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Intraplantar Injection of Monosodium Iodoacetate Produces Hyperalgesia in Rats and the Mechanism Underlying the Effect

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Abstract

Objective: To observe the effect of intraplantar injection of monosodium iodoacetate (MIA) on pain perception in rats and to investigate the role of transient receptor potential vanilloid type 1 (TRPV1) in the effect. **Methods:** Adult male Wistar rats were used in the experiment. 1) MIA was injected subcutaneously into the right hindpaw of rats, the low, medium, and high doses of MIA were 0.11, 0.33, and 1 mg, respectively, then the changes of paw withdrawal thermal latency, paw withdrawal mechanical threshold, and dynamic weight bearing within 4 hours after MIA injection were measured. 2) Capsazepine (TRPV1 antagonist, 30 μg) was injected subcutaneously into the right hindpaw of rats at 2 hours after intraplantar injection of MIA (1 mg), then the changes of paw withdrawal thermal latency, paw withdrawal mechanical threshold, and dynamic weight bearing within 1 hour after capsazepine injection were measured. **Results:** 1) The paw withdrawal thermal latency, paw withdrawal mechanical threshold, and dynamic weight bearing decreased after intraplantar injection of MIA in rats and the effect lasted for at least 4 hours. 2) The MIA-induced reduction in paw withdrawal thermal latency, paw withdrawal mechanical threshold, and dynamic weight bearing were significantly alleviated after intraplantar injection of capsazepine in rats. **Conclusion**: Intraplantar injection of MIA can produce thermal pain, mechanical pain, and spontaneous pain for more than 4 hours, which may be due to the TRPV1 activation caused by MIA.

Keywords

TRPV1, Monosodium Iodoacetate, Capsazepine, Hyperalgesia

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1. Introduction

Our previous study found that intraplantar injection of monosodium iodoacetate (MIA) produced 4 - 6 days of thermal pain, mechanical pain, and spontaneous pain, thus establishing a novel animal model of chronic inflammatory pain. The study showed that the hyperalgesia of this model was at least partially mediated by TRPV1 [1]. We began measuring the pain perception one day after MIA injection in the previous study, we are wondering whether MIA has a pain-inducing effect during the subacute stage (within a few hours after the MIA injection). If yes, what is the mechanism underlying the hyperalgesia?

TRPV1 is one of the transient receptor potential ion channels [2] and is distributed in primary sensory neurons [3]. Studies have shown that TRPV1 can be activated by heat (>42˚C), protons, inflammatory factors, and bacterial toxins [4] [5]. Thus, TRPV1 is associated with thermal and nociceptive sensations. TRPV1 plays an important role in the transmission of pain [2] [6]. We hypothesized that TRPV1 might be involved in the subacute hyperalgesia induced by MIA injection in rat paws. In the present study, we injected capsazepine (CPZ), an antagonist of TRPV1, into rat paws to investigate the role of TRPV1 in the subacute hyperalgesia induced by MIA injection.

2. Materials and Methods

2.1. Animals

In this study, adult male Wistar rats weighing 240 - 300 g were purchased from Tianqin Biotechnology Co., Ltd., Changsha, Hunan, China. The feeding environment was kept at 21˚C - 25˚C, and a 12 hr light/12 hr dark cycle was maintained. The rats ate and drank freely. The animal experiments adopted in this research were in accordance with the relevant regulations of experimental animal ethics of Youjiang Medical University for Nationalities.

2.2. Reagents

Monosodium iodoacetate was purchased from Shanghai Lianshuo Biotechnology Co., Ltd. (Cat. No. 64-69-7) and was dissolved in 0.9% NaCl. Capsazepine was purchased from MedChemExpress (Cat. No.HY-15640) and was dissolved in 0.9% NaCl with 0.5% ethanol.

2.3. Animal grouping and drug injection method

The rats used for MIA phenomenon experiment were divided into 4 groups: low dose MIA group (0.11 mg), medium dose MIA group (0.33 mg), high dose MIA group (1 mg), and control group (saline 100 μl), with 8 rats in each group. The injection method was that MIA was injected subcutaneously into the sole of right hindpaw, and the injection volume was 100 μl. The paw withdrawal thermal latency, paw withdrawal mechanical threshold, and dynamic weight bearing were measured every hour after MIA injection.

