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ORIGINAL ARTICLE

PARTICIPATION OF CANNABINOID RECEPTORS IN ANTIPRURITIC ACTIVITY INDUCED BY SYSTEMIC DIPYRONE IN MICE

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ABSTRACT

Aims: The cannabinoid system has been shown to contribute to the antinociceptive effects of nonsteroidal anti-inflammatory drugs. Considering the similar pathophysiological mechanisms underlying pain and itching, we aimed to observe whether dipyrone has an antipruritic effect and whether cannabinoid receptors are involved in this effect.

Methods: In this project, we produced scratching behavior in BALB/c mice, intradermally administering the well-known pruritic agent compound 48/80. After observing the anti-scratching effect of dipyrone with increasing doses, we administered AM-251 (1 mg/kg, intraperitoneal) and AM-630 (3 mg/kg, intraperitoneal) to determine whether the endocannabinoid system was associated with this effect of dipyrone.

Results: Dipyrone reduced scratching behavior at its highest dose used in this study (600 mg/kg); however, neither AM-251 nor AM-630 changed the antipruritic action of dipyrone.

Conclusion: Our findings indicate that dipyrone, at higher doses, attenuates compound 48/80-induced scratching behavior in mice. Cannabinoid receptors have been found not to be involved in the antipruritic effect of dipyrone. Further experiments are required to delineate the mechanisms underlying this high-dose dipyrone effect.

Keywords: AM-251, AM-630, dipyrone, pruritus

INTRODUCTION

Cannabinoids have been known to reduce pain for centuries; however, several reasons preclude their use as effective analgesics (1). These chemical compounds include synthetic cannabinoids, those found in the Cannabis plant (phytocannabinoids), and those distributed throughout the body (endocannabinoids) (1). All cannabinoids activate cannabinoid receptors (CB1 and CB2) and take place in many pathophysiological processes (1). CB1 receptors are distributed widely in the central nervous system and appear to play the predominant role in both the therapeutic and the central adverse effects of these drugs (1). Despite a great deal of research, cannabinoids are only used in limited indications. Synthetic cannabinoids, dronabinol and nabilone, are used in the treatment of chemotherapy-associated emesis, and nabilone is also approved for anorexia associated with acquired immunodeficiency syndrome (1). Nabiximol (Δ9-tetrahydrocannabinol + cannabidiol) is recommended for use in neuropathy and spasticity associated with multiple sclerosis and cancer pain (2, 3). These cannabinoids are still alternatives in aforementioned indications and are approved only in some countries; however, numerous ongoing clinical

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trials seem to enhance their usage in different pathological conditions in addition to its use for pain reduction (2, 3).

Nonsteroidal anti-inflammatory drugs (NSAIDs) are a group of medicines used worldwide to reduce pain, fever, and inflammation (4). They block the cyclooxygenase enzymes (COX-1, COX-2) and inhibit the production of prostaglandins (4). Distinct from traditional NSAIDs, paracetamol and dipyrone appear not to act on peripheral COX enzymes; a recent research indicates that they do not have a direct significant effect on endocannabinoid levels in a rat's brain and spinal cord (4). Yet, there are previous studies indicating that both increases in endocannabinoid tonus and cannabinoid receptors are associated with the antinociceptive effects of paracetamol and dipyrone (5-7). The cannabinoid system has also been proposed to participate in antipyretic, anxiolytic, and anticonvulsive effects of these drugs, although there are contradictory data (7-10).

Pruritus, also known as itching, is an uncomfortable, irritating sensation that provokes the urge to scratch (11). It is the predominant symptom of many diseases and can affect the quality of life (11). Overall, pruritus and pain are two sensations showing many similarities, especially in pathological and chronic conditions (11, 12). Accordingly, similar to pain, involvement of the cannabinoid system in the development of pruritus and the probable therapeutic effect of cannabinoids in pruritic states have been suggested (13, 14). Cannabinoid agonists have been shown to attenuate histamine-induced responses, while rimonabant, CB1 receptor antagonist, induced scratching behavior (13, 14). Similarly, we presented that activation of cannabinoid receptors using the synthetic cannabinoid agonist WIN 55,212-2 and enhancing endocannabinoid tonus via blockade of endocannabinoid degrading enzymes, fatty acid amide hydrolase, and monoacylglycerol lipase exhibit dose-dependent antipruritic activity (15-17).

This study aims to examine whether dipyrone reduces itching behavior induced by compound 48/80 (C 48/80) and to observe whether cannabinoid receptors mediate dipyrone's effects in case of any antipruritic action.

