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Involvement of TRPV1 in Acute Inflammatory Pain Induced by Intraplantar Injection of Monosodium Iodoacetate in Rats

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Abstract

Objective: To investigate whether intraplantar injection of monosodium iodoacetate (MIA) in rats produces acute inflammatory pain and to explore whether the hyperalgesia is mediated by transient receptor potential vanilloid type 1 (TRPV1). **Methods:** Male Wistar rats were used in the study. MIA was injected intraplantarly into the sole of the left hindpaw of rats and 30 minutes later, the paw skin color, paw volume, paw temperature, and serum IL-1β level of rats were measured. MIA was injected intraplantarly into the sole of the left hindpaw of rats and the changes of thermal pain latency, mechanical pain threshold, and dynamic weight bearing were measured after injection for one hour. TRPV1 antagonist capsazepine and MIA were co-injected intraplantarly into the sole of the left hindpaw of rats, and the changes in thermal pain latency, mechanical pain threshold, and dynamic weight bearing were measured for one hour after MIA injection. MIA was injected intraplantarly into the sole of the left hindpaw of rats and 30 minutes later, TRPV1 agonist capsaicin was injected intraplantarly to record capsaicin-induced guarding behavior. **Results:** The paw skin became red, the paw was swollen, and the paw temperature increased for the injected paw and the IL-1β level in the serum was elevated at 30 minutes after intraplantar injection of MIA in rats. Intraplantar injection of MIA in rats reduced the thermal pain latency, mechanical pain threshold, and dynamic weight bearing. The hyperalgesia lasted for at least one hour and the effect was dose-dependent. Intraplantar co-injection of capsazepine and MIA reversed the thermal hyperalgesia, mechanical hyperalgesia, and spontaneous pain induced by MIA. Rats showed more guarding behaviors such as paw lifting and paw licking in response to intraplantar injection of capsaicin at 30 minutes after intraplantar injection of MIA. **Conclusion:** Acute inflam-

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matory pain can be produced by intraplantar injection of MIA in rats, which leads to the establishment of a novel animal model of acute inflammatory pain. MIA-induced acute inflammatory pain may be due to TRPV1 upregulation.

Keywords

Monosodium Iodoacetate, Transient Receptor Potential Vanilloid Type 1, Inflammatory Pain

1. Introduction

For whether intraplantar injection of monosodium iodoacetate (MIA) in rats can produce hyperalgesia, we conducted studies at two periods: first, we measured nociceptive changes starting on the 1st day after intraplantar injection of MIA in rats, and observed that the rats produced hyperalgesia for 4 - 6 days, thus producing a novel animal model of chronic inflammatory pain [1]; next, we measured nociceptive changes starting at the 1st hour after intraplantar injection of MIA in rats and observed that the rats produced hyperalgesia for more than 4 hours, thus producing a subacute inflammatory pain animal model [2]. The purpose of the present study is to further explore whether intraplantar injection of MIA produces inflammatory pain during the acute phase (within one hour of MIA injection).

Different pain models are used for pain studies with different time courses. For the study of chronic inflammatory pain, there are bee venom model [3], turpentine model [4], complete Freund's adjuvant model [5], etc. For the study of subacute inflammatory pain, there is a carrageenan model [6]. The monosodium iodoacetate chronic pain model [1] and the monosodium iodoacetate subacute pain model [2] developed by us provide new options in animal models for chronic pain study and subacute pain study, respectively. A formalin model is used to study acute inflammatory pain [7]. However, the acute pain exhibited by this model is often unstable and sometimes even inconsistent with each other [8] [9]. Our present research is expected to provide a novel model with stable pain for studying acute inflammatory pain.

Transient receptor potential vanilloid type 1 (TRPV1), a non-selective cation channel expressed in primary afferent neurons [10], has been shown to play an important role in the occurrence and maintenance of hyperalgesia in rats with complete Freund's adjuvant inflammatory pain [11], carrageenan inflammatory pain [12], and formalin inflammatory pain [13]. Our previous studies also showed that TRPV1 mediated the development of hyperalgesia in MIA-induced chronic inflammatory pain [1] and subacute inflammatory pain [2]. So, we propose a hypothesis that if MIA can induce acute inflammatory pain, TRPV1 may also be involved in the development of hyperalgesia. In the present study, we investigated the role of TRPV1 in acute inflammatory pain by giving TRPV1 antagonist capsazepine and TRPV1 agonist capsaicin.

2. Materials and Methods

2.1. Animals

Male Wistar rats, weighing 210 - 260 g, were used in the study, which were purchased from Changsha Tianqin Biotechnology Co., Ltd., Hunan, China. The feeding temperature was maintained at 23 - 26˚C with a 12 hr light/12 hr dark cycle, and the animals were free to eat and drink. All animal experiments were conducted at 23 - 26˚C and complied with the relevant regulations on laboratory animal ethics of Youjiang Medical University for Nationalities.