The rats used for MIA mechanism experiment were divided into 2 groups: control group and capsazepine group, with 8 rats in each group. The dose of capsazepine, a TRPV1 antagonist, was 30 μg in 100 μl, the choice of which was based on our prior study [1]. The injection method was that at first MIA was injected subcutaneously into the sole of right hindpaw of rats, 2 hours later, capsazepine was injected subcutaneously into the sole of the same hindpaw. The paw withdrawal thermal latency, paw withdrawal mechanical threshold, and dynamic weight bearing were measured every 10 minutes after capsazepine injection.

2.4. Measurement of Paw Withdrawal Thermal Latency

The instrument used to measure paw withdrawal thermal latency of rats was Plantar Test (Hargreaves's Apparatus) (Ugo Basile, Cat. No. 37370). Five days of acclimatization training was needed for rats before experiments. When doing experiments, the rat was put into a transparent box with a perforated cover, after switching on, a radiant heat source was applied to the plantar surface of the rat through a glass plate, meanwhile, the instrument was timed. After heat irradiation for a period of time, the rat showed an evasive behavior by withdrawing its paw when it could not bear the heat. At the time when the rat withdrew its paw, the instrument automatically stopped timing and displayed the time from heat irradiation to paw withdrawal, which was paw withdrawal thermal latency (thermal pain latency).

2.5. Measurement of Paw Withdrawal Mechanical Threshold

The instrument used to measure paw withdrawal mechanical threshold of rats was Dynamic Plantar Aesthesiometer (Ugo Basile, Cat. No. 37450). Five days of acclimatization training was needed for rats before experiments. When doing experiments, the rat was put into a transparent box with an empty bottom on a wire mesh plate, after switching on, a needle of the instrument extended upward and acted on the plantar surface of the rat's paw with increasing force. A period of time later, the rat showed an evasive behavior by withdrawing its paw when it could not bear the needle's force. At the time when the rat withdrew its paw, the protruded needle sprang down and the instrument screen automatically displayed the greatest force applied, which was paw withdrawal mechanical threshold (mechanical pain threshold).

2.6. Measurement of Dynamic Weight Bearing

The instrument used to measure dynamic weight bearing of rats was Bipedal Balance Tester (Techman, Cat. No. PH-200). Dynamic weight bearing was used to reflect spontaneous pain of rats. Five days of acclimatization training was needed for rats before experiments. When doing experiments, the rat was put into a black box and the left and right hindpaws stood on left and right weight sensors, respectively, the instrument automatically displayed the value of the weight bearing of each handpaw. The percentage of the weight bearing of inflammatory hindpaw to total weight bearing of both hindpaws was regarded as dynamic weight bearing.

2.7. Data Analysis

Data were analyzed using software SPSS and were presented as mean ± standard error. Student *t*-test was used to analyze the differences between the two groups. $P < 0.05$ was considered significantly different.

3. Results

3.1. Effect of Intraplantar Injection of Monosodium Iodoacetate on the Paw Withdrawal Thermal Latency in Rats

The purpose of this experiment was to observe the changes in paw withdrawal thermal latency (thermal pain latency) after intraplantar injection of monosodium iodoacetate (MIA) in rats (**Figure 1**). The thermal pain latency reflects the degree of thermal pain. The lower the thermal pain latency, the stronger the thermal pain. MIA was injected intraplantarly into the right hindpaw of rats. The injection doses of MIA were as follows: low dose MIA group (MIA, 0.11 mg), medium dose MIA group (MIA, 0.33 mg), and high dose MIA group (MIA, 1 mg), the volume of injection solution was 100 µl, and the thermal pain latency was measured every hour after MIA injection. Compared with the control group (intraplantar injection of 100 µl saline), the thermal pain latency of the low dose MIA group did not change; the thermal pain latency of medium dose MIA group decreased 1 hr after MIA injection and the decreasing effect lasted for at least 4 hr ($P < 0.05$); the thermal pain latency of high dose MIA group decreased more significantly 1 hr after MIA injection and the effect lasted for at least 4 hr ($P < 0.05$). These results indicated that intraplantar injection of MIA could produce more than 4 hours of thermal pain, and the effect was dose-dependent.

