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Descending serotonergic and noradrenergic systems do not regulate the antipruritic effects of cannabinoids*

Todurga ZG, Gunduz O, Karadag CH, Ulugol A. Descending serotonergic and noradrenergic systems do not regulate the antipruritic effects of cannabinoids.

Background: For centuries, cannabinoids have been known to be effective in pain states. Itch and pain are two sensations sharing a lot in common.
Objective: The goal of this research was to observe whether the cannabinoid agonist WIN 55,212-2 reduces serotonin-induced scratching behaviour and whether neurotoxic destruction of descending serotonergic and noradrenergic pathways mediate the antipruritic effect of WIN 55,212-2.
Material and methods: Scratching behaviour was induced by intradermal injection of serotonin (50 µg/50 µl/mouse) to Balb/c mice. The neurotoxins 5,7-dihydroxytryptamine (5,7-DHT, 50 µg/mouse) and 6-hydroxydopamine (6-OHDA, 20 µg/mouse) are applied intrathecally to deplete serotonin and noradrenaline in the spinal cord. WIN 55,212-2 (1, 3, 10 mg/kg, i.p.) dose-dependently attenuated serotonin-induced scratches. Neurotoxic destruction of neither the serotonergic nor the noradrenergic systems by 5,7-DHT and 6-OHDA, respectively, had any effect on the antipruritic action of WIN 55,212-2.
Conclusion: Our findings indicate that cannabinoids dose-dependently reduce serotonin-induced scratching behaviour and neurotoxic destruction of descending inhibitory pathways does not mediate this antipruritic effect.

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Keywords: cannabinoid system; descending inhibition; WIN 55,212-2; pruritus

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Significant outcomes

- The cannabinoid agonist, WIN 55,212-2, attenuated serotonin-induced scratches.
- Unlike its antinociceptive effect, neurotoxic destruction did not influence the antipruritic action of WIN 55,212-2.

Limitations

- Monoamine levels may be measured to verify the degree of depletion of serotonin and noradrenaline in the spinal cord.

Introduction

Pruritus (itch), an unpleasant sensation that evokes the desire to scratch, is a significant clinical problem. It is a common symptom not only of skin diseases, such as atopic dermatitis, contact dermatitis, urticaria and psoriasis, but also of systemic disorders, such as uraemia and cholestasis. Therefore, treating pruritus will improve the quality of life for many patients. Itch and pain sensations have much in common. Similar to pain, itch sensation is transmitted via primary

afferent C fibres from the skin to the superficial layer of the spinal cord dorsal horn and then to the thalamus by spinothalamic pathways (1, 2). Recently, descending inhibitory system has been shown to exert a tonic inhibition on the itch signalling (3), supporting the notion that the spinal cord is very important in modulation of pruritus (4–7). As a result, mediators and receptors involved in itch signal transmission in the spinal cord's dorsal horn are gaining more attention for the development of new drugs for patients with pruritic disease (4,6,8).

The analgesic effects of cannabimimetic compounds have been known for centuries; however, they cannot be used effectively in clinical settings because of their abuse potential and unwanted central effects, including tolerance and physical dependence (9–12). Cannabinoid drugs have only recently been approved for medicinal purposes, mainly for neuropathic pain, multiple sclerosis, etc. (13–15). Recent experimental research also indicates that cannabinoids elicit antipruritic effects (16–18). Participation of descending serotonergic and noradrenergic systems in spinal modulation of pain has been known for a long time (19,20). Moreover, descending inhibitory pathways are indicated to modulate antinociceptive action of cannabinoids (10,19,21, 22).

Aims of the study

Taking into account the similarities between pain and itch, we aimed to observe attenuation of serotonin-induced itch responses with the cannabinoid agonist WIN 55,212-2 and determine whether neurotoxic destruction of descending serotonergic and noradrenergic pathways play a role in the antipruritic effect of WIN 55,212-2.

Materials and methods

Animals and ethics

Male Balb/c mice (Center of the Laboratory Animals, Trakya University), weighing 20–30 g, were used in the experiments. Mice were maintained under 12–12 h light-dark cycles at the temperature of 21 ± 2°C with water and food available *ad libitum*. The local 'Animal Care Ethics Committee' approved all experimental protocols of this study.

Serotonin-induced scratching behaviour and rotarod assessment

Scratching behaviour was produced by intradermal injection of 50 µg/50 µl of serotonin into the pre-shaved rostral part of the back of the mice. Immediately after intradermal serotonin injection, the animals were put back into transparent acrylic cages individually and scratching of the injected site by the hind-paws was videotaped for 30 min. Typically, the mice produced several scratches per second and such response is counted as one bout of scratching. The videotape was played back to count the number of bouts of scratching.

Motor performance was tested using a rotarod apparatus (Commat, Ankara, Turkey). Latencies to fall of the mice were recorded. A cut-off time of 180 s and a speed of 16 rpm were adapted before assessments.