2.2. Reagents

Monosodium iodoacetate (MIA, Shanghai Lianshuo Biotechnology Co., Ltd., Cat. No. 64-69-7) was dissolved in 0.9% NaCl. Capsaicin (MedChemExpress, Cat. No. HY-10488) was dissolved in 0.9% NaCl containing 0.5% ethanol. Capsazepine (MedChemExpress Inc., Cat. No. HY-15640) was dissolved in 0.9% NaCl containing 0.5% ethanol.

2.3. Intraplantar Injection and Drug Dosages

Monosodium iodoacetate (MIA), capsazepine, and capsaicin were injected subcutaneously into the sole of the left hindpaw of rats. The doses of MIA were 0.11, 0.33, and 1 mg in 100 μL. The dose of capsazepine was 30 μg in 100 μL. The dose of capsaicin was 4.5 μg in 50 μL. The dosages were chosen from the following studies [1] [2].

2.4. Measurement of Paw Skin Color

Rats were injected intraplantarly with MIA, 30 minutes later, the rat was placed in a plastic cone and the injected paw was stretched out to observe the change in skin color of the paw and photographs were taken to record the change. The red degree of the paw skin was obtained by measuring the gray values using Image J.

2.5. Measurement of Paw Volume

Rats were injected intraplantarly with MIA, 30 minutes later, the rat was placed in a plastic cone and the injected paw was stretched out. The volume of the injected paw was measured by using Plethysmometer (Ugo Basile, Cat. No. 37140).

2.6. Measurement of Skin Temperature of Rat Paw

Rats were injected intraplantarly with MIA, 30 minutes later, the rat was placed in a plastic cone and the injected paw was stretched out. The temperature of the injected paw was measured with an Infrared Thermometer (Benetech, Cat. No. GT303B).

2.7. Measurement of Serum IL-1β by Enzyme Linked Immunosorbent Assay (ELISA)

Rats were injected intraplantarly with MIA, 30 minutes later, rats were anesthetized with isoflurane, blood was collected from the abdominal aorta, and serum

was collected by centrifugation after blood coagulation. Serum IL-1β levels were measured by ELISA following the protocol of IL-1β ELISA kit (Elabscience, Cat. No. E-EL-R0012).

2.8. Measurement of Paw Withdrawal Thermal Latency

The paw withdrawal thermal latency of rats was measured using Plantar Test (Hargreaves's Apparatus) (Ugo Basile, Cat. No. 37370). Before the experiment, rats were acclimatized for 5 days in a transparent plexiglass box with a glass floor to reduce stressful reactions such as walking, probing, grooming, and frequent defecation. During the experiment, the rats were placed in the plexiglass box. After the rats were kept quiet, the pain measuring meter was turned on, and a beam of thermal infrared light was shone on the soles of the hindpaw of the rats. As the thermal stimulation time proceeds, the rat would feel pain in its paw and withdraw its paw. The time (s) from the issuance of the thermal beam to the occurrence of rat's paw withdrawal was recorded as paw withdrawal thermal latency (thermal pain latency).

2.9. Measurement of Paw Withdrawal Mechanical Threshold

The paw withdrawal mechanical threshold of rats was measured using Dynamic Plantar Aesthesiometer (Ugo Basile, Cat. No. 37450). Before the experiment, rats were acclimatized for 5 days in a transparent plexiglass box with a metal mesh floor to reduce stressful reactions such as walking, probing, grooming, and frequent defecation. During the experiment, the rats were placed in the plexiglass box. After the rats were kept quiet, the pain measuring meter was turned on, and a needle was extended and pressed on the plantar surface of the hindpaw of the rats. The needle force exerted on the plantar surface of the paw was gradually increased. As the stimulation time progresses and the mechanical force on the sole increases, the rat would feel pain in its paw and withdraw its paw. The minimum force (g) of the needle stimulus that caused the rat to withdraw its paw was recorded as the paw withdrawal mechanical threshold (mechanical pain threshold).

2.10. Test of Dynamic Weight Bearing

Dynamic weight bearing of rats was measured using Bipedal Balance Tester (Techman, Cat. No. PH-200). Before the experiment, rats were acclimatized for 5 days in a black plexiglass box to reduce stressful reactions such as walking, probing, grooming, and frequent defecation. During the training, the left and right hindpaws of the rats were placed respectively on the left and right pressure sensing plates of the pain measuring meter. During the experiment, the rats were placed in the black box. After the rats were kept quiet and stood in the correct posture, the pain measuring meter was turned on to measure the weight-bearing of two hindpaws of the rats, and the ratio of the weight-bearing of the inflammatory hindpaw to the total weight-bearing of left and right hindpaws was calculated and expressed in the form of a percentage, which was regarded as the dynamic weight bearing, and represented to the degree of the spontaneous pain of the rats.