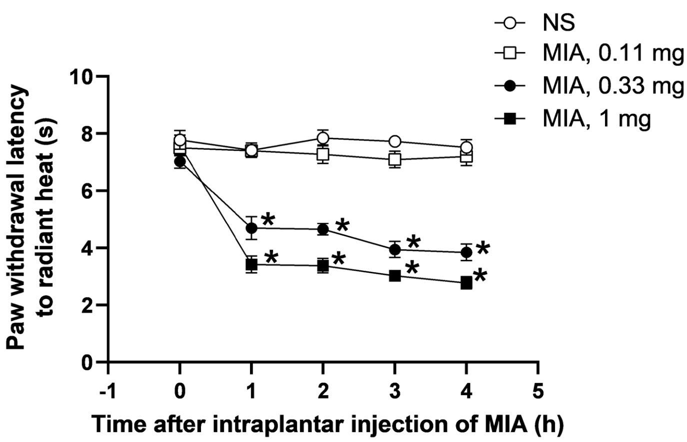

Figure 1. Effect of intraplantar injection of monosodium iodoacetate (MIA) on thermal pain latency in rats. The horizontal coordinate represented the time after intraplantar injection of MIA, and the vertical coordinate represented the paw withdrawal thermal latency (thermal pain latency). The doses of MIA were 0.11, 0.33, and 1 mg, respectively, and the injection volume was 100 µl. Data were expressed as mean ± standard error. The number of animals in each group was 8. The statistical difference at time points between groups was evaluated by Student's *t*-test. * meant $P < 0.05$ compared with the control group (NS group).

3.2. Effect of Intraplantar Injection of Monosodium Iodoacetate on the Paw Withdrawal Mechanical Threshold in Rats

The purpose of this experiment was to observe the changes in paw withdrawal mechanical threshold (mechanical pain threshold) after intraplantar injection of monosodium iodoacetate (MIA) in rats (**Figure 2**). The mechanical pain threshold reflects the degree of mechanical pain. The lower the mechanical pain threshold, the stronger the mechanical pain. MIA was injected intraplantarly into the right hindpaw of rats. The injection doses of MIA were as follows: low dose MIA group (MIA, 0.11 mg), medium dose MIA group (MIA, 0.33 mg), and high dose MIA group (MIA, 1 mg), the volume of injection solution was 100 μl, and the mechanical pain threshold was measured every hour after MIA injection. Compared with the control group (intraplantar injection of 100 μl saline), the mechanical pain threshold of the low dose MIA group did not change; the mechanical pain threshold of medium dose MIA group decreased 1 hr after MIA injection and the decreasing effect lasted for at least 4 hr ($P < 0.05$); the mechanical pain threshold of high dose MIA group decreased more significantly 1 hr after MIA injection and the effect lasted for at least 4 hr ($P < 0.05$). These results indicated that intraplantar injection of MIA could produce more than 4 hours of mechanical pain, and the effect was dose-dependent.