Experimental procedures and drugs

In order to determine the effect of cannabinoid treatment on serotonin-induced scratches, different doses of WIN 55,212-2 (1, 3, 10 mg/kg) were administered intraperitoneally (i.p.) 30 min before serotonin administrations. In different groups, the neurotoxins 5,7-dihydroxytryptamine (5,7-DHT, 50 µg/mouse) and 6-hydroxydopamine (6-OHDA, 20 µg/mouse) were given intrathecally to deplete serotonin and noradrenaline and to degenerate noradrenergic and serotonergic terminals in the spinal cord. Desipramine (25 mg/kg, i.p.) is given 30 min before 5,7-DHT in order to prevent the uptake of 5,7-DHT into noradrenergic terminals. In another set of experiments, to observe the effects of lesioning of descending noradrenergic and serotonergic pathways on cannabinoid-induced antipruritic action WIN 55,212-2 was administered 3 days after 5,7-DHT and 6-OHDA injections. The doses, treatment times of the drugs and experimental procedures were similar to our previous studies (18,23,24).

Serotonin hydrochloride, WIN 55,212-2, 5,7-DHT and 6-OHDA were purchased from Sigma, whereas desipramine hydrochloride from Tocris. WIN 55,212-2 was given in 20% DMSO, 5% Tween 80, 5% ethanol and 70% saline, while other drugs were dissolved in 0.9% sterile saline.

Statistical analysis

To determine statistical differences between groups, two-way analysis of variance, followed by Bonferroni's *t*-test, were carried out. Values of $p < 0.05$ were considered to be significant. All data was expressed as mean ± SEM.

Results

Effects of the cannabinoid agonist WIN 55,212-2 on serotonin-induced scratches

Intradermal serotonin injection (50 µg/50 µl) elicited marked scratching of the injection site (Fig. 1). Systemic administration of the cannabinoid agonist,

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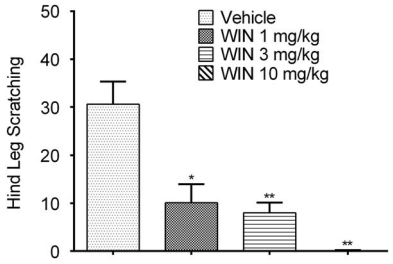

Fig. 1. Effects of systemic (1, 3, 10 mg/kg) injections of the cannabinoid agonist, WIN 55 212-2, on serotonin-induced scratches (two-way analysis of variance, followed by Bonferroni's *t*-test, $*p < 0.005$, $**p < 0.001$, $n = 10$ for each group).

WIN 55,212-2 (1, 3, 10 mg/kg, i.p.), significantly and dose-dependently attenuated serotonin-induced scratches. Its anti-scratching activity was stronger with its higher doses (3, 10 mg/kg), as compared with the lowest dose (1 mg/kg) ($p < 0.005$ and <0.01, respectively, Fig. 1). No scratches were observed with 10 mg/kg dose of WIN 55,212-2 (Fig. 1).

The influence of spinal noradrenalin and serotonin depletion on the anti-scratching activity of WIN 55,212-2

Spinal 5,7-DHT (50 µg/mouse) and 6-OHDA (20 µg/mouse) injections are known to deplete serotonin and noradrenaline in the spinal cord. Reduction of monoamine levels in the spinal tissues with administrations of these neurotoxins is mentioned to be 85% and 90–95% in two similar studies (25,26). Intrathecal pretreatment with neither 5,7-DHT nor 6-OHDA reduced anti-scratching activity of WIN 55,212-2 (Figs 2, 3).

Effects of WIN 55,212-2 on locomotor performance

WIN 55,212-2 impaired locomotor performances only at the highest dose administered, 10 mg/kg ($p < 0.05$, Fig. 4).

Discussion

As mentioned in the introduction, a large number of experimental and clinical researches have been done on the analgesic effects of cannabinoids. On the other hand, although pain is known to share many similarities with itch, cannabinoid effects on pruritus has not attracted much attention and there are very few studies in this area. Previously, Darmani and Pandya (16,27) showed that cannabinoid receptor agonists attenuated scratching responses while cannabinoid antagonists induced scratching behaviours in mice. Similarly, the cannabinoid CB1 receptor antagonist rimonabant-induced pruritic responses,

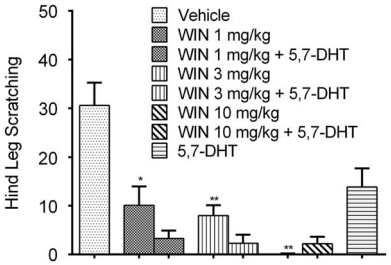

Fig. 2. Effects of intrathecal pretreatment with the neurotoxin 5,7-dihydroxytryptamine (5,7-DHT, 50 µg/mouse) on attenuation of serotonin-induced scratches by WIN 55,212-2 (two-way analysis of variance, followed by Bonferroni's *t*-test, $*p < 0.005$, $**p < 0.001$, $n = 10$ for each group). WIN versus WIN + 5,7-DHT comparisons in the respective groups were not statistically significant.