2.11. Observation of Capsaicin-Induced Guarding Behavior

Before the experiment, rats were required to undergo 5 days of acclimatization in a transparent plexiglass cage. During the experiment, the rats were acclimatized in the transparent plexiglass cage for 30 minutes. After the rats were kept quiet, the rat was placed in a plastic cone and the left hindpaw was stretched out to receive intraplantar injection of capsaicin (4.5 μg/50 μL) into the sole of the left hindpaw, and then the rats were immediately transferred to the transparent plexiglass cage for behavioral observation. The time when the rats showed guarding behaviors (including paw lifting, paw licking, etc.) within 5 min after capsaicin injection was recorded.

2.12. Data Analysis

The present study used SPSS 26.0 to statistically analyze the experimental data, and the measurement data were expressed as mean ± standard error. The independent sample *t*-test method was used when the comparisons were made between two groups; and one-way analysis of variance (ANOVA) was used to assess the comparisons between three and more independent groups. The differences between groups were determined to be statistically significant when $P < 0.05$.

3. Results

3.1. Effect of Intraplantar Injection of MIA on the Skin Color of Rat Paw

The purpose of this experiment was to observe the changes in the skin color of rat paw after intraplantar injection of MIA (Figure 1). Rats in the MIA group were injected intraplantarly with MIA (1 mg/100 μL) in the left hindpaw, and rats in the control group were injected intraplantarly with 100 μL of saline in the left hindpaw. The skin color of the injected hindpaw was measured 30 minutes after injection in the two groups. The skin color of the rat paw in the MIA group became redder compared with that of the control group ($P < 0.05$).

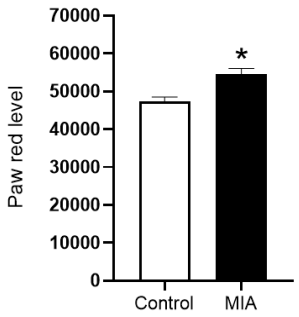

Figure 1. Effects of intraplantar injection of MIA on the skin color of rat paw. Rats in the MIA group and the control group were injected intraplantarly in the left hindpaw with MIA (1 mg/100 μL) and 100 μL of saline, respectively. The skin color of the injected paws was measured 30 minutes after injection. Data were expressed as mean ± standard error. The number of animals in each group was 8. Compared with the control group, * indicates $P < 0.05$.

3.2. Effect of Intraplantar Injection of MIA on the Paw Volume in Rats

The purpose of this experiment was to observe the changes in rat paw volume after intraplantar injection of MIA (Figure 2). Rats in the MIA group were injected intraplantarly with MIA (1 mg/100 μL) in the left hindpaw, and rats in the control group were injected intraplantarly with 100 μL of saline in the left hindpaw. The volume of the injected hindpaw was measured 30 minutes after injection in the two groups. Compared with the control group, the paw volume of rats in the MIA group increased significantly ($P < 0.05$).

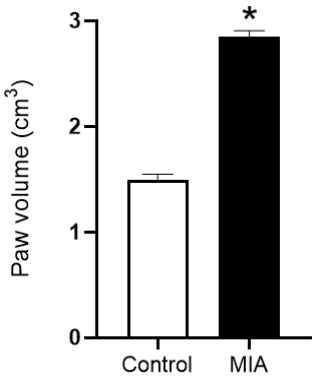

Figure 2. Effect of intraplantar injection of MIA on the paw volume in rats. Rats in the MIA group and the control group were injected intraplantarly in the left hindpaw with MIA (1 mg/100 μL) and 100 μL of saline, respectively. The paw volume of the injected paws was measured 30 minutes after injection. Data were expressed as mean ± standard error. The number of animals in each group was 8. Compared with the control group, * indicates $P < 0.05$.

3.3. Effect of Intraplantar Injection of MIA on the Skin Temperature of Rat Paw

The purpose of this experiment was to observe the changes in skin temperature of rat paw after intraplantar injection of MIA (Figure 3). Rats in the MIA group were injected intraplantarly with MIA (1 mg/100 μL) in the left hindpaw, and rats in the control group were injected intraplantarly with 100 μL of saline in the left hindpaw. The skin temperature of the injected hindpaw was measured in both groups of rats 30 minutes after injection. The skin temperature of the rat paw in the MIA group was elevated compared with that in the control group ($P < 0.05$).