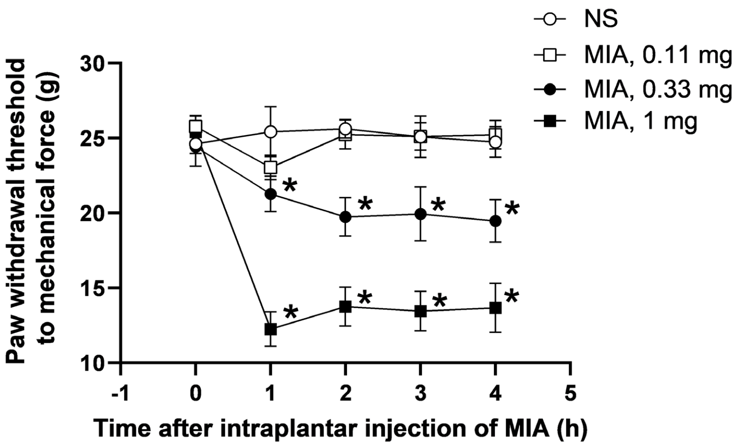

Figure 2. Effect of intraplantar injection of monosodium iodoacetate (MIA) on mechanical pain threshold in rats. The horizontal coordinate represented the time after intraplantar injection of MIA, and the vertical coordinate represented the paw withdrawal mechanical threshold (mechanical pain threshold). The doses of MIA were 0.11, 0.33, and 1 mg, respectively, and the injection volume was 100 μl. Data were expressed as mean ± standard error. The number of animals in each group was 8. The statistical difference at time points between groups was evaluated by Student's *t*-test. * meant $P < 0.05$ compared with the control group (NS group).

3.3. Effect of Intraplantar Injection of Monosodium Iodoacetate on the Dynamic Weight Bearing in Rats

The purpose of this experiment was to observe the changes in dynamic weight bearing after intraplantar injection of monosodium iodoacetate (MIA) in rats (**Figure 3**). The dynamic weight bearing reflects the degree of spontaneous pain.

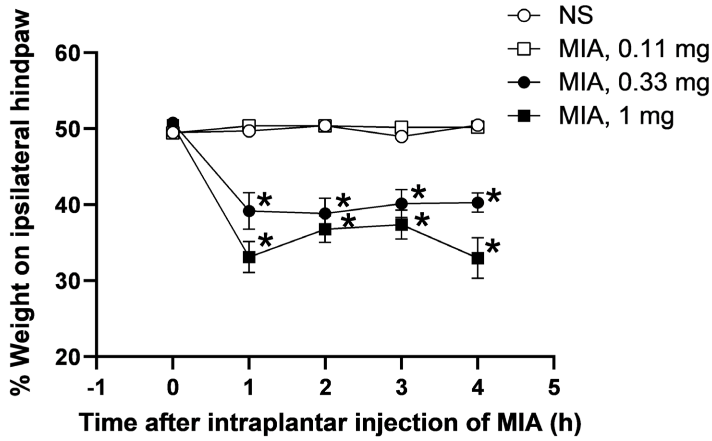

Figure 3. Effect of intraplantar injection of monosodium iodoacetate (MIA) on dynamic weight bearing in rats. The horizontal coordinate represented the time after intraplantar injection of MIA, and the vertical coordinate represented the dynamic weight bearing. The doses of MIA were 0.11, 0.33, and 1 mg, respectively, and the injection volume was 100 μl. Data were expressed as mean ± standard error. The number of animals in each group was 8. The statistical difference at time points between groups was evaluated by Student's *t*-test. * meant $P < 0.05$ compared with the control group (NS group).

The lower the dynamic weight bearing, the stronger the spontaneous pain. MIA was injected intraplantarly into the right hindpaw of rats. The injection doses of MIA were as follows: low dose MIA group (MIA, 0.11 mg), medium dose MIA group (MIA, 0.33 mg), and high dose MIA group (MIA, 1 mg), the volume of injection solution was 100 μl, and the dynamic weight bearing was measured every hour after MIA injection. Compared with the control group (intraplantar injection of 100 μl saline), the dynamic weight bearing of the low dose MIA group did not change; the dynamic weight bearing of medium dose MIA group was decreased 1 hr after MIA injection and the decreasing effect lasted for at least 4 hr ($P < 0.05$); the dynamic weight bearing of high dose MIA group decreased more significantly 1 hr after MIA injection and the effect lasted for at least 4 hr ($P < 0.05$). These results indicated that intraplantar injection of MIA could produce more than 4 hours of spontaneous pain, and the effect was dose-dependent.

