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Research Article

The role of Cannabinoid Receptors in Visceral Pain Sensation of the Rat: An Interventional Study

Seyed Mohammad Nabavi, Mohammadreza Rahimnejad, Ahmad Asadi Ardebili, and Ramin Hajikhani*

Department of Pathobiology, Faculty of Veterinary Medicine, Islamic Azad University of Karaj, Karaj, Iran

*** *Corresponding author:*** Ramin Hajikhani, Department of Pathobiology, Faculty of Veterinary Medicine, Islamic Azad University of Karaj, Karaj, Iran. Email: hajikhani@kiau.ac.ir

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ABSTRACT

Introduction: Visceral pain, originating from internal organs, represents a challenging aspect of pain management due to its intricate mechanisms and often debilitating nature. Understanding the underlying pathways involved in visceral pain perception is crucial for developing effective therapeutic strategies. The current study aimed to delve into recent advancements in the understanding of cannabinergic modulation of visceral pain perception, focusing on findings from interventional studies utilizing animal models, particularly rats.

Materials and methods: A total of 30 male rats aged 3 months, with an average weight of 220 g were randomly divided into 3 groups. The groups contained the control group which received an intraperitoneal injection of normal saline, the second group received an intraperitoneal injection of anandamide (2 mg/kg), and the third group received an intraperitoneal injection of tramadol (20 mg/kg). The pain in all groups was assessed by an acetic acid test.

Results: The data obtained from the intraperitoneal injection of anandamide to the rats of the experimental group showed a significant decrease in the amount of perceived visceral pain compared to the control group. In addition, the results showed that tramadol injection significantly decreased visceral pain in experimental group 2 compared to the control group. A comparison of the mean experimental groups 1 and 2 showed tramadol as an opioid agonist reduced visceral pain perception to a greater extent than anandamide.

Conclusion: The current study provides evidence for the involvement of cannabinoid receptors in the modulation of visceral pain sensation in rats.

1. Introduction

Pain, an intricate physiological response, serves as a crucial indicator of tissue damage or dysfunction within the body. It is a multifaceted phenomenon, encompassing various sensory and emotional dimensions, deeply entrenched in the intricate web of neurobiological mechanisms[1]. Understanding the mechanisms underlying pain perception is pivotal for developing effective therapeutic interventions, particularly in conditions where pain becomes chronic and debilitating[2].

Pain perception is facilitated by a complex interplay of signaling pathways involving specialized receptors distributed throughout the body[3]. The cannabinergic system has garnered substantial attention among these receptors due to its modulatory role in pain sensation[4]. Cannabinoid receptors, notably CB1 and CB2, are integral components of this system, exerting profound effects on pain processing within the central and peripheral nervous systems[5].

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The visceral pain, which originates from the internal organs, and the involvement of cannabinergic receptors remains a subject of intense investigation[6]. Visceral pain presents a unique challenge in clinical management due to its diverse etiology and complex underlying mechanisms. Unlike somatic pain, which arises from the skin, muscles, or joints, visceral pain often manifests as a diffuse, poorly localized sensation, making it particularly challenging to diagnose and treat[7]. Furthermore, visceral pain can be categorized into distinct subtypes based on its underlying pathology, ranging from inflammatory to neuropathic mechanisms[8]. Inflammatory visceral pain arises from the activation of immune cells and the release of pro-inflammatory mediators within the visceral organs, contributing to hypersensitivity and heightened pain perception[9]. On the other hand, neuropathic visceral pain results from nerve injury or dysfunction, leading to aberrant neuronal signaling and chronic pain states[10]. In this context, elucidating the role of cannabinergic receptors in mediating visceral pain sensation holds significant promise for developing targeted therapeutic strategies[11]. By dissecting the intricate interactions between cannabinoids and pain pathways, researchers aim to uncover a novel approach for pain management, potentially offering relief to millions suffering from visceral pain-related disorders[12].

Expanding upon the distinct types of visceral pain, it's important to note that nociceptive pain, another category, arises from the activation of specialized receptors known as nociceptors in response to harmful stimuli[13]. This type of pain is typically acute and serves a protective function, alerting the organism to potential tissue damage[14]. However, when nociceptive signaling becomes dysregulated, it can contribute to chronic visceral pain conditions, further complicating clinical management[1].