3.4. Effect of Intraplantar Injection of MIA on Serum IL-1β Levels in Rats

The purpose of this experiment was to observe the changes in serum IL-1β levels after intraplantar injection of MIA (Figure 4). Rats in the MIA group were injected intraplantarly with MIA (1 mg/100 μL) in the left hindpaw, and rats in the control group were injected intraplantarly with 100 μL of saline in the left hindpaw. The serum IL-1β levels of the two groups were measured 30 minutes after

injection. Compared with the control group, the level of serum IL-1β was significantly increased in the MIA group ($P < 0.05$).

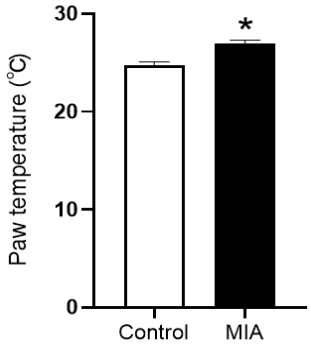

Figure 3. Effect of intraplantar injection of MIA on the skin temperature of rat paw. Rats in the MIA group and the control group were injected intraplantarly in the left hindpaw with MIA (1 mg/100 μL) and 100 μL of saline, respectively. The skin temperature of the injected paws was measured 30 minutes after injection. Data were expressed as mean ± standard error. The number of animals in each group was 8. Compared with the control group, * indicates $P < 0.05$.

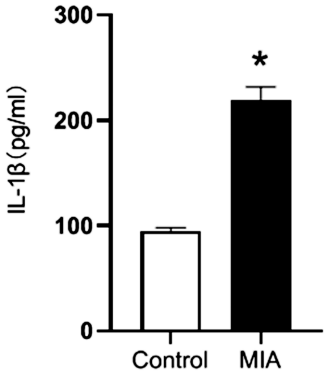

Figure 4. Effect of intraplantar injection of MIA on serum IL-1β levels in rats. Rats in the MIA group and the control group were injected intraplantarly in the left hindpaw with MIA (1 mg/100 μL) and 100 μL of saline, respectively. The serum IL-1β levels in both groups were measured 30 minutes after injection. Data were expressed as mean ± standard error. The number of animals in each group was 5. Compared with the control group, * indicates $P < 0.05$.

3.5. Effect of Intraplantar Injection of MIA 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) within one hour after intraplantar injection of MIA in rats (**Figure 5**). Rats in the MIA groups were injected intraplantarly with MIA in the left hindpaw. The doses of low, medium, and high MIA were 0.11, 0.33, and 1 mg, respectively, and the injection volume was 100 μL. Rats in the control group were injected intraplantarly in the left hindpaw with 100 μL of saline. Compared with the control group, the thermal pain la-

tency of rats in the low-dose MIA group did not change; the thermal pain latency of rats in the medium-dose MIA group decreased at the 10th minute after the injection, and this decrease persisted for at least one hour ($P < 0.05$); the thermal pain latency of rats in the high-dose MIA group decreased at the 10th minute after the injection with a greater decrease, which persisted for at least one hour ($P < 0.05$). These results suggested that intraplantar injection of MIA could produce acute thermal hyperalgesia in rats, and this thermal hyperalgesia effect was dose-dependent.

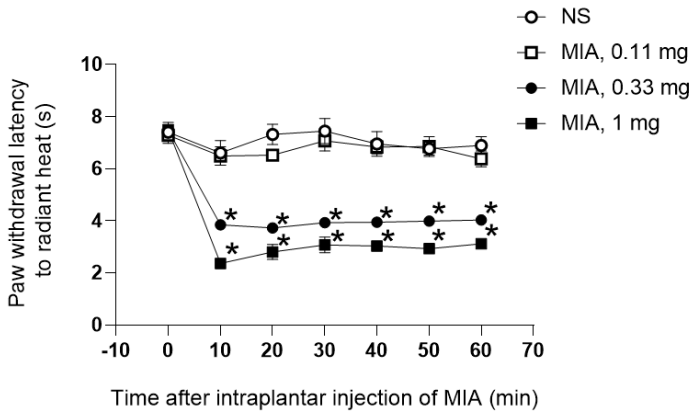

Figure 5. The effect of intraplantar injection of MIA on thermal pain latency in rats. The horizontal coordinate represented the time after intraplantar injection of MIA, and the vertical coordinate represented the paw withdrawal thermal latency (thermal pain latency). The rats in the low, medium, and high dose MIA groups were injected with 100 μL of solution containing 0.11, 0.33, and 1 mg MIA in the left hindpaw, respectively. The rats in the control group were injected with 100 μL of saline (NS) in the left hindpaw. Data were expressed as mean ± standard error. The number of animals in each group was 8. Compared with the control group (NS group) at the same time point, * indicated $P < 0.05$.