3.4. Effect of Intraplantar Injection of Capsazepine on the Thermal Pain Induced by Monosodium Iodoacetate in Rats

The purpose of this experiment was to observe the effect of blocking TRPV1 on monosodium iodoacetate (MIA)-induced thermal pain in rats (**Figure 4**). Firstly, MIA (1 mg) was injected intraplantarly into the right hindpaw. Two hours later, the TRPV1 antagonist capsazepine (30 μg) was injected intraplantarly into the right hindpaw. The volume of the injection solution was 100 μl, and the thermal pain latency was measured every 10 minutes. The control group was rats receiving intraplantar injection of 100 μl saline at 2 hr after MIA injection. Both the thermal pain latency in the capsazepine group and the thermal pain latency in the control group decreased after MIA injection and kept at a low

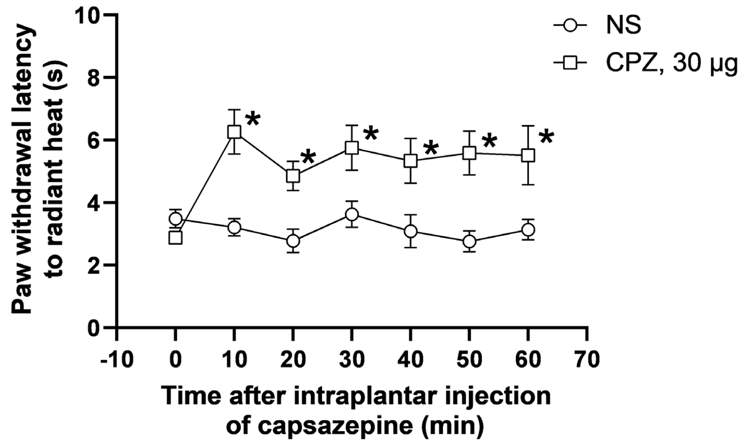

Figure 4. Effect of intraplantar injection of capsazepine on the thermal pain induced by monosodium iodoacetate in rats. The horizontal coordinate represented the time after intraplantar injection of capsazepine (CPZ) or saline at the 2nd hr after intraplantar injection of monosodium iodoacetate, and the vertical coordinate represented the thermal pain latency. The dose of CPZ was 30 μg and the injection volume was 100 μl. Data were expressed as mean ± standard error. The number of animals in each group was 8. The statistical difference at time points between groups was evaluated by Student's *t*-test. * meant $P < 0.05$ compared with the control group (NS group).

value at 2 hours after MIA injection. However, the thermal pain latency in the capsazepine group started to increase after intraplantar injection of capsazepine and in fact ascended to a high level that was close to the baseline value before MIA injection (Figure 1), meanwhile, the thermal pain latency in the control group kept low levels unchanged after intraplantar injection of saline. The capsazepine-induced effect of increasing the thermal pain latency lasted for at least 1 hour ($P < 0.05$). These results indicated that blocking TRPV1 could alleviate MIA-induced thermal pain, suggesting that the thermal pain induced by intraplantar injection of MIA might be achieved by activating TRPV1 in the peripheral nerve endings of primary sensory neurons in the skin of rat paw.

3.5. Effect of Intraplantar Injection of Capsazepine on the Mechanical Pain Induced by Monosodium Iodoacetate in Rats

The purpose of this experiment was to observe the effect of blocking TRPV1 on monosodium iodoacetate (MIA)-induced mechanical pain in rats (Figure 5). Firstly, MIA (1 mg) was injected intraplantarly into the right hindpaw. Two hours later, the TRPV1 antagonist capsazepine (30 μg) was injected intraplantarly into the right hindpaw. The volume of the injection solution was 100 μl, and the mechanical pain threshold was measured every 10 minutes. The control group was rats receiving intraplantar injection of 100 μl saline at 2 hr after MIA injection. Both the mechanical pain threshold in the capsazepine group and the mechanical pain threshold in the control group decreased after MIA injection and were kept at a low value at 2 hours after MIA injection. However, the mechanical pain threshold in the capsazepine group started to increase after intraplantar injection of capsazepine and in fact ascended to a high level that was close to the baseline value before MIA injection (**Figure 2**), meanwhile, the mechanical pain threshold in the control group kept low levels unchanged after intraplantar injection of saline. The capsazepine-induced effect of increasing the mechanical pain threshold lasted for at least 1 hour ($P < 0.05$). These results indicated that blocking TRPV1 could alleviate MIA-induced mechanical pain, suggesting that the mechanical pain induced by intraplantar injection of MIA might be achieved by activating TRPV1 in the peripheral nerve endings of primary sensory neurons in the skin of rat paw.