In recent years, preclinical studies utilizing animal models, particularly rats, have provided invaluable insights into the role of cannabinergic receptors in modulating visceral pain perception[15]. By employing various interventional techniques, such as pharmacological manipulation and genetic modification, researchers have been able to dissect the specific contributions of CB1 and CB2 receptors to different aspects of visceral pain processing[16]. These studies have not only elucidated the analgesic potential of cannabinoid-based therapies but have also shed light on the intricate mechanisms underlying their efficacy.

The current study aimed to delve into recent advancements in the understanding of cannabinergic modulation of visceral pain perception, focusing on findings from interventional studies utilizing animal models, particularly rats.

2. Materials and Methods

2.1. Ethical approval

All procedures were approved by the Animal Care Committee of Veterinary Medicine, Karaj Branch, Islamic Azad University. All principles of laboratory animal care and specific international laws for studding on animals were followed.

2.2. Study design

A total of 30 male Wistar rats aged 3 months, with an average weight of 220 g have been bought from Pasteur Institute, Iran, and kept in laboratory conditions with free access to water and commercial pellet food daily. Experimental animals have been kept in standard cages with a minimum of 50 percent humidity, 24°C temperature, and 12 hours of dark/light cycle with appropriate ventilation in a particular cage. The rats were divided into three groups accidently. The groups contained the control group which received intraperitoneal injection of normal saline plus pain assessment by acetic acid test. The second group received an intraperitoneal injection of anandamide plus pain assessment by acetic acid test. the third received an intraperitoneal injection of tramadol plus pain assessment by acetic acid test.

2.3. Intraperitoneal injection

For intraperitoneal injection, a 22-gauge needle was used. The skin of the site of the injection (abdomen) was first disinfected with 70% alcohol, and the animal's head was kept downward so that the intestines entered the anterior region of the abdomen and the injection was performed correctly. In this study, one hour before the start of the test, anandamide (cannabinoid agonist) was injected into the rats of the experimental group at 2 mg/kg by IP method[17] and the rats of the control group were injected with the equivalent of normal saline. In the opioid test group, tramadol was administered intraperitoneal to the rats at 20 mg/kg[18].

2.4. Examining nerve reflexes

To determine the effects of the cannabinergic system on visceral pain, an intraperitoneal injection of 0.6% acetic acid at a dose of 4 mg/kg[19], was used and the number of reflex contractions, each of which lasted a few seconds and was completely recognizable, was observed and counted for 90 minutes. The data every 5 minutes were averaged and statistically analyzed as primary data[20].

2.5. Statistical analysis

The obtained data from the histopathological study was analyzed by using SPSS software (version 19). The results were analyzed in one-way variance analysis (ANOVA) and reported as mean +_standard division. The statistical differences between the treatments and the control groups were checked via T-test at the significance level of $p < 0.05$.

3. Results

The data obtained from the intraperitoneal injection of

anandamide to the rats of the experimental group 1 and 2 showed a significant decrease in the amount of perceived visceral pain compared to control group, which indicated a decrease in nerve stimulation caused by the injection of acetic acid ($p < 0.05$). In addition, the results showed that tramadol injection significantly decreased visceral pain in experimental group 2 compared to the control group ($p < 0.05$). A comparison of the mean experimental groups 1 and 2 showed tramadol as an opioid agonist reduced visceral pain perception to a greater extent than anandamide ($p < 0.05$, Table 1 and Figure 1).

Table 1. Visceral pain results in treated male rats aged 3 months

Case number	Control	Opioid	Cannabinoid
1	133	112	128
2	137	120	129
3	130	119	125
4	132	115	129
5	135	115	123
6	131	120	126
7	136	117	125
8	137	119	127
9	136	120	123
10	137	121	128
Mean ± Standard deviation	134.40^{c} ± 2.675	117.80^{a} ± 2.936	126.30^{b} ± 2.263
P-value	< 0.05	< 0.05	< 0.05

