, including its antioxidant and anti-inflammatory effects. Additionally, capsaicin has been shown to lower blood pressure, aid in weight loss, relieve pain, and even possess potential cancer-preventive properties (5-7). Capsaicin binds to the transient receptor potential vanilloid 1 (TRPV1) receptor, formerly known as the vanilloid receptor, which is predominantly found in sensory neurons (8).

This research aims to investigate the potential of capsaicin in reducing neuropathic pain associated with diabetes. By employing a rodent model of streptozotocin-induced diabetes, we examined the effects of direct capsaicin administration on pain sensitivity in this condition, which is characterized by peripheral nerve dysfunction.

MATERIALS AND METHODS

Experimental subjects and ethical standards

The research team meticulously selected 24 male Wistar rats, each weighing between 250 to 300 grams, from the Pasteur Institute of Iran, located in Tehran. These animals were housed in groups within a rigorously controlled environment throughout the experimental period. To ensure their well-being, the rats were subjected to a precisely regulated 12-hour light-dark cycle, with lights automatically turning on at 7:00 AM and off at 7:00 PM, closely mimicking natural daylight patterns. The ambient temperature was consistently maintained between 22 to 25 degrees Celsius, creating a stable and comfortable thermal environment for the subjects.

The rats were provided with unrestricted access to a standard rodent chow diet and clean, sterile drinking water during the study. This setup was designed to minimize any potential stress and maintain the animals' health.

All experimental procedures were conducted in strict compliance with the guidelines outlined in the National Institutes of Health's Guide for the Care and Use of Laboratory Animals (NIH Publications No. 8023, revised 1978).

Surgical technique and recovery period

The surgical procedure commenced with the induction of a profound state of anesthesia in the Wistar rats. This was accomplished via an intraperitoneal injection of sodium pentobarbital, a powerful anesthetic, administered at a dosage of 10 mg/kg of the rat's body weight. To further mitigate any potential pain and minimize bleeding during the surgery, a local anesthetic solution was also applied. This solution comprised lidocaine, a numbing agent, and epinephrine, a vasoconstrictor, in a volume of 0.2 milliliters. The researchers meticulously injected this anesthetic mixture around the designated surgical site on the rat's skull.

Once the rats were fully anesthetized and the surgical site numbed, they were positioned in a specialized stereotaxic apparatus from Stoelting, USA, which ensured precise and controlled placement of the surgical instruments. A linear incision was then made along the midline of the scalp to expose the skull surface. The researchers identified and cleaned two cranial landmarks, the bregma and lambda, which served as anatomical reference points. Using these landmarks, a stainless steel guide cannula was implanted, targeting the ventrolateral periaqueductal gray (vlPAG) area of the midbrain. The stereotaxic coordinates relative to the bregma were: anterior-posterior = -7.8 mm, medial-lateral = +0.8 mm, and dorsal-ventral = 6.4 mm, according to the standardized rat brain atlas (9).

To secure the guide cannula, two additional stainless steel screws were used, and dental cement was applied over the entire assembly. After the surgery, the rats were given a one-week recovery period before the scheduled experiments began.

Diabetes induction

To induce diabetes in the experimental group of Wistar rats, the researchers administered a single intraperitoneal injection of streptozotocin (STZ) at a dose of 60 mg/kg of body weight. This freshly prepared pharmacological agent was injected into the abdominal cavity. Three days post-injection, blood samples were collected from the tail veins of the rats and analyzed using the AVAN AGM01® digital glucometer from Iran to measure blood glucose levels. Rats with blood glucose levels above 250 mg/dl were classified as diabetic, marking the start of the study.

The control group received an equivalent volume of citrate buffer solution, the vehicle for

STZ. Throughout the study, the researchers monitored and recorded the body weights and blood glucose levels of all rats at both the beginning and end of the experimental period, allowing them to track the progression of diabetes in the experimental group compared to the control group.