3.6. Effect of Intraplantar Injection of MIA 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) within one hour after intraplantar injection of MIA in rats (Figure 6). Rats in the MIA groups were injected intraplantarly with MIA in the left hindpaw. The doses of low, medium, and high MIA were 0.11, 0.33, and 1 mg, respectively, and the injection volume was 100 μL. Rats in the control group were injected intraplantarly in the left hindpaw with 100 μL of saline. Compared with the control group, the mechanical pain thresholds of rats in the low-dose MIA group did not change; those of rats in the medium-dose MIA group decreased at the 60th minute after injection ($P < 0.05$); those of rats in the high-dose MIA group decreased from the 10th to the 60th minute after injection ($P < 0.05$), and the decreases were more pronounced than those in the medium-dose MIA group. These results suggested that intraplantar injection of MIA can produce acute mechanical hyperalgesia in rats, and this mechanical hyperalgesia effect was dose-dependent.

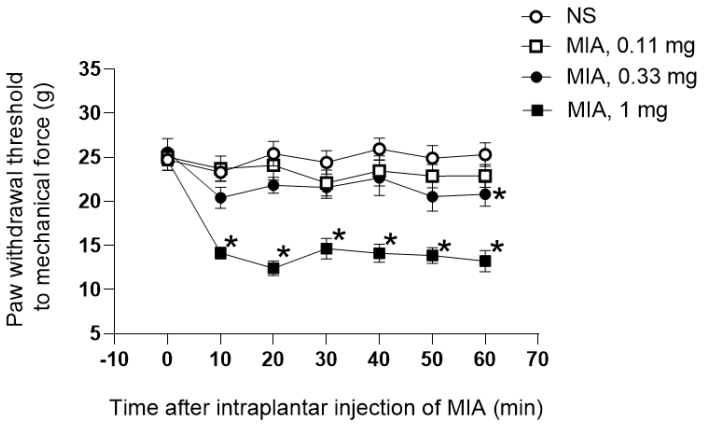

Figure 6. The effect of intraplantar injection of MIA on mechanical pain threshold in rats. The horizontal coordinate represented the time after intraplantar injection of MIA, and the vertical coordinate represented the paw withdrawal mechanical threshold (mechanical pain threshold). The rats in the low, medium, and high dose MIA groups were injected with 100 μL of solution containing 0.11, 0.33, and 1 mg MIA in the left hindpaw, respectively. The rats in the control group were injected with 100 μL of saline (NS) in the left hindpaw. Data were expressed as mean±standard error. The number of animals in each group was 8. Compared with the control group (NS group) at the same time point, * indicated $P < 0.05$.

3.7. Effect of Intraplantar Injection of MIA on Dynamic Weight Bearing in Rats

The purpose of this experiment was to observe the changes in dynamic weight bearing in rats within one hour after intraplantar injection of MIA (**Figure 7**). Rats in the MIA groups were injected intraplantarly with MIA in the left hindpaw.

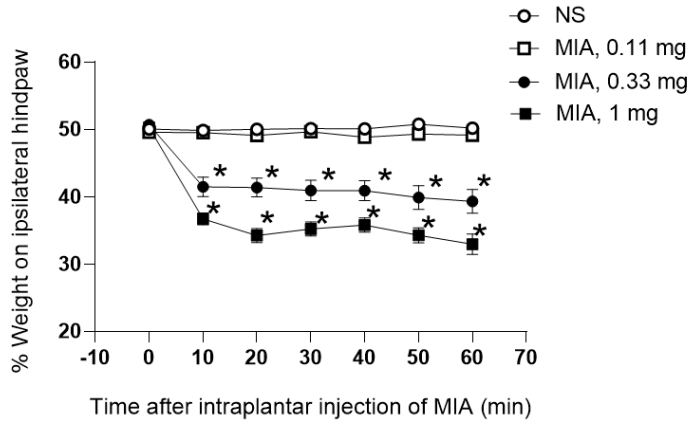

Figure 7. Effect of intraplantar injection of MIA on dynamic weight bearing in rats. The horizontal coordinate represented the time after intraplantar injection of MIA, and the vertical coordinate represented the dynamic weight bearing. The rats in the low, medium, and high dose MIA groups were injected with 100 μL of solution containing 0.11, 0.33, and 1 mg MIA in the left hindpaw, respectively. The rats in the control group were injected with 100 μL of saline (NS) in the left hindpaw. Data were expressed as the mean ± standard error. The number of animals in each group was 8. Compared with the control group (NS group) at the same time point, * indicated $P < 0.05$.

The doses of low, medium, and high MIA were 0.11, 0.33, and 1 mg, respectively, and the injection volume was 100 μL. Rats in the control group were injected intraplantarly in the left hindpaw with 100 μL of saline. Compared with the control group, there was no change in the dynamic weight bearing of the rats in the low-dose MIA group; the dynamic weight bearing of the rats in the medium-dose MIA group decreased at the 10th minute after injection, and this decrease persisted for at least one hour ($P < 0.05$); the dynamic weight bearing of the rats in the high-dose MIA group decreased at the 10th minute after injection with a greater decrease, and this decrease persisted for at least one hour ($P < 0.05$). These results suggested that intraplantar injection of MIA can produce acute spontaneous pain in rats, and this hyperalgesia effect was dose-dependent.