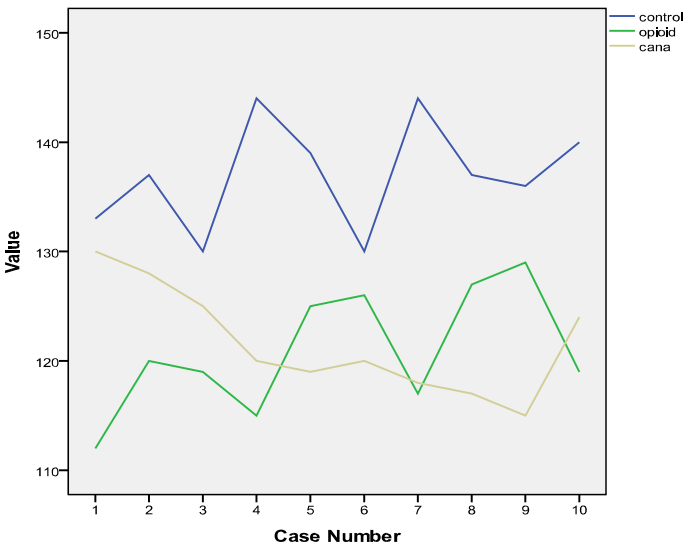

Figure 1. Comparison of visceral pain in treated male rats aged 3 months

4. Discussion

Based on the analysis of the data obtained from the intraperitoneal injection of anandamide to the rats, a significant reduction in visceral contractions caused by the pain induced by the injection of acetic acid was observed, which indicates the reduction of inflammation caused by the injection of acetic acid in the experimental group. In other it can be understood that by administration of cannabinoids, the inflammation in rat decreased. These results were in line with previous studies that demonstrated that cannabinoids can reduce pro-inflammatory and increase anti-inflammatory cytokines in pathophysiological processes[21,22]. It is believed that the anti-inflammatory effects and other effects of cannabinoids on the immune system are due to the presence of CB1 CB2 receptors (cannabinoid receptors 1 and 2) in the cells of the immune system, including lymphocytes[23]. Reducing the production of cytokines and chemokines and increasing the activity of regulatory T cells, which leads to the reduction of immune reactions, are the main anti-inflammatory mechanisms of cannabinoids[24,25]. The endocannabinoid system has a clear immune regulation effect and can be used to suppress immunity and treat immune-mediated injuries in organs such as the liver[26,27]. Also, the cytotoxic, anti-proliferative and immunosuppressive effects of some cannabinoid compounds have made these compounds to be considered as potential candidates for administration in malignancies and autoimmune diseases[28,29].

The results of this study also showed that the stimulation of cannabinoid receptors by the cannabinoid agonist anandamide had anti-inflammatory effects. This finding confirmed the results of other studies on the role of the cannabinoid system in pain relief [30,31]. The anti-inflammatory properties of cannabinoids make these compounds clinically useful because they reduce both inflammation and narcolepsy at the central and peripheral levels[32].

The comparison between anandamide and tramadol highlights the differential efficacy of cannabinoids and opioid agonists in alleviating visceral pain. While both compounds demonstrated significant analgesic effects, tramadol exhibited superior potency in reducing pain perception. This disparity may reflect differences in the underlying mechanisms of action, with tramadol likely exerting its analgesic effects through opioid receptor activation and modulation of pain processing pathways[33]. Furthermore, the observed differences in efficacy between cannabinoid and opioid agonists may be attributed to their distinct mechanisms of action and receptor signaling pathways[34]. Cannabinoid receptors modulate pain signaling through the activation of G-protein-coupled receptors and subsequent regulation of neurotransmitter

release[22], while opioids primarily act on μ-opioid receptors to inhibit pain transmission at the spinal and supraspinal levels[35]. Additionally, cannabinoid receptor activation may exert anti-inflammatory effects, contributing to the attenuation of visceral pain associated with inflammatory processes[21].

5. Conclusion

In conclusion, the current study provides evidence for the involvement of cannabinoid receptors in the modulation of visceral pain sensation in rats. The observed reduction in pain perception following cannabinoid receptor activation highlights their potential as therapeutic targets for managing visceral pain conditions. Future research studies aimed at unraveling the intricacies of cannabinoid receptor-mediated pain modulation hold promise for advancing the understanding of pain mechanisms and developing novel treatments for visceral pain disorders.

Declarations

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Ramin Hajikhani, Seyed Mohammad Nabavi, and Ahmad Asadi Ardebili designed the study and performed the sampling and practical procedures. Seyed Mohammad Nabavi wrote the draft of the manuscript. Mohammadreza Rahimnejad performed the statistical analysis, revise the draft of the manuscript, remove the language errors and check the final version of the article. All authors check the final draft of the article and the statistical results.

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Ethical considerations

This study is written originally without any copy from other published papers. The data are collected based on experimental rules and authors did not use a part of raw or analyzed data in other articles.

Availability of data and materials

All data and related findings of the thesis are prepared for publishing in the present journal.

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