Drugs and mode of application

In this study, two primary pharmacological agents were employed:

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

Capsaicin: Also obtained from Sigma–Aldrich Co. (USA), capsaicin was dissolved in 10% ethanol for preparation. For intracerebral microinjections, a uniform volume of 0.5 µl was used across all experimental groups, regardless of whether the injection contained the drug (capsaicin; 10 nmol/0.5 µl) or the vehicle (10% ethanol). These microinjections were administered 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). The injections were delivered precisely over 50 seconds, with the injection cannulas left in place for an additional 60 seconds to ensure optimal delivery of the substances into the target brain region, the vlPAG.

Pain assay (tail-flick test)

The animals' nociceptive responses were evaluated using the tail-flick test, conducted with a specialized device (Poya Armaghan Apparatus, Iran). In this 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 taken for the animal to withdraw its tail after the thermal stimulus, known as the tail-flick latency (TFL), was carefully recorded. TFL measurements were taken at several intervals during the 60-minute experimental period: at 5, 15, 30, 45, and 60 minutes from the experiment's onset. To avoid potential tissue damage, a maximum response time of 10 seconds was set for the tail-flick reaction.

Experimental design

The animals were randomly assigned to either the control group or the diabetic group. Each of these groups was further divided into two subgroups: one receiving vehicle treatment and the other receiving capsaicin treatment. This study aimed to explore the impact of intra-vlPAG administration of capsaicin on pain tolerance duration. The tail-flick test was conducted after securely restraining the animals. Five minutes prior to the tail-flick test, the animals were microinjected with either capsaicin or ethanol.

Histology

Upon completion of the tail-flick test, the animals were humanely euthanized. This was achieved by administering a deep anesthesia mixture of Ketamine and Xylazine intraperitoneally. Post-euthanasia, each animal's brain was meticulously extracted and placed in a 10% formalin solution. The brain samples were stored in this solution for a minimum of four days to ensure adequate fixation. Subsequently, the fixed brain tissues were coronally sectioned into thin slices, each 50 micrometers thick. These brain sections were then analyzed with reference to the Paxinos and Watson rat brain atlas. Only data from animals with accurately positioned microinjection cannulae within the vlPAG region were included in the final statistical analysis.

Statistical analysis

All data are presented as the mean ± standard error of the mean (mean ± S.E.M.). To evaluate differences between the 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 vlPAG increases pain tolerance in healthy rats

The statistical analysis demonstrated a significant difference in tail-flick latency among the experimental groups in healthy rats. At the 5-minute mark post-test initiation, the control group

treated with capsaicin showed a significantly longer tail-flick latency compared to the control group treated with the vehicle ($p < 0.001$). This significant difference in tail-flick latency between the capsaicin and vehicle groups was also observed at the 15-minute mark ($p < 0.001$). However, no significant differences were noted between the two groups at the 30, 45, and 60-minute time points (Fig. 1).

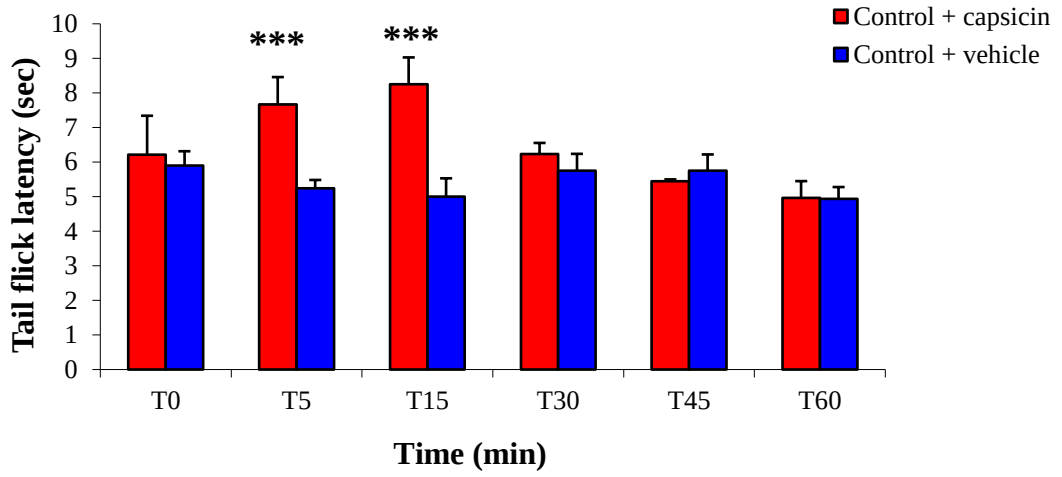

Fig. 1. The bar graph illustrates the impact of microinjection of capsaicin into the ventrolateral periaqueductal gray (vlPAG) region on tail flick latency in healthy animals (n=6). Data represent mean ± SEM. ***p < 0.001