MATERIAL AND METHODS

Animals & Ethics

In this study, male BALB/c mice (2-3 months) weighing 20-30 g were used (Center of the Laboratory Animals, Trakya University). All animals were housed in a light (12/12 h day/night cycles) and temperature-controlled (21±2 °C) room where food and water were available *ad libitum*. A total of 48 mice were used in this study. Separate groups, each involving 6 mice were used for each set of experiments. The experiments were conducted after approval of the local "Animal Care Ethics Committee" (protocol code: TÜHADYEK-2019/27, date: 25.10.2019). Moreover, during all procedures, the Ethical Committee of the International Association for the Study of Pain guidelines were followed strictly (18).

Study Design

Itching behavior was evoked by intradermal injection of 100 µg/50 µL of C 48/80 into the rostral part of the back of each mouse. Several scratches per second were accepted as one bout of scratching. These scratches were videotaped and counted for 30 minutes, beginning just after administering C 48/80. To examine the antipruritic effect of the drug, different doses of dipyrone [150, 300, 600 mg/kg, intraperitoneal (i.p.)] were tested. Subsequently, effects of the cannabinoid CB1 receptor antagonist AM-251 (1 mg/kg, i.p.) and the cannabinoid CB2 receptor antagonist AM-630 (3 mg/kg, i.p.) on the antipruritic activity of dipyrone were analyzed. Dipyrone was injected 30 minutes before C 48/80 administration, and cannabinoid receptor antagonists were administered 10 minutes before dipyrone.

Drugs

Compounds 48/80 and AM-630 were supplied from Sigma-Aldrich (St Louis, MO, USA); AM-251 was obtained from Tocris (UK); and dipyrone was purchased from Cayman (Ann Arbor, MI, USA). C 48/80 and dipyrone were dissolved in physiological saline, while AM-251 and AM-630 were administered in 20% dimethyl sulfoxide, 1% Tween 80, 1% ethanol, and 78% saline. Doses of each drug and treatment schedules were chosen from our previous studies (19-24).

Statistical Analysis

Graphpad Prism 8.4 was used for statistical analysis and to plot the graphs. To assess the significance of any difference between groups, the Kruskal-Wallis test, then Dunn's Multiple Comparisons test were performed. In all analyses, $p<0.05$ was considered statistically significant.

RESULTS

Reduction of Compound 48/80-induced Scratching Behavior by Dipyrone

Dipyrone (150, 300, 600 mg/kg) administration attenuated C 48/80-induced scratches at its highest dose (30 mg/kg; *$p<0.001$, compared to vehicle); but treatment with lower dipyrone doses had no effect on scratching behavior (Figure 1).

Influence of Cannabinoid Receptor Antagonism on Dipyrone-induced Antipruritic Activity

Neither AM-251 (1 mg/kg) nor AM-630 (3 mg/kg) had any influence on the antipruritic activity of dipyrone (Figure 2).

Effect of Cannabinoid Receptor Antagonists on Compound 48/80-induced Scratching Behavior When Administered Alone

When administered alone, AM-251 (1 mg/kg) had no effect on C 48/80-induced scratching activity whereas AM-630 (3 mg/kg) reduced C 48/80-induced scratches (*p<0.001, compared to the vehicle; Figure 3).

DISCUSSION

Dipyrone is one of the most extensively used non-opioid analgesic drugs (25). Unlike traditional NSAIDs it has low anti-inflammatory activity but exerts substantial analgesic efficacy (25). It has been suspected for a long time that the central nervous system plays role in the antinociceptive effect of dipyrone and the contribution of the endocannabinoid system is one of the topics attracting attention to explain its mechanism of action (25). In this study, we investigated whether dipyrone produced antipruritic effects in mice who developed scratching behavior following C 48/80 administration and observed the contribution of cannabinoid receptors to its antipruritic action. In this study, dipyrone was found to be alleviating scratching at higher doses, but blockade of cannabinoid receptors was not found to be changing this effect.

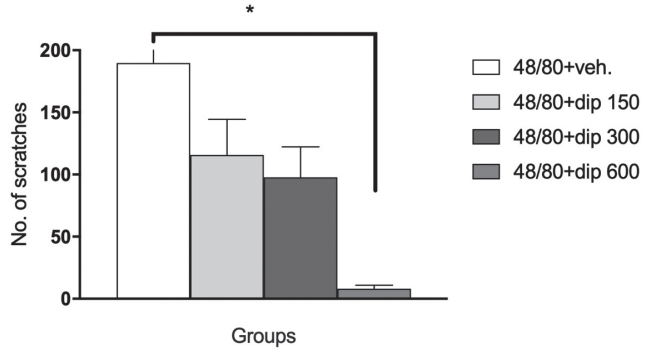

Figure 1: Effects of dipyrone (150, 300, 600 mg/kg, i.p.) on compound 48/80-induced scratches (*p<0.001, compared to vehicle).