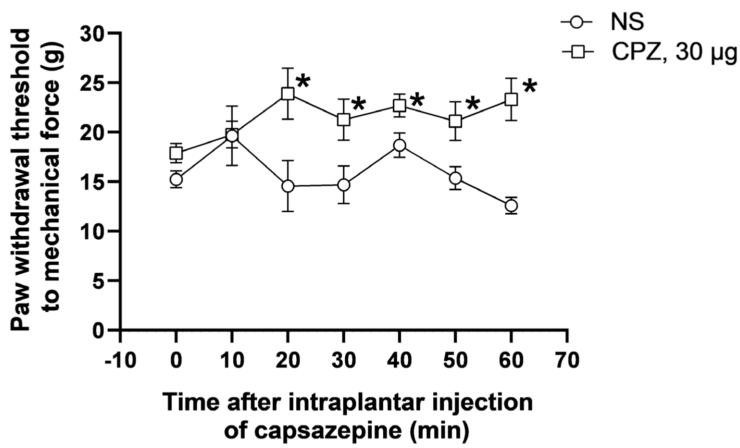

Figure 5. Effect of intraplantar injection of capsazepine on the mechanical pain induced by monosodium iodoacetate in rats. The horizontal coordinate represented the time after intraplantar injection of capsazepine (CPZ) or saline at the 2nd hr after intraplantar injection of monosodium iodoacetate, and the vertical coordinate represented the mechanical pain threshold. The dose of CPZ was 30 μg and the injection volume was 100 μl. Data were expressed as mean ± standard error. The number of animals in each group was 8. The statistical difference at time points between groups was evaluated by Student's *t*-test. * meant $P < 0.05$ compared with the control group (NS group).

3.6. Effect of Intraplantar Injection of Capsazepine on the Spontaneous Pain Induced by Monosodium Iodoacetate in Rats

The purpose of this experiment was to observe the effect of blocking TRPV1 on monosodium iodoacetate (MIA)-induced spontaneous pain in rats (**Figure 6**). Firstly, MIA (1 mg) was injected intraplantarly into the right hindpaw. Two hours later, the TRPV1 antagonist capsazepine (30 μg) was injected intraplantarly into the right hindpaw. The volume of the injection solution was 100 μl, and the dynamic weight bearing was measured every 10 minutes. The control group was rats receiving intraplantar injection of 100 μl saline at 2 hr after MIA injection. Both the dynamic weight bearing in the capsazepine group and the dynamic weight bearing in the control group decreased after MIA injection and were kept at a low value at 2 hours after MIA injection. However, the dynamic weight bearing in the capsazepine group started to increase after intraplantar injection of capsazepine and in fact ascended to a high level which was almost the same as the baseline value before MIA injection (**Figure 3**), meanwhile, the dynamic weight bearing in the control group kept low levels unchanged after intraplantar injection of saline. The capsazepine-induced effect of increasing the dynamic weight bearing lasted for at least 1 hour ($P < 0.05$). These results indicated that blocking TRPV1 could alleviate MIA-induced spontaneous pain, suggesting that the spontaneous pain induced by intraplantar injection of MIA might be achieved by activating TRPV1 in the peripheral nerve endings of primary sensory neurons in the skin of rat paw.