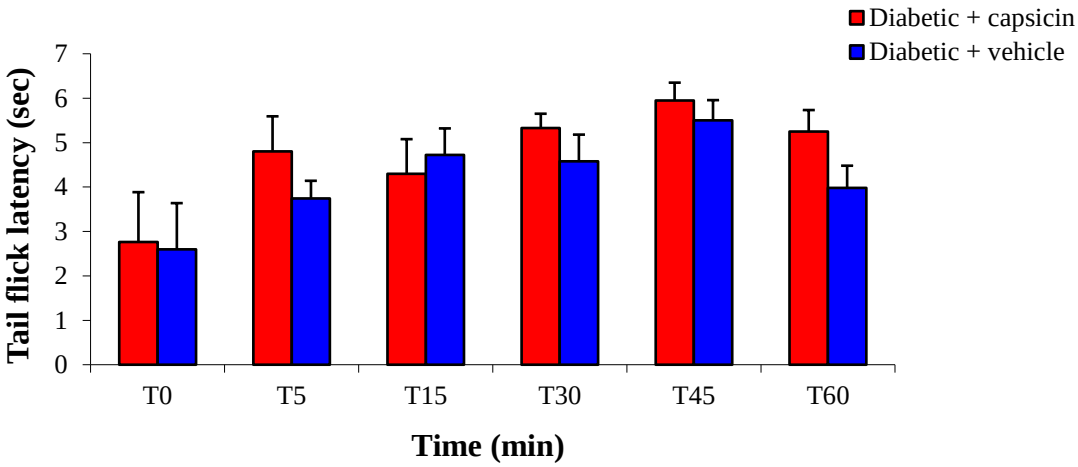

Figure 2. The bar graph illustrates the effect of directly microinjecting capsaicin into the ventrolateral periaqueductal gray (vlPAG) region on tail flick latency in diabetic animal groups (n=6). Data are presented as mean ± SEM.

Capsaicin microinjection into the vlPAG does not change pain tolerance in neuropathic rats

Statistical analysis revealed no significant difference in tail-flick latency between the diabetic group treated with capsaicin and the diabetic control group receiving vehicle treatment (Fig. 2).

DISCUSSION

In this study, we demonstrated that microinjections of capsaicin into the PAG significantly reduced nociceptive behaviors in the tail-flick test in healthy animals. However, this effect was absent in the diabetic neuropathy model, and the analgesic effects in healthy animals were not sustained over a longer duration. These results differ from earlier studies that indicated capsaicin was beneficial as a supplementary pharmacological treatment for managing pain. For instance, Bodnar et al. discovered that intraventricular administration of capsaicin diminished the analgesic effects of both morphine and cold-water swim (10, 11). Additionally, capsaicin injections into the dPAG increased paw withdrawal latencies in response to noxious thermal stimuli (12). Another study revealed that capsaicin initially heightened pain sensitivity in animals, but after a few hours, it induced a phase where the animals no longer responded to the painful stimulus (13). Also previous research has shown that capsaicin treatment is effective for a range of painful conditions, such as complex regional pain syndromes, neuropathic pain, postsurgical neuropathic pain, post-herpetic neuralgia, and painful diabetic peripheral neuropathy (14-21). Nevertheless, our findings reveal that capsaicin microinjections fail to produce analgesic effects in diabetic animals. This ineffectiveness might be attributed to the particular dosage or the administration frequency applied in our experiments.

CONCLUSION

Since our study did not demonstrate notable anti-nociceptive effects on tail-flick latency through capsaicin receptor activation in diabetic rats, it is advisable to administer higher doses of these compounds over an extended period for more precise results. Additionally, further research into the underlying molecular mechanisms is recommended.

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DECLARATIONS

Authors have no conflict of interest to declare.

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