3.8. Effect of Intraplantar Injection of Capsazepine on MIA-Induced Thermal Hyperalgesia

The purpose of this experiment was to observe the effect of antagonizing TRPV1 in the paw on MIA-induced thermal hyperalgesia in rats (**Figure 8**). The TRPV1 antagonist used was capsazepine (CPZ). The groups of rats and the drugs injected in each group were: saline group (100 μL of saline), capsazepine group (CPZ 30 μg/100 μL), MIA group (MIA 1 mg/100 μL), and MIA + capsazepine group (MIA 1 mg/50 μL + CPZ 30 μg/50 μL). The changes in thermal pain latency were observed within one hour after intraplantar injection of corresponding drugs in the left hindpaw of rats. The thermal pain latency of saline group and capsazepine group did not change. The thermal pain latency of MIA group and MIA + capsazepine group decreased significantly at 10 minutes after injection, and the thermal pain latency of MIA group continued to be low within the next 50 minutes, whereas those of the MIA + capsazepine group were elevated to the values of basal thermal pain latency at the 20th minute after injection, and persisted for at least one hour ($P < 0.05$). These results indicated that antagonizing TRPV1 alleviated MIA-induced acute thermal hyperalgesia, suggesting that intraplantar injection of MIA induces acute thermal hyperalgesia in the rats by activating TRPV1.

3.9. Effect of Intraplantar Injection of Capsazepine on MIA-Induced Mechanical Hyperalgesia

The purpose of this experiment was to observe the effect of antagonizing TRPV1 in the paw on MIA-induced mechanical hyperalgesia in rats (**Figure 9**). The TRPV1 antagonist used was capsazepine (CPZ). The groups of rats and the drugs injected in each group were: saline group (100 μL of saline), capsazepine group (CPZ 30 μg/100 μL), MIA group (MIA 1 mg/100 μL), and MIA + capsazepine group (MIA 1 mg/50 μL + CPZ 30 μg/50 μL). The changes in mechanical pain threshold were observed within one hour after intraplantar injection of corresponding drugs in the left hindpaw of rats. The mechanical pain threshold of saline group and capsazepine group did not change. The mechanical pain threshold of MIA group and MIA + capsazepine group decreased significantly at 10 minutes

after injection, and the mechanical pain threshold of MIA group continued to be low within the next 50 minutes, whereas those of the MIA + capsazepine group were elevated to the values of basal mechanical pain threshold at the 20th minute after injection, and persisted for at least one hour ($P < 0.05$). These results indicated that antagonizing TRPV1 alleviated MIA-induced acute mechanical hyperalgesia, suggesting that intraplantar injection of MIA induces acute mechanical hyperalgesia in the rats by activating TRPV1.

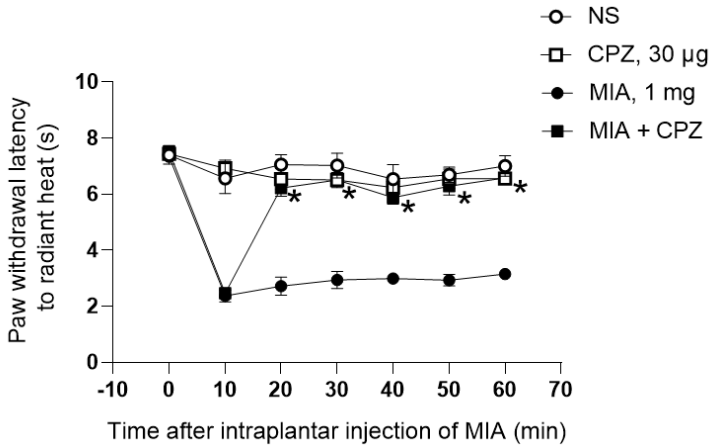

Figure 8. Effect of intraplantar injection of capsazepine (CPZ) on thermal hyperalgesia induced by MIA. The horizontal coordinate represented the time after intraplantar injection of CPZ, and the vertical coordinate represented the thermal pain latency. The doses of CPZ and MIA were 30 μg and 1 mg, respectively, and the volume of injection was 100 μL. The NS group was injected with 100 μL of saline. Data were expressed as mean ± standard error. The number of animals in each group was 8. Compared with MIA group at the same time point, * indicates $P < 0.05$.