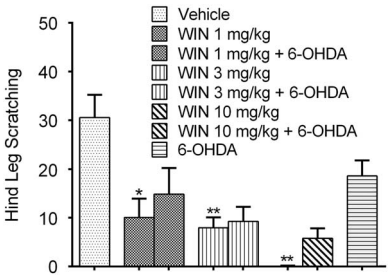

Fig. 3. Effects of intrathecal pretreatment with the neurotoxin 6-hydroxydopamine (6-OHDA, 20 µg/mouse) on attenuation of serotonin-induced scratches by WIN 55,212-2 (two-way analysis of variance, followed by Bonferroni's *t*-test, $*p < 0.005$, $**p < 0.001$, $n = 10$ for each group). WIN versus WIN + 6-OHDA comparisons in the respective groups were not statistically significant.

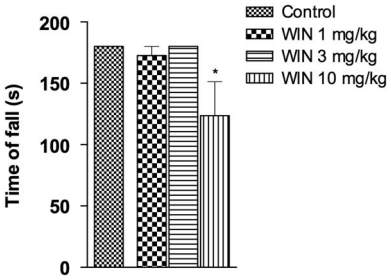

Fig. 4. Effects of different doses of WIN 55,212-2 on spontaneous locomotor activity (one-way analysis of variance, followed by Bonferroni's *t*-test, $*p < 0.05$, $n = 10$ for each group).

and reversal of this effect with mixed cannabinoid agonists have also been indicated (17,28). Moreover, histamine-induced responses are diminished by the potent synthetic cannabinoid HU-210 in human skin (29). Recent research also indicates that scratching behaviour is attenuated by inhibition of endocannabinoid degradative enzymes, fatty acid amide hydrolase and monoacylglycerol lipase, pointing to the

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importance of the endocannabinoid system in modulation of scratching response (18,30). Here, in line with these previous researches, we showed that the cannabinoid agonist WIN 55,212-2 decreased serotonin-induced scratches dose-dependently, no scratches are observed with the highest used dose of WIN 55,212-2. It should be taken into consideration that WIN 55,212-2 impaired motor function at the dose of 10 mg/kg and its anti-scratching activity at the highest dose may be due to impairment in motor control (31–33).

CB1 receptors are known to be the principal receptor in cannabinoid action for both pain and itch responses (17,34). CB1 receptor antagonists induce scratching behaviour, and CB1 receptors are necessary to produce rimonabant-induced scratching (17,30). On the other hand, recent research suggests that cannabinoid CB2 receptors also play role in the modulation of scratching behaviour. CB2, but not the CB1, receptor antagonist has recently been found to reverse the antipruritic effect of the FAAH inhibitor URB597 (18). Spradley et al. (35) indicated that both CB1 and CB2 receptors mediate endocannabinoid modulation of serotonin-induced scratching behaviour. Moreover, new CB2 selective agonists are considered to be promising orally active antipruritic agent candidates (36,37). Thus, both CB1 and CB2 receptors are now implicated to play active roles in reduction of scratching behaviour. Since WIN 55,212-2 is a mixed CB1/CB2 agonist, both CB1 and CB2 receptors may be involved in the measured anti-scratching effect of WIN 55,212-2. Observing the effects of specific CB1 and CB2 agonists and antagonists will improve understanding of the individual roles cannabinoid receptors play in scratching behaviour.

The involvement of the descending monoaminergic systems in the regulation of pain signalling in the spinal cord has been known for many years (19). Similarly, the descending noradrenergic, but not serotonergic, system has been shown to exert a tonic inhibition of itch signalling through α-adrenoceptors (3). Stimulation of both α1-and α2-adrenoceptors and inhibition of noradrenaline reuptake has been found to attenuate scratching behaviour (38–40). Gutierrez et al. (21) demonstrated that cannabinoid antinociception is diminished following neurotoxic destruction of descending noradrenergic pathways. Our findings, showing that neurotoxic destruction of neither serotonergic nor noradrenergic pathways influence the efficiency of cannabinoids to attenuate itch behaviour, are not consistent with these reports. However, we speculate that cannabinoid-induced antinociception and antipruritic activities may develop via different mechanisms.

Our results indicate that cannabinoid receptor agonists attenuate serotonin-induced scratching behaviour. Moreover, we suggest that, unlike their antinociceptive action, neurotoxic destruction of monoaminergic pathways does not affect the antipruritic action of cannabinoids. We conclude that, regardless of the mechanism of action in the spinal cord, cannabinoid receptor agonists might be effective for the treatment of pruritus in mice.

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Conflicts of Interest

None.

Ethical Standards

The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional guides on the care and use of laboratory animals.

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