veh.: Vehicle, dip: Dipyrone

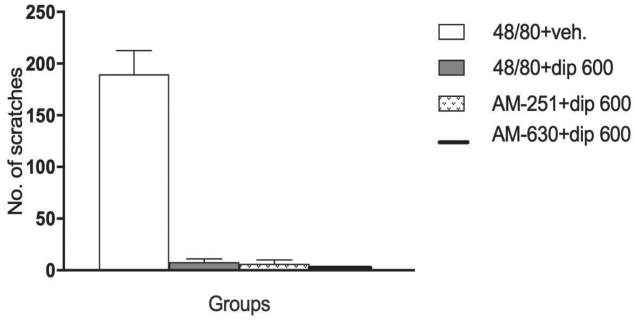

Figure 2: Effects of the cannabinoid CB1 receptor antagonist AM-251 (1 mg/kg, i.p.) and the cannabinoid CB2 receptor antagonist AM-630 (3 mg/kg, i.p.) on the antipruritic activity of dipyrone (600 mg/kg, i.p.).

veh.: Vehicle, dip: Dipyrone

There are studies indicating that cannabinoid agonists and drugs elevating endocannabinoid tonus locally exert antipruritic effects (13-17). However, only a very limited number of studies have been conducted on the effects of NSAIDs, the non-opioid analgesic dipyrone and paracetamol, on pruritus. Systemic administration of two classical NSAIDs, tenoxicam and diclofenac, significantly reduced the incidence and severity of postoperative pruritus in patients receiving epidural opioids (26, 27). Moreover, paracetamol has been shown to reduce scratching behavior, especially at higher doses, but antagonism of CB1 and CB2 receptors have been shown not to prevent the antipruritic effects of systemic paracetamol (28-30). Similar to observations on paracetamol studies, our results indicate that dipyrone elicited antipruritic effects at the highest dose. Since many analgesic drugs dose-dependently blocked serotonin and C 48/80-induced scratching behaviors, with some exhibiting complete inhibition at certain doses, further experiments are needed to discriminate whether our findings will be evaluated as false-positive responses or not (29).

In this study, to clarify the mechanism of systemic dipyrone's antipruritic effect, the cannabinoid receptors were antagonized with AM-251 and AM-630. We observed that cannabinoid receptor antagonists had no influence on the anti-scratching effect of dipyrone, signaling that mechanisms other than the cannabinoid receptors are mediating its antipruritic action. In addition to COX inhibition, the classical mechanism of NSAIDs' effects such as the release of endogenous opioids, participation of nociceptin/orphanin FQ receptors, L-arginine/NO/cGMP/K_{ATP}, and/or the glutamatergic systems are among the suggested mechanisms for the peripheral and central

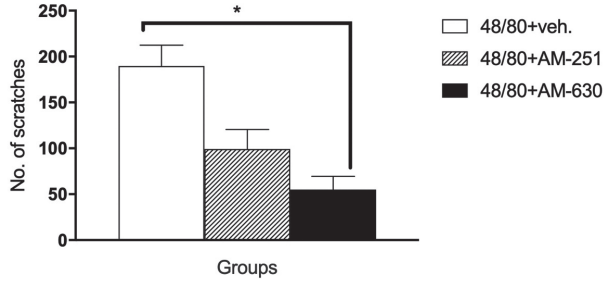

Figure 3: Effects of the cannabinoid CB1 receptor antagonist AM-251 (1 mg/kg, i.p.) and the cannabinoid CB2 receptor antagonist AM-630 (3 mg/kg, i.p.) on compound 48/80-induced scratches when administered alone (*p<0.001, compared to the vehicle).

veh.: Vehicle, dip: Dipyrone

antinociceptive effects of dipyrone (21, 31-35). Similar to its antinociceptive activity, all of these mechanisms may also mediate the antipruritic action of dipyrone, but detailed experiments on this area should be conducted.

Another interesting finding of our study was that the CB2 receptor antagonist AM-630 had no effect on the antipruritic effect of dipyrone but unexpectedly exerted anti-scratching properties when administered alone. Since CB2 receptors are mainly expressed in the periphery and do not mediate the central effects of cannabinoids, this appears to be an exceptional result. In our earlier studies, we found that AM-630, when injected intrathecally, did not exert any effect on its own or altered the antinociceptive action of diclofenac (36, 37). When other pruritus models, different techniques, and/or different routes of administration are used, we hope to see similar results as AM-630 having no effect when administered alone.

CONCLUSION

We observed that the non-opioid analgesic drug dipyrone reduces C 48/80-induced scratching behavior in mice when given systemically, but only when it is used at the highest dose. Blockade of cannabinoid receptors has not participated in this antipruritic activity of high-dose dipyrone.

Ethics Committee Approval: This work was approved by Trakya University Local Ethics Committee of Animal Experiments (protocol code: TÜHADYEK-2019/27, date: 25.10.2019).

Informed Consent: N/A

Conflict of Interest: The authors declared no conflict of interest.

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