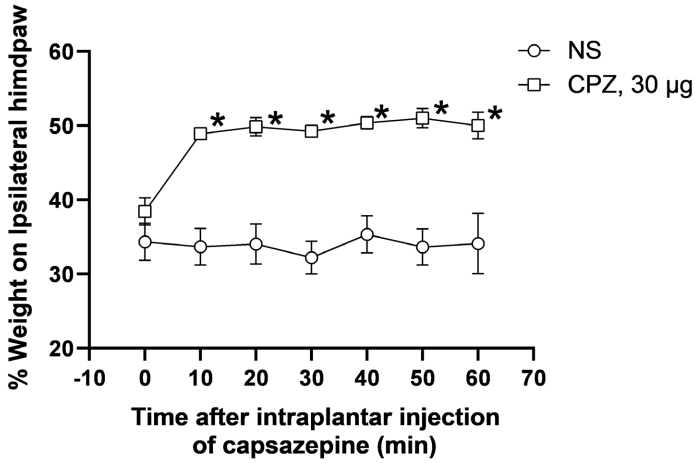

Figure 6. Effect of intraplantar injection of capsazepine on the spontaneous pain induced by monosodium iodoacetate in rats. The horizontal coordinate represented the time after intraplantar injection of capsazepine (CPZ) or saline at the 2nd hr after intraplantar injection of monosodium iodoacetate, and the vertical coordinate represented the dynamic weight bearing. The dose of CPZ was 30 μg and the injection volume was 100 μl. Data were expressed as mean ± standard error. The number of animals in each group was 8. The statistical difference at time points between groups was evaluated by Student's *t*-test. * meant $P < 0.05$ compared with the control group (NS group).

4. Discussion

Our previous studies reported that intraplantar injection of monosodium iodoacetate (MIA) in rats produced thermal pain, mechanical pain, and spontaneous pain on the second day after MIA injection, and the hyperalgesia lasted for 4-6 days, thereby establishing a new animal model of medium-range chronic inflammatory pain [1]. The goal of the present study was to refine the inflammatory pain model from the perspective of pain perception in animals during the subacute stage (within a few hours after monosodium iodoacetate injection) after MIA injection. We observed that: 1) high dose of MIA produced obvious thermal pain, mechanical pain, and spontaneous pain, the hyperalgesia occurred in the 1st hour after MIA injection and lasted up to at least the 4th hour after MIA injection; 2) medium dose of MIA induced thermal pain, mechanical pain, and spontaneous pain to a certain extent, the hyperalgesia occurred at the 1st hour after MIA injection and lasted up to at least the 4th hour after MIA injection; 3) low dose of MIA did not produce thermal pain, mechanical pain, and spontaneous pain. These results indicated that the rats developed hyperalgesia in the subacute stage after intraplantar injection of MIA and the effect was dose-dependent.

TRPV1 is distributed in primary sensory neurons and their peripheral and central nerve endings, and is responsible for sensing and transmitting nociceptive stimuli from the internal and external environment in pain transduction pathways [3], thus participating in the occurrence and development of various inflammatory pain, such as formalin-induced inflammatory pain [7], carrageenan-induced inflammatory pain [8], and complete Freund's adjuvant-induced inflammatory pain [9]. Our mechanical study on the chronic inflammatory pain induced by intraplantar injection of MIA also revealed that the occurrence and development of this kind of chronic pain was at least partially mediated by TRPV1 [1]. Capsazepine, a selective antagonist of TRPV1, was often used in the study of TRPV1-mediated hyperalgesic mechanism [10] [11]. In our present study, we used capsazepine to block TRPV1, and found that the hyperalgesia induced by intraplantar injection of MIA in the subacute stage was alleviated or even reversed by capsazepine, suggesting that the thermal pain, mechanical pain, and spontaneous pain caused by intraplantar injection of MIA in the subacute stage may be achieved partially by activating TRPV1 in the peripheral nerve endings of primary sensory neurons in the skin of rat paw.

Our previous study showed that intraplantar injection of capsaicin (a TRPV1 agonist) induced more nocifensive behavior in MIA rats during the chronic hyperalgesia stage than in control rats, further proving that TRPV1 may be up-regulated during chronic hyperalgesia induced by MIA. Similar experiments are planned to be performed in MIA rats in the subacute stage in the future, aiming to strengthen the findings that TRPV1 plays a key role in mediating MIA-induced subacute hyperalgesia.

In conclusion, intraplantar injection of monosodium iodoacetate in rats can produce thermal pain, mechanical pain, and spontaneous pain for more than 4 hours, which may be caused by the activation of TRPV1 located in the peripheral nerve ending of primary sensory neurons by monosodium iodoacetate. Our data may provide a novel animal model for the exploration of the occurrence of chronic pain, and might offer a reference for the development of analgesic drugs targeting TRPV1.

Acknowledgements

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Conflicts of Interest

The authors declare that there are no conflicts of interest.

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