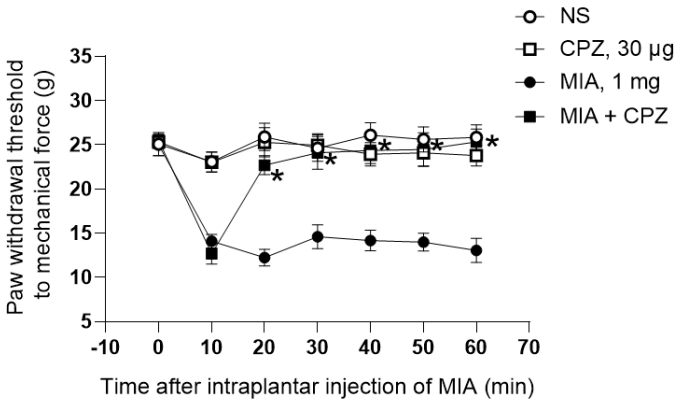

Figure 9. Effect of intraplantar injection of capsazepine (CPZ) on mechanical hyperalgesia induced by MIA. The horizontal coordinate represented the time after intraplantar injection of CPZ, and the vertical coordinate represented the mechanical pain threshold. The doses of CPZ and MIA were 30 μg and 1 mg, respectively, and the volume of injection was 100 μL. The NS group was injected with 100 μL of saline. Data were expressed as mean ± standard error. The number of animals in each group was 8. Compared with MIA group at the same time point, * indicates $P < 0.05$.

3.10. Effect of Intraplantar Injection of Capsazepine on Spontaneous Pain Induced by MIA

The purpose of this experiment was to observe the effect of antagonizing TRPV1 in the paw on MIA-induced spontaneous pain in rats (Figure 10). The TRPV1 antagonist used was capsazepine (CPZ). The groups of rats and the drugs injected in each group were: saline group (100 μL of saline), capsazepine group (CPZ 30 μg/100 μL), MIA group (MIA 1 mg/100 μL), and MIA + capsazepine group (MIA 1 mg/50 μL + CPZ 30 μg/50 μL). The changes in dynamic weight bearing were observed within one hour after intraplantar injection of corresponding drugs in the left hindpaw of rats. The dynamic weight bearing of saline group and capsazepine group did not change. The dynamic weight bearing of MIA group and MIA + capsazepine group decreased significantly at 10 minutes after injection, and MIA group continued to be low within the next 50 minutes, whereas MIA + capsazepine group were elevated to the values of basal dynamic weight bearing at the 20th minute after injection, and persisted for at least one hour ($P < 0.05$). These results indicated that antagonizing TRPV1 alleviated MIA-induced acute spontaneous pain, suggesting that intraplantar injection of MIA induces acute spontaneous pain in the rats by activating TRPV1.

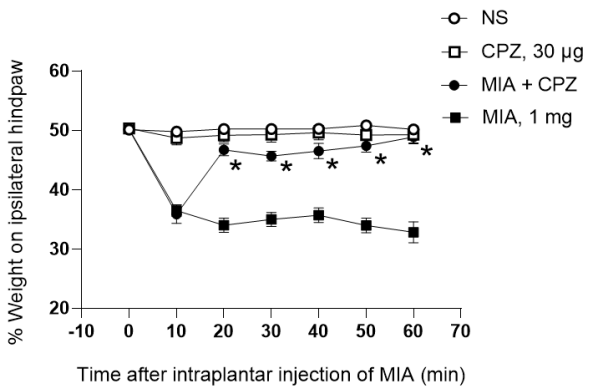

Figure 10. Effect of intraplantar injection of capsazepine (CPZ) on spontaneous pain induced by MIA. The horizontal coordinate represented the time after intraplantar injection of CPZ, and the vertical coordinate represented the dynamic weight bearing. The doses of CPZ and MIA were 30 μg and 1 mg, respectively, and the volume of injection was 100 μL. The NS group was injected with 100 μL of saline. Data were expressed as mean±standard error. The number of animals in each group was 8. Compared with MIA group at the same time point, * indicates $P < 0.05$.

3.11. Effect of Intraplantar Injection of MIA on Capsaicin-Induced Guarding Behavior

The purpose of this experiment was to observe the effect of intraplantar injection of MIA on capsaicin-induced guarding behavior (Figure 11). Capsaicin is an agonist of TRPV1. Rats in the MIA group were injected intraplantarly with MIA (1 mg/100 μL) in the left hindpaw, and rats in the control group were injected intraplantarly with 100 μL of saline in the left hindpaw. 30 minutes later, rats in the

two groups were injected intraplantarly with capsaicin (4.5 μg/50 μL) in the left hindpaw, and the durations of the appearance of guarding behaviors such as paw lifting and paw licking were recorded within five minutes after capsaicin injection. Compared with the control group, rats in MIA group showed more guarding behaviors ($P < 0.05$). Capsaicin induces guarding behavior by activating TRPV1, and the duration of this behavior might be positively correlated with the TRPV1 expression level. This result suggested that TRPV1 expression level was upregulated in the paws of rats with acute inflammatory pain induced by MIA.

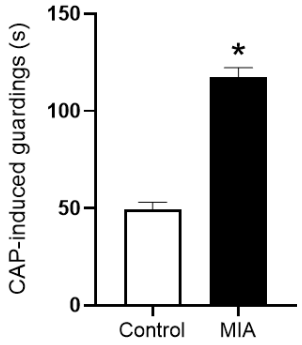

Figure 11. Effect of intraplantar injection of MIA on capsaicin-induced guarding behavior. Rats in the MIA group and the control group were injected intraplantarly in the left hindpaw with MIA (1 mg/100 μL) and 100 μL of saline, respectively. 30 minutes later, rats in both groups were injected intraplantarly with capsaicin (4.5 μg/50 μL) in the left hindpaw. Data were expressed as mean ± standard error. The number of animals in each group was 8. Compared with the control group, * indicates $P < 0.05$.

4. Discussion

In the present study, it was observed that the skin color of the paw became red, the paw became 1.9 times larger, the paw temperature increased by about 2˚C, and the level of serum IL-β increased 30 minutes after intraplantar injection of MIA, which indicated that the rat paw showed obvious inflammatory reactions such as redness, swelling, and heat after MIA injection. The thermal pain latency, mechanical pain threshold, and dynamic weight bearing were significantly reduced after intraplantar injection of MIA and the reduction occurred as early as 10 minutes after MIA injection and lasted at least 60 minutes after MIA injection, which indicated that acute thermal hyperalgesia, mechanical hyperalgesia, and spontaneous pain appeared after intraplantar injection of MIA into the rat paw and the degree of hyperalgesia showed a dose-dependent pattern. The above changes in inflammatory response indexes and pain indexes indicate that an acute inflammatory pain model can be successfully established by intraplantar injection of MIA in rats. In addition to injecting 3 doses of 0.11, 0.33, and 1 mg MIA, we also injected 2 doses of 0.03 and 3 mg MIA to measure the pain response. It was shown that 0.03 mg MIA did not produce pain and the hyperalgesia produced by 3 mg MIA was not significantly different from that of 1 mg MIA (data not shown). Therefore, on the one hand, we concluded that 1 mg MIA could be used as the

optimal dose for this acute inflammatory pain model; on the other hand, 1 mg MIA was applied when conducting mechanism experiments using capsazepine and capsaicin.

After co-injection of MIA and TRPV1 antagonist capsazepine in the rat paw, the decrease of thermal pain latency, mechanical pain threshold, and dynamic weight bearing induced by MIA in rats was alleviated, indicating that blockade of TRPV1 can significantly alleviate the thermal hyperalgesia, mechanical hyperalgesia, and spontaneous pain induced by MIA. Why the capsazepine's pain-relieving effect occur 20 minutes after CPZ injection, not 10 minutes after CPZ injection? The possible reason may be related to the time required for competitive binding of TRPV1 by capsazepine. The injection of capsazepine alone in normal rats had no effect on thermal pain latency, mechanical pain threshold, and dynamic weight bearing, suggesting that TRPV1 is not involved in basal nociception in normal rats, which is consistent with Nguyen's finding that injection of capsazepine alone had no effect on thermal pain latency [14]. The above results suggested that acute hyperalgesia induced by intraplantar injection of MIA may be achieved by activating TRPV1. When the TRPV1 agonist capsaicin was given after an intraplantar injection of MIA, rats showed more guarding behaviors such as paw lifting and paw licking. Taking into account that the longer time for the guarding behavior caused by capsaicin might be due to the higher level of the TRPV1 expression, this result suggested that there was an upregulation of TRPV1 expression in the rat paw after MIA injection. Therefore, it was proved that acute hyperalgesia induced by intraplantar injection of MIA may be achieved by activation of TRPV1 from perspectives of antagonism of TRPV1 and activation of TRPV1. As for the reason for the upregulation of TRPV1, since it was observed that the level of serum IL-Iβ increased after MIA injection, it may be that MIA injection stimulated the production of inflammatory factors such as IL-1β in the rat paw, which subsequently activated TRPV1 in the paw, leading to hyperalgesia.

In order to investigate the relationship between inflammatory response and pain perception in acute inflammatory pain induced by MIA, we plan to record the time course of changes in inflammatory markers after MIA injection and to use IL-1β receptor antagonist to observe whether the upregulation of TRPV1 could be prevented and MIA-induced hyperalgesia could be reversed in future experiments.

In conclusion, acute inflammatory hyperalgesia can be induced by intraplantar injection of MIA in rats, whereby a novel animal model of acute inflammatory pain can be established. Acute inflammatory pain induced by MIA may be related to the upregulation of TRPV1.

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Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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